Endophytic *Beauveria bassiana*: a tool for comprehensive plant protection

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DEDICATION

This dissertation is dedicated to all that have supported me in my journey at The University of Tennessee, Knoxville, specially, to my parents, my father Venkateswara Rao Yerukala, and mother, Narasamma Yerukala; my sisters, Bindhu Madavi Yerukala, Geetha Yerukala, and Jayasree Yerukala; my brother, Sushanth Yerukala; and my grandparents: grandmother Balamma and grandfather Durgaiya; grandmother Timmama and grandfather Narasappa. I am fortunate and lucky to study my doctorate at this wonderful university. I would like to thank my major professor, Dr. Bonnie Ownley, for continuous support and encouragement. I could not have done it without her support.

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ABSTRACT

Beneficial endophytes live inside plants and establish a mutualistic relationship with the host plant, obtaining nutrition and shelter, and providing protection to the host against biotic and abiotic stresses. Beneficial endophytes have emerged as alternatives to chemical pesticides for control of plant pests and diseases. The insect pathogenic fungus, *Beauveria bassiana* (Bb), is endophytic in some plant species. Remarkably, endophytic Bb can protect host plants against both insect pests and plant pathogens. However, less is known about the effectiveness of Bb against phytopathogens, especially plant-parasitic nematodes. Therefore, to better understand the body of published research on endophytic Bb, three separate meta-analyses were performed to determine: 1) the rate of colonization of various plant species by endophytic Bb; 2) the efficacy of endophytic Bb against insect pests, and 3) the suppression of plant pathogens by endophytic Bb. Furthermore, the efficacy of Bb against root-knot nematodes was tested with *in vitro* and *in plantae* bioassays. Meta-analysis revealed that Bb most effectively colonized eudicots, angiosperms, annuals, the plant family Brassicaceae and the edible crop, faba bean. Colonization was most effective in leaves and plants with fibrous or tap root systems, and when Bb was applied to foliage of whole plants. Meta-analysis of endophytic Bb efficacy against insects revealed that Bb was most effective against the insect order Thysanoptera, family Crambidae, especially the spotted stem borer. Meta-analysis on phytopathogens indicated that endophytic Bb significantly suppressed plant disease especially white mold and *Zucchini yellow mosaic virus*, an aphid-borne potyvirus. In nematode assays, endophytic Bb was not effective in inhibition of *Meloidogyne incognita* egg hatch or nematode number (immobile or mobile) in both greenhouse and *in vitro* assays. In fact, root galling and egg count/root system was higher than without Bb. In summary, endophytic Bb has highly effective in colonization of diverse plant species, causing mortality of many insect pests and suppressing plant pathogens, but Bb was not effective against southernroot-knot nematode. Overall, the meta-analyses provided comprehensive assessments of the current knowledge on endophytic Bb research in suppressing pests and pathogens, and are valuable tools in further development of Bb as a sustainable plant management strategy.

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LITERATURE REVIEW

Introduction

Microorganisms have evolved different mechanisms to ameliorate the effects of environmental stresses. One such adaptation is an endophytic lifestyle. Endophytes are ubiquitous organisms that live in plant tissues establishing a mutual long-lasting relationship with their host (Yan et al., 2015), obtaining nutrition and shelter, and providing protection to the host against biotic and abiotic stresses. They can occur in any part of the plant including leaves, stems, roots, flowers, and seeds. Endophytes can be bacterial or fungal (Bacon and Hinton, 2014).

The mechanisms of stress protection are poorly understood, but may be either direct or indirect. Direct mechanisms involve the production of antifungal, antibacterial, and antinematicidal compounds (Schwarz et al., 2004), alterations in host physiology (Bultman et al., 2004), competition (Mejia et al., 2004), and mycoparasitism (Zabalgogeazcoa, 2008; Ownley et al., 2008), whereas indirect mechanisms involve priming and regulating innate defense mechanisms of the host plant (Neha et al., 2014). Due to their ability to provide protection against biotic stresses, endophytes have emerged as important alternatives to chemical pesticides for the control of pests and diseases affecting agricultural and horticultural crops.

More recently it has been discovered that some well-known insect-pathogenic fungi that are commercially used in biological control of insect pests are endophytic in certain plants (Vega et al., 2008). One such example is *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Ascomycota), a well-known entomopathogenic fungus. Remarkably, as an endophyte, *B. bassiana* not only protects the host plant against insect pests (Rondot and Reineke, 2013), but it can also provide protection against plant diseases such as root rot (Ownley et al., 2008; 2004; 2010). Plants can be inoculated with *B. bassiana* artificially through seed treatment (Ownley et

al., 2008; Quesada-Moraga et al., 2006) or foliar spray (Russo et al., 2015). These approaches offer exciting possibilities for developing sustainable plant protection strategies utilizing endophytic entomopathogens for management of multiple pests and diseases simultaneously.

As an endophyte, *B. bassiana* can provide season-long protection against tunneling, sucking, mining, and chewing insects, as well as borers, weevils, and stem gall wasps (Akutse et al., 2013; Bing and Lewis, 1991; Lewis and Cossentine, 1986; Bing and Lewis, 1993; Cherry et al., 2004; Quesada-Moraga et al., 2009; Gurulingappa et al., 2010; Akello et al., 2007; Powell et al., 2007; Biswas et al., 2013; El-Deeb et al., 2012; Reddy et al., 2009; Maketon et al., 2013). Endophytic *B. bassiana* can also be effective against soilborne plant pathogenic fungi, and foliar bacterial and viral plant pathogens (Ownley et al., 2008; Griffin, 2007; Jaber et al., 2014). However, less is known about the effectiveness of endophytic *B. bassiana* against bacteria and viruses, and little is known of its efficacy against plant-parasitic nematodes. Also, little is known about the durability of *B. bassiana*-based plant protection when challenged by multiple pests and pathogens simultaneously. Therefore, this research is aimed to address some of these knowledge gaps and to investigate the potential mechanisms of action of endophytic *B. bassiana* against root-parasitic nematodes.

General review on endophytes

Current research has mainly focused on identifying a single endophyte effective against multiple pests, which will likely require identification of endophytes with multiple mechanisms for development of comprehensive, integrated, and sustainable plant protection. The information available on selected entomopathogenic fungi used as inundative biological control agents and shown to act as endophytes, is summarized in this chapter.

Beneficial endophytes protect their host plants from various abiotic and biotic stresses through direct and indirect mechanisms. In the direct mechanism, they produce a diversity of compounds and antimicrobial metabolites like alkaloids, peptides, steroids, terpenoids, phenols, quinones, and flavonoids (Yu et al., 2010), aromatic compounds such as 6,8-Dimethoxy-3-(2'-oxo-propyl)-coumarin and 2,4-dihydroxy-6-[(1'E,3'E)-penta-1', 3'-dienyl]-benzaldehyde, and periconicin B (Teles et al., 2006), mannitol and other carbohydrates, and proline (antioxidant capacity) (White and Monica, 2010) that protect the host plant from biotic or abiotic stress. In the indirect mechanism, the defense mechanisms of plants are activated, including induced systemic resistance (ISR) against herbivores, pathogens, and other enemies (Rodrigues et al. 2008; Saikkonen et al., 1998), and abiotic stresses such as drought (Bae et al., 2009; 2011), high concentrations of heavy metals (White and Monica, 2010; Li et al., 2011) and unfavorable temperatures (Tellenbach and Thomas, 2012). The following brief review is focused on fungal endophytes.

Endophytic fungi

Fungal endophytes are diverse and occur in almost all plants including agricultural crops such as wheat (Larran et al., 2002a), bananas (Cao et al., 2002), soybeans (Larran et al., 2002b), and tomato (Larran et al., 2001). Fungal endophytes can play multiple roles in protecting plants from enemies such as plant pathogens (Wicklow et al., 2005), herbivorous insects (Clement et al., 1994), and plant parasitic nematodes (Elmi et al., 2000) without harming natural enemies. Known endophytic fungi are classified as clavicipitaceous, such as *Neotyphodium coenophialum*, *Cordyceps* spp., *Balansia* spp., *Epichloe* spp., and *Claviceps*, and are found in grasses (Bacon et al., 1977; Bacon and White, 2000), or nonclavicipitaceous, like *Phoma* sp., *Mycophycia*

ascophylli, Arthrobotrys spp., *Fusarium culmorum, Colletotrichum magna*, *Curvularia protuberate*, (Rayner, 1951; Lopez-Llorca et al., 2006; Rodriguez et al., 2008) and *Trichoderma amazonicum* (Chaverri et al., 2011), and found in found in non-vascular plants, ferns, allies, conifers and angiosperms.

The endophytic fungi-mediated plant defense mechanisms against pests and pathogens can be broadly categorized as structural or physical, and biochemical. Structural mechanisms include deposition of compounds like callose, lignin, and phenolic compounds (Jeun et al., 2004), and formation of tyloses, and gum deposition that form physical barriers to restrict pathogen entry (Olivain and Alabouvette, 1999; Schmelzer 2002). The biochemical mechanisms may be direct or indirect.

The pre-existing biochemical mechanism involves *de novo* production of antimicrobial compounds, e.g. phytoalexins, PR proteins, chitinases, β 1, 3-glucanases, β 1, 4-glycosidases, peroxidases (POX), and phenylalanine ammonia - lyase (PAL) (Duijff et al., 1988; Bargabus et al., 2004; Belgrove, 2007; Neha et al., 2014). Many of these induced products are involved in regulation of systemic acquired resistance (SAR), ISR, and other pathways. Endophytes also protect the host plant through other mechanisms such as competition with pathogens for nutrients and space, and mycoparasitism (Zabalgogeazcoa, 2008; Ownley et al., 2008), which restrict pathogen entry and growth.

Entomopathogenic Beauveria bassiana (Bb)

Beauveria bassiana (Balsamo) Vuillemin is a ubiquitous entomopathogenic fungus belonging to the order Hypocreales (Ascomycota). It causes white muscardine disease in insects. The Italian entomologist Agostino Bassi was the first to report the disease in 1763, and he coined the term

muscardine in 1835 (Nirupama, 2014). Initially the causal agent was named *Botrytis bassiana* by Balsamo-Crivelis in honor of Agostino Bassi. Later Veillmuin replaced the genus name with *Beauveria* (Porter, 1973). *Beauveria bassiana* is an anamorph of *Cordyceps bassiana* (teleomorph), a member of the Ascomycota.

Endophytic nature and host crop

Beauveria bassiana can act as an endophyte in different plants. Bing and Lewis (1991) reported on the endophytic nature of *B. bassiana* in *Zea mays* and its ability to colonize the plant. Since then many studies have demonstrated the endophytic nature of *B. bassiana* by artificially introducing it into a plant and determining its efficacy against insects and pathogens. Posada et al. (2007) inoculated *B. bassiana* in coffee seedlings using different methods and isolated the fungus from distant parts after inoculation, further confirming that *B. bassiana* can act as an endophyte. They also detailed endophyte movement in the plant. Akutse et al. (2013) evaluated the endophytic nature of *Beauveria* and *Hypocrea*, on *Vicia faba* and *Phaseolus vulgaris* and found that they successfully colonized both plant species. Similarly, Rondot and Reineke (2013) attempted to establish *B. bassiana* in grape, by spraying a spore suspension on the grape leaf surface, and they obtained successful colonization. In addition, other researchers have demonstrated the endophytic ability of *B. bassiana* in other plants. These include: potato, jimsonweed and cocklebur (Jones 1994), tomato (Leckie, 2002; Ownley et al., 2008; Gurulingappa et al., 2010), snap bean (Ownley et al., 2008), cotton (Jones 1994; Ownley et al., 2008; Gurulingappa et al., 2010), *Theobroma gileri* (Evans et al., 2003), wheat (Gurulingappa et al., 2010; Russo et al., 2015) tobacco and soybean (Russo et al., 2015), bark of *Carpinus carolinata* (Bills and Polishook, 1991), *Theobroma cacao* (Posada and Vega, 2005), common

bean (Parsa et al., 2013), banana (Akello et al., 2007), bean, corn, pumpkin (Gurulingappa et al., 2010), date palm (Gomez- Vidal et al., 2006; Arab and El-Deeb, 2012), jute (Biswas et al., 2012), opium poppy (Quesada - Moraga et al., 2006), radiata pine (Brownbridge et al., 2012), sorghum (Tefera and Vidal, 2009), *Pinus monticola* (Ganley and Newcombe, 2006), artichoke (Guesmi-jouini et al., 2014), and cowpea (Maketon et al., 2013).

Colonization pattern

In nature, plant colonization by *B. bassiana* begins when a conidium lands directly on the host cuticle followed by attachment, germination, and penetration or entry through natural openings into the host plant. Thereby *B. bassiana* proliferates inside the plant and establishes a mutual relationship with the host crop (Ownley et al., 2008). Exact movement and mechanism inside the plant is still unclear. Quesada-Moraga et al. (2006) used electron microscopy to study the colonization pattern of *B. bassiana* following a foliar spray on opium poppy. They discovered proliferation of *B. bassiana* inside the plant 24 to 48 h after inoculation. Only a few conidia germinated and successfully penetrated the leaf. There were no hyphae near the stomatal region. Few hyphae were noted in xylem vessels. However, in maize, *B. bassiana* hyphae colonized xylem vessels, and conidia germinated and entered the plant through the cuticle (Wagner and Lewis, 2000). Further, Maketon et al. (2013) noted *B. bassiana* conidia and hyphae in parenchyma cells and vascular tissue.

Metabolites produced by B. bassiana

Beauveria bassiana produces a diverse array of secondary metabolites, including bassianolides, bassianolone, beauvericin, beauverolides, cyclosporine A, oosporein, oxalic acid. Antibacterial, antifungal, and cytotoxic activity have been reported for these compounds (Grove and Pople,

1980; Genthner et al., 1994; Lopez et al., 2006). However, not all isolates produce all compounds.

Effects of endophytic B. bassiana on plant pathogens

The role of *B. bassiana* as an endophyte and its ability to control plant pathogens, such as *Rhizoctonia solani* and *Pythium myriotylum* in tomato (Ownley et al., 2004, Clark, 2006, and cotton have been studied (Griffin, 2007, Ownley et al., 2008) and reductions in disease severity and disease symptoms of endophyte-treated plants were reported. Similarly, Jaber (2015) found that *B. bassiana* was effective against the downy mildew pathogen *Plasmopara viticola* in grape, where disease severity and symptom expression were reduced. Bishop (1999) and Seth (2001) reported *B. bassiana* controlled *R. solani* in tomato, and Batson et al. (2000) noted season-long protection against *R. solani* in cotton due to endophytic *B. bassiana*.

Jaber et al. (2014) conducted a detailed study on the efficacy of *B. bassiana* against *Zucchini yellow mosaic virus* (ZYMV) in squash and found that disease severity and symptom expression was suppressed in plants inoculated with *B. bassiana*. They also noted that multiplication and accumulation of virus in the endophyte-inoculated plants was reduced, compared to other treatments. This implied that induced resistance was a prime mechanism in the process.

Effects of endophytic B. bassiana on insects

Akutse et al. (2013) evaluated the efficacy of endophytic *B. bassiana* against pea leaf miner (*Liriomyza huidobrensis*) on *Vicia faba* and *Phaseolus vulgaris* and discovered that in endophyte-treated plants there was a noticeable decrease in leaf miner population compared to the control. Rondot and Reineke (2013) demonstrated the endophytic nature of *B. bassiana*

in grape in the greenhouse and showed its effectiveness against vine mealy bug *Planococcus ficus* and black vine weevil *Otiorhynchus sulcatus*. Bing and Lewis (1991) studied the endophytic nature of *B. bassiana* in field conditions with foliar spray and injection methods at the whorl-stage of maize against European corn borer larvae, *Ostrinia nubilalis,* for two consecutive years. Although *B. bassiana* successfully colonized maize with both methods, foliar application was more effective in reducing tunneling by *O. nubilalis* and provided season-long protection to the plant. Lewis and Cossentine (1986) obtained similar results of reduced tunneling by *O. nubilalis* in maize when plants were treated with *B. bassiana* (foliar application) at whorl and pollen shedding stages. Vega et al. (2008) isolated B*. bassiana* from coffee plant, which was effective against coffee berry borer *in vitro*. *Beauveria bassiana* was evaluated against larvae of the stem borer, *Sesamia calamistis*, as a seed dressing (field), topical application (field) and stem injection under greenhouse, laboratory, and field conditions in maize (Cherry et al., 2004). The fungus reduced the population of stem borer, stem tunneling, tunnel length, and dead hearts, but there was no fungal infection of dead larvae, which suggested that death may be due to metabolites released by the endophyte inside the plant (i.e. toxicosis). Quesada-Moraga et al. (2009) studied the effect of endophytic *B. bassiana* against stem gall wasp, *Iraella luteipes*, in opium poppy under field conditions for 3 years and found that the endophyte reduced the population of stem gall wasp. Under greenhouse conditions with plants colonized by *B. bassiana,* reproduction rate and growth of the aphid, *Aphis gossypii,* was lower, and mortality of the locust, *Chortoicetes terminifera* was higher, compared to noninoculated (healthy) plants (Gurulingappa et al., 2010). Endophytic *B. bassiana* reduced oviposition rate, egg hatchability and populations of banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae)

in banana (Akello et al., 2007). Similarly, Powell et al. (2007) reported efficacy against *Helicoverpa zea* in tomato and Guesmi-jouini et al. (2014) reported efficacy against artichoke aphid (*Capitophorus elaeagni*; hemipteran; Aphididae) in artichoke. In addition, effective action of the endophyte was reported against bark beetle, *Hylastes ligniperda,* adults and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae (Ganley and Newcombe, 2006); against stem weevil, *Apion corchori,* in white jute (Biswas et al., 2013); against *Bemisia tabaci* (vector of *Tomato leaf curl virus*) in tomato (El-Deeb et al., 2012); against stem borer (*Chilo partellus*) in *Sorghum bicolor* (Reddy et al., 2009); and against cowpea aphid (*Aphis craccivora*) in cowpea (Maketon et al., 2013).

Compatibility of B. bassiana with Bacillus thuringiensis

Beauveria bassiana alone, and in combination with *Bacillus thuringiensis* and carbofuran was studied for two consecutive years in corn against European corn borer; *B. bassiana* successfully colonized corn, reduced the insect population, and reduced tunneling of first- and second-generation larvae (Lewis et al., 1996). A greater decrease in the population of larva was noticed when all three treatments were used, suggesting that endophytic *B. bassiana* was compatible with *B. thuringiensis*. Its ability to control insect pests was enhanced when used in combination with other organisms and chemicals (Lewis et al., 1996). Lewis et al. (2001) reported that *B. bassiana* could colonize transgenic corn lines.

Role of B. bassiana in induced resistance against plant pathogens

Ownley et al. (2010) provided a detailed explanation of the endophytic nature of the entomopathogenic fungi *B. bassiana*, *Lecanicillium* spp., and *Trichoderma* (Ascomycota: Hypocreales), and their role in controlling plant disease by using induced systemic resistance

(ISR) as one of the mechanisms for defense. Ownley et al. (2008) also reported that ISR was a possible mechanism for reduction of the incidence and severity of disease caused by *X. axonopodis* pv. *malvacearum* in cotton, and *R. solani* and *P. myriotylum* in tomato plants colonized with *B. bassiana*.

Beauveria bassiana against nematodes

Liu et al. (2008) found that soil drenching of tomato plants with cultural filtrates of *B. bassiana* showed nematicidal effects against northern root-knot nematode in both *in vitro* and *in vivo* experiments. *Beauveria bassiana* filtrates reduced egg hatch, and increased nematode mortality under *in vitro* conditions, while in *in vivo* studies, it reduced the population of nematodes in soil and in roots and reduced gall formation and egg-mass production. Lijie et al. (2008) also noted nematicidal activity of *B. bassiana* against four nematodes, i.e. *Meloidogyne incognita*, *Heterodera glycines*, *Aphelenchoides besseyi* and *Caenorhabditis* species under *in vitro* conditions, when cultural filtrates of the fungus were applied to nematodes.

Inoculation methods

Russo et al. (2015) evaluated different inoculation methods, i.e. foliar spray, and seed and root inoculation of *B. bassiana* to establish its endophytic nature in tobacco, corn, wheat, and soybean, and found that the leaf spray method resulted in significant colonization compared to other methods. Posada et al. (2007) evaluated different inoculation methods (foliar, stem injection, soil drench) and found that in coffee, stem injection was the best method. Parsa et al. (2013) found that different parts of the plant colonized by the fungus varied according to the inoculation method used to treat common bean. They observed more colonization of leaf via foliar application and more colonization in roots when soil drench was used, whereas in stem,

the extent of colonization was moderate with both methods. Jaber et al. (2014) evaluated different strains of *B. bassiana* by foliar spray of squash plants with a conidial suspension, and found that all *Bb* strains successfully colonized squash plants. Wagner and Lewis (2000) used conidial suspensions as foliar sprays on the abaxial surface of leaves in maize to study the ability of *B. bassiana* to act as an endophyte.

Ownley et al. (2008) found that seed treatment was effective method for snap bean, cotton, and tomato. A foliar spray was better than a stem injection method to inoculate corn with *B. bassiana* (Bing and Lewis, 1991). Other inoculation methods include soil drenches and immersions (Tefera and Vidal, 2009; Posada et al., 2007), seed coatings (Brownbridge et al., 2012), radicle dressings (Posada and Vega, 2005; Posada et al., 2006), root and rhizome immersions (Akello et al., 2007; Akello et al., 2009), stem injections (Posada et al., 2007), foliar sprays (Tefera and Vidal, 2009; Posada et al., 2007), foliar and seed treatment in opium poppy (Quesada-Moraga et al., 2006), and flower sprays (Posada et al., 2010).

Identification methods for B. bassiana

Generally, microscopic methods play a key role in detection of the location and movement of the endophyte inside the plant. Transmission (TEM) and scanning electron microscopy (SEM), fluorescent microscopy and confocal microscopy has enabled visual tracking of microbes in plants more feasible. Wagner and Lewis in 2000, first visualized Bb penetration inside plant leaf tissues of maize by light and electron microscopy and reported that 3% of conidia germinated on the leaf surface and less than 1% of conidia were able to penetrate via stomata on the leaf surface. Visualization of Bb entering plant tissues from stomata or by wounds has been noted (Quesada-Moraga et al., 2006; Wagner and Lewis, 2000), as well as Bb hyphae between plant

parenchyma cells or in xylem vessels (Wagner and Lewis 2000; Quesada-Moraga et al. 2006; Gomez-Vidal et al. (2006). Appressorium-like structures were not observed in any Bb plant infections, unlike insect infections where it is common (Gomez-Vidal et al., 2006).

Several studies have reported using PCR for identification of B. bassiana from plants (Posada et al. 2007; Quesada-Moraga et al., 2006; Reddy et al. (2013). Castillo et al. (2008) developed a real time PCR assay to detect and quantify *B. bassiana* in ash trees. Landa et al. (2013) developed a two-step nested PCR technique, which increased detection of endophytic *B. bassiana* in opium poppy by 10,000-fold over single step PCR. Development of a GFP-tagged transformant facilitated visualization of Bb colonizing aerial plant tissues of opium poppy, especially intercellular spaces, and leaf trichomes (Landa et al., 2013).

Research Objectives

This research has the following objectives.

Objective 1: Conduct a meta-analysis on the efficacy of endophytic colonization of *Beauveria bassiana* across the plant kingdom.

Objective 2: Conduct a meta-analysis on the efficacy of endophytic *B. bassiana* against insect pests.

Objective 3: Conduct a meta-analysis on the efficacy of endophytic *B. bassiana* against plant diseases.

Objective 4: Assess the impact of endophytic *B. bassiana* on root-knot nematode (*Meloidogyne incognita*) in greenhouse grown tomato plants, and the effect of *B. bassiana* conidia on root-knot nematode hatch and juvenile survival in *in vitro* assays.

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CHAPTER 1

META-ANALYSIS ON THE COLONIZATION EFFICACY OF THE ENDOPHYTIC INSECT PATHOGENIC FUNGUS, *BEAUVERIA BASSIANA*, ACROSS THE PLANT KINGDOM

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Abstract

The insect pathogenic fungus *Beauveria bassiana* (Bb) colonizes several plant species as an endophyte. In the past two decades, many studies have been conducted on endophytic colonization by Bb. Despite the accumulated research literature, the extent of Bb colonization of plants, and the diversity of plant taxa colonized by this fungus is not clear. Additionally, consistency, synthesis, and validation of the research conducted to date has not been quantitatively verified. To fill these knowledge gaps, a meta-analysis was conducted on studies reported from 2002 to 2018 on percentage plant colonization by endophytic Bb under a variety of experimental conditions. For all studies, the analysis was based on a comparison of endophytic Bb treatment versus non-Bb controls. The agricultural database Web of Science was used to identify research publications, and in total, 232 results (published articles) were extracted and screened. From these publications, 1,051 studies (individual treatments) were extracted and analyzed with a random-effects model and Comprehensive Meta-Analysis, Version 3 (CMA) software. Several influential factors that impacted endophytic *Bb* colonization efficacy in plants were determined and analyzed with moderator analysis. There were 33 moderators, including endophytic *Bb* factors (13), plant factors (12) and other experimental factors (8). Overall, the collective research studies had a significant ($P \leq 0.05$) endophytic colonization rate by Bb of 29% across different plant taxa, thus the null hypothesis was

rejected, and the 1,051 studies collected do not share a common effect. Moreover, the collected studies had high heterogeneity (I^2 = 96.93), and no consistency across studies. Moderator analysis revealed that the highest plant colonization was recorded with strain Bb1022 with 66%. Endophytic colonization rates were high with native Bb isolates (30%), and isolates from insects (34%), especially the pine shoot moth (66%). Among research locations, reports from Australia had the highest Bb endophytic colonization rate (53%). Application of endophytic Bb with solid substratum was reported as highly effective with significant colonization in plants (35%). High rates of plant colonization (52%) were found with high concentrations of Bb conidia applied (1 x 10^{12} conidia per ml), and horizontal transmission (29%) of the fungus was more effective than vertical transmission. For plant moderators, colonization by Bb was most effective for faba bean (62%), and the plant family Brassicaceae (58%). Overall, colonization was most effective in angiosperms - eudicots (34%), 36% in annuals, 46% in plants classified with a growth habit classified as forb/herb/vine, and 41% for plants with fibrous or tap root systems. Based on crop distribution, such as agricultural and horticultural classification, Bb colonization was highly effective in horticultural crops (29%), industrial crops (45%), oilseeds (58%), and pulse crops (46%). In regards to different plant parts that had been treated with Bb; colonization was highest (57%) when the fungus was applied to the whole plant. Colonization was 61% when applied to plants at the leaf-stage. Regarding the number of days after inoculation, i.e. incubation period, after 120 to 140 days, colonization was 51%. Colonization was greatest when experiments were performed in growth chambers (37%). Recovery of endophytic Bb from plant tissues plated on culture medium was 64% at 15 days after plating. Confirmation of recovery of Bb from colonized plants was greatest with molecular

techniques (56%), including use of the ITS1/ITS4 primer pair for identification with PCR (71%). Furthermore, higher Bb colonization (57%) was reported when cross-sections of leaf mid-rib tissue were examined for intercellular hyphal growth. Journal articles published during 2007-2011 reported the highest levels of Bb colonization (40%). In these articles, the most studied isolates were Bb GHA (120), native isolates (859), Bb isolated from India (236), isolates from soil from banana plantations (165), and isolates from insects (583), especially from chrysomelid beetle (120). The specific insect source of the Bb isolate used in many (505) studies was not reported. The most studied application method was foliar (397) with horizontal transmission of the fungus (1,035). The concentration of Bb applied to plants most often (549 studies) was 1 x 10^8 spores/ml. The amount of Bb applied to plants was not reported (333) in many research publications, followed by an application amount of Bb of 5 ml (144). Most studies had a colonization range by Bb of 0 to 10% (375), and leaves were the most commonly reported part of the plant that was colonized (432). For plant-related moderators, the most studied included: crop species = maize (163), plant family = Poaceae (271), Eudicots (497), Angiosperms - Eudicots (497), perennials (647), plant growth habit = herb (381), plants with fibrous root systems (426), horticultural crops (685), edible crops (821), fruit crops (320), and 'general' food crops (435). The most studied plant age at inoculation was 28 days (165), leaves were chosen most often for application of Bb (233), the most studied incubation period following application of Bb was 1 to 20 days (374), under greenhouse conditions (547). In most reports, fungal outgrowth from plant tissue on culture medium was recorded at 20 days (200). Identification and confirmation of Bb was most often done with microscopy (692), with confirmation by observations on the morphology of conidia and fungal colony (1,030). Most research publications did not report use

of molecular methods to confirm Bb in the plant (692), but of those that did, elongation factor PCR primers EF349 and EF1685R were reported for 90 studies. However, colonization detected with those primers was very low (<5%) and non-significant. Most studies on endophytic Bb were published during the years 2012-2016 (667), and the majority were published in the journal *Biological Control* (256). Thus, overall research results confirm large variability in endophytic colonization by Bb across a diverse plant kingdom. Hence these findings act as a valuable tool to enhance current understanding and knowledge on the endophytic lifestyle of Bb, and provides new information for development of novel, sustainable, and ecofriendly disease management strategies. These results may also point to future research directions that need to be explored involving plant protection with endophytic Bb.

Introduction

Microorganisms that have evolved endophytic lifestyles are protected from unfavorable environmental conditions and have found new nutritional niches. In the endophyte mode, microbes live within plant tissues without causing any detrimental symptoms (Porras-Alfaro and Bayman, 2011) and establish a mutualistic long-lasting relationship with the host plant (Yan et al., 2015), obtaining nutrition and shelter, and providing protection to the host against biotic and abiotic stresses (Singh et al., 2011).

In this endophytic relationship, microbes and plants have developed a complex ecological habitat, which is often a highly specific symbiotic association (Bacon and White, 2000). In addition, recent research studies suggest that in native ecosystems, all plants are in symbiotic association with microbes including fungi, bacteria, yeast, and others, either in the internal tissues of plants, or on the surfaces of leaves, stems, or roots that can influence plant

performance (Ganley et al., 2004; Van der et al., 2006). Further, plants frequently harbor fungi, either during part or all of their life cycle, or over multiple host generations without causing any symptoms in plants (Wilson,1995; McKinnon et al., 2017); these fungi are known as endophytic.

Fungal endosymbionts or endophytes can be defined as microbes that colonize and live partially or entirely in the internal tissues of their host plant and protect the plant from biotic and abiotic stresses (Singh et al., 2011). Biotic stress tolerance includes herbivorous insect pests and plant diseases. Abiotic stress tolerance includes heat, cold, soil salinity, drought, oxidative stress, and heavy metal toxicity. Endophytic fungi operate by multiple mechanisms and promote crop growth and nutrient acquisition (Singh et al., 2011). These fungi have emerged as a valuable tool and resource in agriculture for crop improvement and protection. Further, endophytes have shown promise as alternatives to chemical pesticides for control of pests and plant diseases due to their ability to protect plants against biotic stresses (Rondot and Reineke, 2013; Arab and El-Deeb, 2012).

In recent years, it was discovered that the well-known insect pathogenic fungus *Beauveria bassiana,* (Balsamo) Vuillemin (Bb) (Hypocreales: Ascomycota), can act as an endophyte and was capable of colonizing different plant taxa (Tefera and Vidal, 2009). Several researchers have established the endophytic habit of Bb in different plant species and tissues, including maize (Ramirez-Rodriguez and Sanchez-Pena, 2016), banana (Akello et al., 2009), sorghum (Reddy et al., 2009), oil seed rape (Lohse et al., 2015; Vidal and Jaber, 2015), opium poppy (Quesada-Moraga et al., 2014), cotton (Lopez et al., 2014), wheat (Sanchez-Rodriguez et al., 2015; Russo et al., 2015), pine trees (Lefort et al., 2016), jute (Biswas et al., 2013) faba bean (Akello and Sikora, 2012), pumpkin (Gurulingappa et al., 2010), tomato (Gurulingappa et al.,

2010), melon (Resquin-Romero et al., 2016), coffee (Posada and Vega, 2006), cocoa (Posada and Vega, 2005), tobacco (Russo et al., 2015), soybean (Russo et al., 2015), and others. As an endophyte, the insect pathogenic fungus Bb not only protects the host plant against insect pests (Rondot and Reineke, 2013), but can also protect against phytopathogens (Ownley et al., 2008). Endophytic Bb can be artificially introduced to plants by various methods, including seed (Sanchez-Rodriguez et al., 2015), foliar spray (Russo et al., 2015), soil application (Tefera and Vidal, 2009), root (Brownbridge et al., 2012), and rhizome (Akello et al., 2007). Bb also exists as a natural endophyte in some plant species, such as pine (Lefort et al., 2016). Thus, there is ample evidence that entomopathogenic Bb can colonize different plant tissues from diverse plant genera. However, the interaction of host and endophyte depend on several external and internal factors, and the relationship can be beneficial to both plant and fungal endophyte, or neutral, antagonistic, or pathogenic (Singh et al., 2011; Mayerhofer et al., 2013; Hardoim et al., 2015; Jumpponen, 2001).

Understanding the functional role of Bb as an endophyte is crucial in determining how the fungus may affect the host plant, its ecology, and its association with other organisms. Plant colonization by endophytic Bb is influenced by several factors, such as experimental conditions, host plant characteristics, inoculation method, environmental conditions, specific interactions between a Bb isolate and a plant species, transmission (vertical or horizontal) of the fungus, differences in rates of colonization of above and below ground plant parts or tissues, plant growth conditions, and interactions with other microorganisms. Other parameters to consider are the nature of Bb formulation, and the consistency of colonization of the host plant. These influential factors (Table 1) on plant colonization by endophytic Bb are addressed through

moderator analysis in this study. To gain a more thorough understanding of the mechanisms underlying the interaction between endophytic Bb and host plant, it is essential to conduct a synthesis of results of different individual studies, which is the overall goal of this research. Furthermore, Bb isolates produce a wide range of bioactive mycotoxins with activity against herbivore insects and phytopathogens (Vey et al., 2001). It is not known if these compounds influence Bb colonization or plant performance, which could cause variability among results and across studies. In addition, the durability of endophytic Bb colonization in different plant species and its long-term effects is unknown. Moreover, Bb formulations and application methods on the plant vary greatly, and variable colonization rates likely depend on environmental conditions, application period, and the instability of conidia exposed to solar rays (Vidal and Jaber, 2015).

A substantial number of reports on endophytic colonization by Bb on different host plants have accumulated. In order to get a broader picture of endophytic Bb research, it is essential to review and assess the evidence for conceptual linkages , including the extent of Bb colonization on different crop species, homogeneity, durability, and the process and development of colonization to further summarize the overall efficacy of endophytic Bb colonization capabilities to draw stronger conclusions for future success.

Individual studies do not provide comparisons or conclusions across all other related studies, and small studies may not provide enough statistical evidence for the observed effect. Different experimental conditions (climate, weather, temperature, plant management, soil) and research methods may be used across many experiments that are focused on the same organism, and the differences may result in diverse results and wide variability of findings

across studies. This variability may lead to different interpretations; and as a result, different conclusions may be drawn or different recommendations may be given. Hence, a common approach is needed to better understand principles, procedures, and practices, including interactions that contribute to the desired effects (Giller et al., 2009). In the current study, the aim was to combine results of statistical analyses from different experiments to identify patterns among studies and relationships that add context to various research studies in the existing literature (Corbeels et al., 2014). This will provide a more comprehensive view of the published research on endophytic Bb and its ability to colonize numerous plant species.

Colonization of plants by endophytic Bb is influenced by environmental factors, which may lead to huge variations between different individual research studies. Understanding the functional role of Bb as an endophyte is crucial in determining how the fungus may affect the host plant, its ecology, and its association with other organisms. Furthermore, Bb isolates produce a wide range of bioactive mycotoxins with activity against herbivore insects and phytopathogens (Vey et al., 2001). It is not known if these compounds influence Bb colonization or plant performance, which could cause variability among results and across studies. In addition, the durability of endophytic Bb colonization in different plant species and its long-term effects is unknown. Moreover, Bb formulations and application methods on the plant vary greatly, and variable colonization rates likely depend on environmental conditions, application period, and the instability of conidia exposed to solar rays (Vidal and Jaber, 2015).

Meta-analysis, which is a quantitative and statistical analysis of the collection of findings from individual studies (Glass,1976; Gurevitch and Hedges, 1999), could be the best solution to

provide evidence-based-findings with systematic, quantitative statistical assessment (Haidich, 2010).

A meta-analysis is also the most convenient way to evaluate and validate components of a model. Meta-analysis is most widely used in medical and social sciences to provide quantitative summaries and to draw overall conclusions from research literature (Morris, 2008; DerSimonian and Laird,1986; Haidich, 2010; Nelson et al., 2015). In the past decade, the application of meta-analysis in agricultural studies has drawn significant attention to obtain a comprehensive conclusion from several research studies (Poeplau and Don, 2015). Thus, this approach was used to quantify Bb colonization efficacy and variability across multiple studies to determine the effect size of Bb treatments, and to identify consistent or inconsistent results across studies (Borenstein et al., 2009). The results of a meta-analysis can be used to provide recommendations on use of an endophytic biological control agent, such as Bb, or provide direction for future research on this topic. Additionally, there are no meta-analysis studies reported on the colonization effect of endophytic Bb isolates on different plant habitats to date.

The aim of this research is to fill various knowledge gaps about endophytic Bb and host plant interactions, and to investigate potential mechanisms of action of endophytic Bb by meta-analysis. The objectives of the study were the following:

1. Utilize a quantitative meta-analysis approach to synthesize and determine the overall summary effect sizes of endophytic Bb colonization in different host plants from published research;
2. Analyze the endophytic percent colonization range in different plant taxa;

3. Determine the influence of various external and internal factors on Bb colonization with moderator analysis;

4. Assess publication bias;

5. Investigate whether the results of endophytic colonization by Bb have been more pronounced or consistent under various experimental conditions over an extended time.

Additionally, in the current study, we aimed to reveal the variation in the interaction of Bb and host plants by selecting moderator factors that influence Bb, the host plant, and the environment in which the Bb-plant interaction occurs. It is essential to identify influential moderator factors to gain an understanding of the relationship between Bb and the host plants it colonizes.

The current research is the first meta-analysis study on Bb focusing on its endophytic nature. By combining the results of different studies on Bb and its plant hosts, these findings may provide direction to future research related to endophytic phytoprotection. More specifically, this quantitative review may enhance our current understanding of Bb as an endophyte, enabling development of sustainable plant protection tools to improve crop productivity and crop protection.

Materials and Methods

Literature search and data extraction (Fig. 1.1)

Data were extracted from the scientific agriculture database “Thompson Reuters” Web of Science on December 3, 2018. Search terms included "*Beauveria bassiana*" AND "endophyte" AND "insects" in the search engine. Eighty-two published articles (results) were extracted from the Web of Science Core Collection. On December 7, 2018, a search was performed with the

terms "*Beauveria bassiana*" AND "endophyte" AND "colonization" AND "plant", and 55 published articles (results) were extracted. An additional 26 published articles were extracted on December 7, 2018 with the search terms "*Beauveria bassiana* AND "endophyte" AND "colonization" AND "plant" AND "crop." In total, 232 published articles were extracted.

The extracted data from published articles were excluded based on the following criteria:

1. absence of colonization data (n=17);
2. data repetition (n=139;
3. non-related or non-relevant data (n=18);
4. bulletins (n=2);
5. review articles (n=5);
6. abstract only (n=2); and
7. book chapters (n=2).

From the final 47 filtered articles, 1,051 studies (treatments) were extracted, and 1,051 effect sizes (n=1051) were obtained. Furthermore, this analysis was focused specifically on data related to Bb endophytic colonization (%) of different plant taxa.

Data extraction

Mean and sample size of each Bb treatment and control (no Bb treatment) for percentage Bb endophytic colonization (%) in plants were extracted from each publication. The sample size was taken as n=2 if LSD/ standard errors were mentioned in the publication. If the sample size was not mentioned in the publication, n=1 was used. If the data were presented in the form of graphs, the software WebPlotDigitizer (Rohatgi, 2011) was used to extract mean values.

Moderator variables

Moderator variables are essential to determine and explain if differences exist across or between studies in the observed output results (Lipsey, 2003). Moderator variables also determine if there is significant heterogeneity in the meta-analysis (Auge et al., 2016). Moderators provide information about why some studies have larger and smaller effect sizes, and they help explain the relation with effect sizes and tell how they are related to each other (Lipsey, 2003).

Endophytic colonization of Bb in different plant taxa was influenced by several external and internal factors or moderators. For moderator analysis, the influencing factors were categorized into three groups; fungal factors (13), host plant factors (12) and other experimental factors (8). Each main category was further divided into several sub-categories that were moderators (33) for the study (Table 1). If moderator mean effect sizes were significant ($P \leq 0.05$) and the Confidence Interval (CI) (95%) did not overlap with 0 or 100; then a common among study variance was assumed (Borenstein et al., 2009; Saxton, A., personal communication, February 20, 2018).

Effect size and meta-analysis

The effect size (ES) term is used in meta-analysis (Cohen, 1969) for standardization of different treatments and computation of group means (Glass, 1976). The means of each study are computed and averaged to provide a mean ES for the findings (Orwin, 1983). Effect size is a measure of the relative magnitude of a relationship between two variables of interest (Møller and Jennions, 2001; Thalheimer and Cook, 2002). Effect sizes explain experimental size effect and allow us to compare the experimental treatment magnitude from one experiment to

another (Thalheimer and Cook, 2002). Effect sizes can be calculated as raw mean differences, or standardized mean differences, or response ratio. For the current study, effect sizes were calculated as raw mean differences and the methodology of Borenstein et al. (2009) was used. Extracted data were analyzed and computed using the statistical package Comprehensive Meta-Analysis Version 3 (CMA) software (Version 3, Biostat, Englewood, USA; 2014).

A random-effects model (REM) was selected for the meta-analyses instead of a fixed effect model (FM). In a FM, the assumptions are: 1) there is one true effect size across all studies; 2) no variation exists between effect sizes; and 3) there is homogeneity (Borenstein et al., 2009). However, in most agricultural studies, the true effect is assumed to vary across the studies, therefore the effect size is variable and heterogeneity exists. Because these are not assumptions in REM, it was considered the model best suited for the current study.

Effect size calculations

Data extracted from each article were analyzed and the effect sizes were calculated as the raw mean difference (MD) by formula (Borenstein et al., 2009).

$$\boldsymbol{MD = Xt - Xc},$$

where Xt is the treatment mean (mean percent colonization of plants treated with Bb) and Xc is the control mean (mean percent colonization of control plants). The non-parametric variance was used to weight variance within studies of each individual study (Auge et al., 2014).

$$\boldsymbol{V} = (\boldsymbol{nt} + \boldsymbol{nc}) / (\boldsymbol{nt} \times \boldsymbol{nc}),$$

where V = Variance, nt = treatment sample size (number of treated plants), and nc = control sample size (number of control plants).

Statistical analysis and heterogeneity analysis

The data were assumed to be non-parametric because several publications did not report standard errors or standard deviations. To determine the presence or absence of heterogeneity, the Q statistic (a measure of weighted squared deviations) was calculated. To quantify heterogeneity, I^2 was calculated as the ratio of true variance (heterogeneity) to total variance (heterogeneity) across the studies (Higgins and Thompson, 2002). Total heterogeneity (Qt) consists of expected variation or within-study heterogeneity (Qw, i.e. sample error mean) and excess variation or between study variation (Qb, i.e. true heterogeneity in the effect sizes among studies).

$$\boldsymbol{I^2 = \left[\frac{Qt - df}{Qt}\right] \times 100\%},$$

where df = degrees of freedom and signifies the expected variation Qw; Qt-df = excess variation or true heterogeneity. The I^2 value may be 0 or negative (-) or positive (+). The I^2 value is set to 0 (%) when df exceeds Qt, i.e. negative I^2 values are set to 0. When I^2 = 0 %, there is no true heterogeneity. The I^2 value is positive (+) when true heterogeneity lies between 0 and 100%. When I^2 values are large, there is larger variation across the studies, and larger I^2 values reflect that a larger proportion of the observed variation is due to true heterogeneity. An I^2 of more than 75% is considered substantial heterogeneity (Haidich, 2010; Higgins and Thompson, 2002). Homogeneity assumptions were considered invalid if the Q-test (P values) for heterogeneity was <0.1 (Iacovelli et al., 2014). The total summary effect, between study variance (T_2), and actual variance (T) were assessed. The summary effect sizes determined were used to illustrate true heterogeneity.

Forest plots were generated from the extracted data to determine the amount of variation between the studies and to estimate the overall result of information from individual studies (Lewis and Clarke, 2001). The P value was used to test the significance of the hypothesis. If $P>0.05$, the studies were considered non-significant and the null hypothesis was accepted, which states that all studies share a common effect. The non-significance could be due to reluctance to publish smaller studies, and they could be assumed as negative results (Haidich, 2010). If $P<0.05$, the studies were significant, and the null hypothesis was rejected, which states that no common effect was shared between studies. These were considered a positive result, and are often seen with larger studies, that were more likely to have been published (Haidich, 2010).

Publication bias

Meta-analysis assumes that the data collected is unbiased (Rosenthal, 1991; Hedges and Olkin, 1985). However, publication bias (PB) occurs when results of published studies are different or not representative of results from unpublished studies (Rothstein et al., 2005; Duval and Tweedie, 2000). PB could occur due to the reluctance of researchers to publish research results that were either non-significant or non-randomized or not interesting (Haidich, 2010). Additionally, PB arises when published and unpublished study findings or strengths or direction of results differ (Møller and Jennions, 2001).

Further, it is the researcher's responsibility to correct or sort out the bias issue of unbiased literature (Møller and Jennions, 2001) in research. However, PB always exists in the meta-analysis one way or another and cannot be eliminated from the dataset. The existence of PB can be identified by meta-analysis, and effect size estimates can be corrected if PB exists in

the data extracted from published articles. The PB can be used to investigate robust conclusions in the sensitivity analysis (Song and Gilbody,1998; Begg and Mazumdar, 1994).

Assessments of PB are mostly indirect (Møller and Jennions, 2001). In the current study, PB was determined using CMA analysis and several parameters were analyzed to confirm whether PB exists in the dataset. Parameters included values and data from a funnel plot, which illustrates whether the dispersion of research studies is symmetrical or asymmetrical in appearance. A funnel plot explains the relationship between effect size and its variance in the form of a graph, plotting sample size or the standard error of the effect size against effect size (Møller and Jennions, 2001). Classic Fail-safe N and Orwin Fail-safe N were used to determine possible missed studies, and their overall effect. The fail-safe number gives information needed to change a given mean effect size adjusted for sample size into an insignificant result, i.e., the number of null results with a mean effect size of zero needed (Møller and Jennions, 2001). Further inclusion of missed studies would shift the effect size toward the null. The significance of Kendall's tau b value was examined and the Egger regression intercept was used to assess bias using precision. Duval and Tweedie's Trim and Fill method was used to determine missing studies. These tests are available through CMA analysis.

Results

According to our meta-analysis results, Bb can live endophytically across a diverse range of plant taxa. In this study, endophytic Bb colonization effect on plants was meta-analyzed by taking into consideration two treatments, endophytic Bb treatment versus without Bb (control). This comparison was screened across all relevant studies published from 2002 to 2018. Overall, 1,051 effect sizes (studies) with 32 moderators were extracted and meta-analyzed, which were

related to colonization of plants resulting from endophytic Bb infection. The overall summary effect size of percent colonization by endophytic Bb was 29% across all plant species studied, with 95% confidence that the true value across studies was expected to fall between 27.24% (Lower Confidence Interval) to 30.78% (Upper Confidence Interval).

Heterogeneity measures

To assess heterogeneity between the studies, several parameters were examined, including Q, T_2, T, and I^2 values. In the current study, the overall P value ≤ 0.05, indicating that there was a significant difference between the studies; this could be due to a high degree of power or larger studies. Significant P values also reflect a minor amount of observed dispersion within precise studies. Additionally, Q statistics were greater than the expected variance (degrees of freedom), evidence that all the studies do not share a common effect and an indication of the presence of heterogeneity. A higher total dispersion of studies might be due to high error within studies or due to differences in the true effect from study to study. Clearly, P and Q values indicate that the null hypothesis, which states that all studies share common effect sizes, can be rejected, thus leading to the conclusion that all 1,051 studies do not share a common true effect size. The 'between study variance' (T_2), an actual true effect, was 782.33, which could be due to excess variation and the effect size index, indicating real differences among studies. The actual variance (T) was 27.97, and the summary effect was 29.01. It is expected that 95% of the true effect will fall in the range of 30.78% ± 27.24%. A high rate of heterogeneity (I^2 = 96.93%) was found among the studies, indicating that 96.93% of the observed variance in the studies is real, and there is a high level of inconsistency among studies.

In the current study, parameters that influenced endophytic colonization of plants by Bb included 32 moderator variables (Table 1).

Endophytic Bb fungus factors

Specific Bb isolates (Fig. 1.2)

Pooling the data for Bb isolates, the effect of isolate Bb1022 was significant with 66% plant colonization, but there were only two studies. However, this isolate was the most effective because other isolates with higher reported colonization rates: ITCC5562 (68%), Bb135 (70%), ITCC 6063 (70%), Bb64 (76%), Bb LPSc 1067 (86%), ATP04 (89%), were non-significant in the meta-analysis. The most studied isolate was GHA (120 studies; 21% plant colonization), followed by G41 (93) and EABb 04/01-Tip (82). For 96 studies, specific isolate identity was not known (NK) or not reported. There was high heterogeneity (I^2) among most isolates and between the studies ($P<0.05$). Isolates with significant differences in percent colonization between control and Bb treatment and smaller confidence intervals (CIs) tended to have larger study numbers (sample sizes), while isolates that were non-significant ($P>0.05$), with no or low heterogeneity, tended have smaller study numbers and large CI compared to others. Overall, Bb colonization was 34% (1,051 studies, $I^2 = 96.93\%$, $P<0.05$).

General Bb isolate type (Fig. 1.3)

Overall, for general isolate type, the most effective isolates were categorized as native isolates (30%) followed by commercial isolates (23%); however, there was not much difference between these. The most studied isolate type was native isolates (859), and there were 192 commercial isolate studies for an overall study number of 1,051. Heterogeneity was found

among and between studies (P<0.05), which had narrow CI and larger sample sizes. Overall, endophytic Bb colonization rate was 27% (1,051 studies, I^2 = 96.93%, P<0.05).

General source of Bb (Fig. 1.4)

In the 1,051 studies, endophytic Bb isolates collected from insects were highly effective and most studied (583), with 34% plant colonization reported overall. Bb isolated from compost had the highest plant colonization rate (68%), but was not significant due to low study number. Other less studied sources of isolates included soil (192), NK-not known (157), plant (110), fruit (8), and compost (1). There was high heterogeneity for most factors, among and between studies with significant differences (P<0.05), narrow CIs, and larger studies with large sample sizes. The exceptions were compost and fruit, which were non-significant P>0.05, with little or no heterogeneity, and smaller study numbers with large CIs compared to others. Overall, Bb colonization was 24% (1,051 studies, I^2 = 96.93%, P<0.05).

Specific source of endophytic Bb (Fig. 1.5)

Endophytic Bb isolated from pine shoot moth were statistically significant with effective colonization in plants (66%). High plant colonization rates were reported for Orthoptera: Tettigoniidae (86%), larva of codling moth (*Cydia pomonella*) (76%), okra (70%), adult European spruce bark beetle (70%), and compost (68%), but these were non-significant due to low study number. Other categories of specific Bb sources were cotton bollworm (64%), white grub (62%), *Timaspis papaveris* (56%), paddy insect (55%), elephant weevil (53%), sorghum chafer beetle (51%), *Busseola fusca* stem borer (50%), soil (49%), monocots (45%), sugarcane borer (43%), maize and sorghum stem tissues (42%), traditional olive orchard (39%), poppy stem gall wasp (38%), boll weevil cadaver (31%), and click beetle (36%). Low colonization rates were reported

when Bb isolates originated from banana plantation soil (29%), NK - not known (27%), legume pod borer (23%), flea beetle (23%), chrysomelid beetle (21%), coffee berries (20%), unidentified curculionid beetle (19%), coffee berry borer (17%), insect (unidentified) (16%), carrot rhizosphere soil (16%), cashew root and stem borer (16%), *Pinus radiata* seedlings (4.7%), mature pine trees (4.3%), oil palm insect (0.0%), black vine weevil adult (0.0%) and dead leaf (unidentified) (0.0%). Most studied included Bb isolated from banana plantation soil (165) followed by NK-not known (157), chrysomelid beetle (120), mature pine trees (90), Busseola fusca stem borer (77), poppy stem gall wasp (71), boll weevil cadaver (52), olive orchard (36), coffee berry borer (32), elephant weevil (30), insect (unidentified) (27), cotton bollworm (25), carrot rhizosphere soil (24), cashew root and stem borer (24), legume pod borer (24), monocots (12), *Timaspis papaveris* (11), flea beetle (11), sorghum chafer beetle (10), click beetle (10), coffee berries (10), traditional unidentified curculionid beetle (6), *Pinus radiata* seedlings (4), Orthoptera: Tettigoniidae (3), soil (3), dead leaf (unidentified) (2), codling moth *Cydia pomonella* larva (2), European spruce bark beetle adult (2), pine shoot moth (2), black vine weevil adult (2), okra (1), white grub (1), sugarcane borer (1), paddy insect (1), maize and sorghum stem tissues (1), oil palm insect (1) and compost (1). Most factors had high heterogeneity among and between studies with significant differences ($P<0.05$), narrow CI, with larger studies and large sample sizes. The exceptions among these sources of Bb were okra, black vine weevil adult, codling moth larva, compost, dead leaf (unidentified), European spruce bark beetle adult, maize and sorghum stem tissues, monocots, oil palm insect, Orthoptera: Tettigoniidae, paddy insect, Pinus radiata seedlings, sugarcane borer, and white grub, which

were non-significant (P>0.05), had no or low heterogeneity, smaller study numbers, and large CI compared to others. Overall, Bb colonization was 35% (1,051 studies, I^2 = 96.93%, P<0.05).

Specific insect source of endophytic Bb (Fig. 1.6)

Pooling all the data for specific insect sources of Bb, the isolate from pine shoot moth was statistically significant with effective colonization (66%) in plants. High colonization rates were reported from Orthoptera: Tettigoniidae (86%), codling moth larva (76%) and European spruce bark beetle adult (70%), but were non-significant. Most studied isolates included NK- not known (505) followed by chrysomelid beetle (120), maize stalk borer (77), poppy stem gall wasp (71), and boll weevil cadaver (52). There were less than 50 studies each in experiments with other insect sources of Bb. Most factors had high heterogeneity among and between studies with significant differences (P<0.05), narrow CI, and larger sample sizes. The exceptions were black vine weevil adult, codling moth larva, European spruce bark beetle adult, white grub, sugarcane borer, oil palm insect, and paddy insect, which were non-significant (P>0.05) with no or low heterogeneity, smaller study numbers and large CI. Overall, Bb colonization was 38% (1,051 studies, I^2 = 96.93%, P<0.05).

Method of application for endophytic Bb (Fig. 1.7)

The solid substratum method of applying Bb (35%) was highly effective and resulted in 35% colonization in plants. In comparison, Bb applications directly to plants had the highest colonization rate (89%), but was non-significant due to low study number. Other application methods and colonization rates were root and rhizome dip (34%), foliar (34%), rhizome injection method (30%), root dip (29%), seed (26%), plant whorl in whorl-stage applications (22%), root drenching (20%), soil (21%) stem injection (20%) and natural colonization (18%).

The most studied methods included foliar (397), seed (196), root drenching (114), and root dip (105). With one exception, all factors had high heterogeneity among and between studies with significant differences (P<0.05), narrow CI, and large sample sizes. The exception was plant, which was non-significant (P>0.05) with no or low heterogeneity and smaller study numbers with large CI compared to others. Overall, Bb colonization was 29% (1,051 studies, I^2 = 96.93%, P<0.05).

Mode of Bb transmission and mode of Bb endophyte presence in the plant (Fig. 1.8A-B)

Endophytic Bb colonization was most effective and most studied with horizontal transmission (29%), with 1,035 studies followed by natural infection (22%) with 12 studies. Both factors had high heterogeneity among and between studies with significant differences (P<0.05), narrow CI, and were larger studies with large sample sizes. In contrast, vertical transmission had a low colonization rate (4.7%) and there were only four studies. It was non-significant (P>0.05), with no or low heterogeneity, and a smaller study number with large CI. Overall, Bb colonization rate was 24% with 1,051 studies (I^2 = 96.93%, P<0.05).

An overview of studies with artificial inoculation and natural infection indicated that Bb was most effective (29% colonization) when plants were artificially inoculated. This method was also the most studied with 1,035 studies compared to natural infection with 16 studies and 18% plant colonization. Both methods had high heterogeneity among and between studies with significant differences (P<0.05), narrow CI and were larger studies with large sample sizes. Overall, Bb colonization was 26% (1,051 studies, I^2 = 96.93%, P<0.05).

Concentration of Bb spores applied (Fig. 1.9)

Endophytic Bb colonization was highly effective and statistically significant with an application concentration of 1×10^{12} spores/ml (64%). Application concentrations with higher colonization rates: 7.5×10^{8} conidia/ml (89%), 5×10^{9} conidia/ml (70%), desirable amount (65%), were non-significant due to low study numbers. The most studied application concentration was 1×10^{8} spores/ml with 549 studies. Most factors had high heterogeneity among studies ($P<0.05$) with narrow CI, with larger studies. Overall, Bb colonization efficacy was 30% (1,051 studies, I^2 = 96.93%, $P<0.05$).

Amount of Bb applied (Fig. 1.10)

Plant colonization with endophytic Bb was highly effective (61%) and statistically significant when 4 ml of a conidial suspension was applied. The only higher colonization rate was described as a desirable amount, which resulted in 65% plant colonization, however this was non-significant due to study number = 1. The most studied amount of Bb applied was described as NK-not known with 333 studies, and 39% colonization. Most factors had high heterogeneity among and between studies ($P<0.05$) with narrow CI and larger studies with large sample sizes. Overall, Bb colonization was 30% (1,051 studies, I^2 = 96.93%, $P<0.05$).

Colonization range (%) of Bb (Fig. 1.11)

Plant colonization with endophytic Bb was highly effective and statistically significant when reported percent colonization values were in the range of 50-60% (53%). There were 115 studies for the 50-60% range. The most studied colonization ranges were 0-10 (375) and 10-20 (125), but these had no heterogeneity, which was not significant ($P>0.05$). Colonization rates in

ranges higher than 50-60% had no heterogeneity and were not significant (P>0.05) due to low study number. Overall, Bb colonization was 50% (1,051 studies, I^2 = 96.93%, P<0.05).

Plant part colonized by Bb (Fig. 1.12)

Plant colonization with endophytic Bb was most effective (47%) and statistically significant in the whole plant factor with 27 studies. Colonization of leaves (432 studies) and roots (213) and stems (172) were studied the most. All four factors had high heterogeneity among and between studies (P<0.05) with narrow CI. Overall, Bb colonization was 26% (1,051 studies, I^2 = 96.93%, P<0.05).

Plant factors

Plant species (Fig. 1.13)

Endophytic Bb colonization most effective in faba bean (62%), with 15 studies, compared to purple coneflower (70%), which had the highest recorded colonization, but was non-significant due to low study number (1 study). Maize was the most studied crop (163), followed by tissue culture banana (141), pine (94), strawberry (90), sorghum (55), tomato (51), and common bean (50). These factors had high heterogeneity among and between studies with significant differences (P<0.05). Overall, Bb colonization was 35% (1,051 studies, I^2 = 96.93%, P<0.05).

Plant family (Fig. 1.14)

Endophytic Bb colonization was most effective in Brassicaceae (58%) followed by Papaveraceae (50%), Cucurbitaceae (45%), Fabaceae (44%), and Solanaceae (40%). The most studied plant family was Poaceae (271), followed by Musaceae (189), Fabaceae (101), Pinaceae (94), Rosaceae (90), and Solanaceae (75). All factors for plant family had high heterogeneity among and between studies with significant differences (P<0.05) and narrow CI with larger studies and

large sample sizes. Overall, Bb colonization was 31% for plant family (1,051 studies, I^2 = 96.93%, P<0.05).

Plant taxa - group (Fig. 1.15)

Endophytic Bb colonization was most effective (34%) and most studied (497) in angiosperms – eudicots, followed by angiosperms – monocots (29%, 460). These factors had high heterogeneity among and between studies with significant differences (P<0.05), and narrow CI and were larger studies with large sample sizes. In contrast, Gymnosperms – Monterey pine (4.3%, 94) was non-significant because the CI extended to < 0. Overall, Bb colonization was 23% (1,051 studies, I^2 = 96.93%, P<0.05).

Plant life span (Fig. 1.16A)

Endophytic Bb colonization was most effective in annuals (36%) followed by perennials (25%). Most studied included perennials (647) followed by annuals (404). Both had high heterogeneity with significant differences (P<0.05) and narrow CI with larger studies and large sample sizes. Overall, Bb colonization was 30% (1,051 studies, I^2 = 96.93%, P<0.05).

Plant growth habit (Fig. 1.16B)

Plant colonization with Bb was highly effective (46%) in the forb/herb/vine group, followed by herb (38%), vine/creepers (33%), graminoids (28%), shrubs (26%), forb or herb (16%), true grasses (12%) and trees (4.3%). Herb was the most studied group (381), followed by graminoid (235), tree (94), forb or herb (90), vine/creepers (81), shrubs (69), forb/herb/vine (65) and true grasses (36). All had high heterogeneity among studies and significant differences (P<0.05) with narrow CI, and were larger studies with large sample sizes. Overall, Bb colonization was 26% (1,051 studies, I^2 = 96.93%, P<0.05).

Root system (Fig. 1.17)

Endophytic Bb colonization was most effective in fibrous or taproots (41%) followed by rhizome (34%), tap roots (31%), fibrous and rhizomatous roots (27%), fibrous roots (24%) and adventitious roots (22%). Most studied included fibrous roots (426), tap roots (343), rhizome (189), fibrous or taproots (51), adventitious roots (34), and fibrous and rhizomatous roots (8). All were characterized with high heterogeneity among and between studies with significant differences (P<0.05), narrow CI, and larger studies with large sample sizes. Overall, Bb colonization of root systems was 30% (1,051 studies, I^2 96.93%, P<0.05).

Crop distribution (Fig. 1.18A)

Endophytic Bb colonization was most effective in horticultural crops with 29.2%, followed closely by agronomic crops (28.7%). Horticultural crops (685) were studied more than agronomic crops (366). Both factors had high heterogeneity among and between studies with significant differences (P<0.05), and narrow CI with larger studies and large sample sizes. Overall, Bb colonization was 29% (1,051 studies, I^2 = 96.93%, P<0.05).

Horticultural classification (Fig. 1.18B)

Endophytic Bb colonization was greatest in (45%) in industrial crops, followed by forage crops (40%), edibles (30%) and ornamentals (6.6%). Most studied included edibles (821) followed by ornamentals (103), industrial crops (91) and forage crops (36). All factors had high heterogeneity among and between studies with significant differences (P<0.05), and narrow CI with larger studies with large sample sizes. Overall, Bb colonization was 30%, (1,051 studies, I^2 = 96.93%, P<0.05).

Horticultural sub-classification based on use (Fig. 1.19)

Endophytic Bb colonization was most effective in oilseeds (58%). There was 70% colonization for plants categorized in temperate gardens and medicinal use; however, colonization was non-significant due to low study number (1). Other groups included drugs and medicinals (50%), vegetables (41%), forage crops (40%), fiber crops (37%), grain crops (28%), fruits (27%), perennial garden and pocket prairie (26%), field and grain crops (12%), beverage crops (10%) and landscape plants (5.2%). The most studied groups were fruits (320), grain crops (235), vegetables (214), and landscape plants (98). These factors had high heterogeneity among and between studies (P<0.05) with narrow CI and were larger studies with larger sample sizes. Overall, Bb colonization was 31% (1,051 studies, I^2 = 96.93%, P<0.05).

Agricultural crop classification (Fig. 1.20)

Endophytic Bb colonization was most effective in pulse crops (46%) followed by cash crops (43%), forage crops (40%), food crops (31%), and grain crops (28%). Food crops (435) were the most studied, followed by grain crops (235), nonfood crops (127), pulse crops (65), and cash crops (67). All factors for agricultural crop classification had high heterogeneity among and between studies with significant differences (P<0.05), and narrow CI with larger studies and large sample sizes. Overall, Bb colonization was 28% (1,051 studies, I^2 = 96.93%, P<0.05).

Plant age at inoculation (Fig. 1.21)

There were many factors for plant age at inoculation, which included time measurements (days to months and plant phenology). Colonization by endophytic Bb was most effective at the leaf-stage, with 61%. Factors with greater colonization rates, such as 112-days-old (100%) and 20-days-old (85%) were non-significant because they both had only one study number. Effective

colonization was found for several factors with high heterogeneity among and between studies (P<0.05), and narrow CI, including 120-140 days (61%), 3-true-leaf stage (60%), 35 days (54%), true leaf stage (44%), 10 days (42%), and 7 days (41%). Most studied were 28 days (165), 15-30-day old seedlings (144), true leaf stage (108), NK - not known (102), seed (98), seedling (61), 60 days (60), 21 days (38), primary shoot stage-sugarcane (36), 9-10 months-old (34), 7 days (30), and leaf-stage (28). Overall, Bb colonization was 30% (1,051 studies, I^2 = 96.93%, P<0.05).

Plant part treated with Bb (Fig. 1.22)

Colonization with endophytic Bb was most effective (57%) when Bb was applied to the whole plant. Colonization rate was higher (58%) when the fungus was applied to the leaf whorl; however, this was non-significant and had low study number. Other plant parts treated with Bb and their colonization rates included leaves (43%), root and rhizome (34%), root (31%), rhizome (29%), and seed (26%). Leaves were studied most often (233), followed by seed (196), seedling (148), root (129), and soil (107). These factors had high heterogeneity among and between studies with significant differences (P<0.05), narrow CI, and larger sample sizes. Overall, Bb colonization efficacy was 30% (1,051 studies, I^2 = 96.93%, P<0.05).

Experimental factors

Country where research was conducted (Fig. 1.23)

Research conducted in Australia reported that colonization of plants with endophytic Bb was highly effective and significant, with 53% colonization. In contrast, research in Denmark had the least colonization (0.00%). The most studied included India (236) followed by Spain (148), and Uganda (141) with plant colonization percentages of 26%, 43%, and 33%, respectively. Most locations recorded high heterogeneity (I^2) among and between studies (P<0.05), with narrow

CI, and larger studies with large sample sizes. Studies from Argentina, Mexico, Kenya, Cuba, and Denmark were insignificant (P>0.05), and had no or low heterogeneity and smaller study numbers with large CI compared to others. Overall, Bb colonization rate for these different locations where the research was conducted was 32% (1,051 studies, I^2 = 96.93%, P<0.05).

Experimental conditions (Fig. 1.24)

Bb colonization of plants was most effective (37%) under growth chamber conditions, followed by screen house (34%), greenhouse (30%), field (29%), laboratory (19%) and glasshouse (17%). Most studied was greenhouse (547), followed by screen house (189), glasshouse (168), growth chamber (76), field (65) and laboratory (6). All factors, except laboratory, had high heterogeneity among and between studies with significant differences (P<0.05) and narrow CI with larger sample sizes. The factor laboratory was non-significant because the CI extended to <0 and the study size was low compared to other factors. Overall, Bb colonization was 29% (1,051 studies, I^2 = 96.93%, P<0.05).

Incubation period after plant inoculation (Fig. 1.25)

Following application of Bb to plants, there was an incubation period ranging from days to years, or based on plant phenology. An incubation period of 120-140 days had the most effective colonization (51%), followed 5-6 true leaves (39%), 1-20 days (38%), 20-40 days (35%), third true leaf stage (24%), and 40-60 days (24%). Most studied included 1-20 days (374), 20-40 days (249), 40-60 days (169), 60-80 days (74), 100-120 days (47), and 80-100 days (31). These factors had high heterogeneity among and between studies with significant differences (P<0.05), and narrow CI with larger studies and large sample sizes. Overall, Bb colonization rate was 21% (1,051 studies, I^2 = 96.93%, P<0.05).

Bb growth observed in culture (after plating) (Fig. 1.26)

The most effective culture period for observation of fungal growth in culture was 15 days with 64% colonization, followed by incubation period NK - not known (54%), 10 days (46%), 7 days (44%), 12 days (42%), 14 days (38%), 45 days (33%), 28 days (32%), 21 days (31%), and 30 days (27%). Most studied included 20 days (200), followed by 14 days (187), daily observation (108), 12 days (115), 10-14 days (90), 10 days (85), 28 days (73), 21 days (44), 30 days (40), 7 days (30), and 15 days (24). Most factors had high heterogeneity among and between studies with significant differences ($P<0.05$), narrow CI and were larger studies with large sample sizes. Non-significant factors were 4 days (10%), 11 days (14.5%) and 35 days (9.5%), which were non-significant ($P>0.05$) with no or low heterogeneity, smaller study numbers with large CI, or CI that extended to <0. Overall, Bb colonization rate was 31% (1,051 studies, $I^2 = 96.93\%$, $P<0.05$).

Confirmation method for endophytic Bb (Fig. 1.27)

The presence of Bb in plants was confirmed with microscopy, molecular tests, or a combination of these two methods. Confirmation with molecular methods revealed 56% colonization, followed by microscopic methods (33%), and a combination of these methods (20%). Most studied was microscopic (692), followed by combined molecular+microscopic (344) and molecular (15). All had high heterogeneity among and between studies with significant differences ($P<0.05$), and narrow CI with larger studies with large sample sizes. Overall, Bb colonization was 34%, (1,051 studies, $I^2 = 96.93\%$, $P<0.05$).

Plant tissue used for confirmation of endophytic Bb (Fig. 1.28)

The most effective and significant colonization (57%) was noted with examination of leaf mid-rib cross sections for intercellular hyphal growth. Examination of leaf epidermal tissue and

vascular bundles for hyphal growth had a higher colonization rate (79%), but was not significant because the confidence interval extended to >100%. Other studies reported confirmation of endophytic colonization of Bb from leaf and root samples (19%), and hypocotyl (4.7%), but neither were both significant. A large majority of studies reported a lower colonization rate (29%) and confirmed endophytic Bb based on morphology of conidia and colonies growing in culture media. Use of fungal characteristics to confirm presence of Bb corresponded to the reported number of studies for microscopic methods of confirmation (1,030). A smaller number of studies reported use of leaf and root samples (6), mid-rib cross-sections of leaves (6), leaf epidermal and vascular bundles (5) and hypocotyl tissue (4), which corresponded to the number of studies that used molecular methods for confirmation of Bb (15, Fig. 2.28). Factors involving 'conidia and colony morphology' and 'intercellular hyphal growth in the leaf mid-rib' had high heterogeneity among and between studies with significant differences ($P<0.05$). Confidence interval width was inversely related to sample size. Overall, Bb colonization efficacy was 38% (1,051 studies, $I^2 = 96.93\%$, $P<0.05$).

PCR primers for Bb detection (Fig. 1.29)

Plant colonization was most effective (71%) for endophytic Bb when ITS1/ITS4 primer pairs were used for detection. The highest colonization was recorded when primer RD1-F (100%) was used, but this was non-significant due to low study number. Others primers included Bt (64%), SCAR (47%), 5'CGGCGGACTCGCCCCAGCCCG 3'/3'CCGCGTCGGGGTTCCGGTGCG 5' (49%), ITS5/ITS4 (33%), NK-not known (30%), EF52F 5'-3'/EF481R 5'-3'v (4.7%), and EF349/EF1685R (4.3%). The most studied was NK-not known (896), which for this study was defined as the absence of molecular methods for detection). Study numbers for primer pairs were:

EF349/EF1685R (90), ITS1/ITS4 (15), ITS5/ITS4 (12), 5'CGGCGGACTCGCCCCAGCCCG 3'/ 3'CCGCGTCGGGGTTCCGGTGCG 5' (12), Bt (12), SCAR primers (9), EF52F 5'-3'/EF481R 5'-3' (4) and RD1-F (1). All factors, with two exceptions, had high heterogeneity among and between studies (P<0.05), narrow CI, with larger studies and larger sample sizes. Primer pair EF52F 5'-3'/EF481R 5'-3' and RD1-F were non-significant (P>0.05) with no heterogeneity, smaller study numbers, and large CI compared to others. Overall, Bb colonization was 42% (1,051 studies, I^2 = 96.93%, P<0.05).

Publication years (Fig. 1.30)

Endophytic Bb colonization was highly effective in studies published in articles during 2007-2011 (40%), followed by 2017-2018 (27%), 2012-2016 (26%), and 2002-2006 (26%). Most studies on endophytic plant colonization with Bb were published during 2012-2016 (667), followed by 2007-2011 (236), 2017-2018 (119), while the lowest number was recorded in 2002-2006 (29). All publication period factors had high heterogeneity among and between studies (P<0.05), with narrow CIs, and were larger studies with large sample sizes. Overall, Bb colonization for these studies was 30% (1,051 studies, I^2 = 96.93%, P<0.05).

Journal (Fig. 1.31)

Endophytic Bb colonization was highly effective in studies published in the journal Asia-Pacific Entomology (64%), compared to *AMB Express* (100%), which recorded the highest colonization rate, but was non-significant due to low study number. Other journals were *Southwestern Entomologist* (75%), *Symbiosis* (70%), *Asia-Pacific Entomology* (64%), *Current Science* (61%), *PloS ONE* (57%), *Microbial Pathogenesis* (50%), *Phytoparasitica* (47%), *Science and Technology* (45), *Insect Science* (42%), *Journal of Invertebrate Pathology* (39%), *Uttar Pradesh Journal of*

Zoology (35%), *Entomological Science* (33%), *BioControl* (31%), *Entomologia Experimentalis et Applicata* (30%), *Scientia Horticulturae* (26%), *Biological Control* (24%), *Journal of Berry Research* (16%), *Fungal Ecology* (16%), *Indian Journal of Microbiology* (15%), *Jove-Journal of Visualized Experiments* (14%), *Environmental Entomology* (15%), *Crop Protection* (12%), *Mycoscience* (10%), *New Zealand Plant Protection* (4.7%) and *Microbial Ecology* (0.0%). While most studied include *Biological Control* (256), *Journal of Invertebrate Pathology* (184), *Indian Journal of Microbiology* (144), *Journal of Berry Research* (90), *BioControl* (74), *Fungal Ecology* (50), *Uttar Pradesh Journal of Zoology* (48), *Current Science* (28), *Asia-Pacific Entomology* (24), *PloS ONE* (22), *Microbial Pathogenesis* (18), *Mycoscience* (16), *Scientia Horticulturae* (16), *Entomologia Experimentalis et Applicata* (13), *Crop Protection* (12), *Environmental Entomology* (9), *Insect Science* (10), *Phytoparasitica* (9), *Jove-Journal of Visualized Experiments* (6), *Mycopathologia* (5), *New Zealand Plant Protection* (4), *Science and Technology* (2), *Microbial Ecology* (2), *Southwestern Entomologist* (2), *Symbiosis* (1) and *AMB Express* (1). However, most factors had high heterogeneity among and between studies with $P<0.05$, and had significant differences, with narrow CI, and were larger studies, with large sample sizes. The exceptions were *AMB Express*, *Crop Protection*, *Environmental Entomology*, *Jove-Journal of Visualized Experiments*, *Microbial Ecology*, *Mycopathologia*, *Mycoscience*, *New Zealand Plant Protection*, *Southwestern Entomologist* and *Symbiosis*, which were non-significant $P>0.05$, had no or low heterogeneity, and were consistent, with smaller study numbers and large CI compared to others. Overall, Bb colonization rate was 35% (1,051 studies, $I^2 = 96.93\%$, $P<0.05$).

Publication bias (PB) (Fig. 1.32)

In the current study, there was no evidence of PB in the data set. According to the funnel plot, the point estimates of the studies were dispersed symmetrically in the graph. Additional tests available through CMA, also indicated no publication bias. Duval and Tweedie's Trim and Fill test suggested that there was little change in trim and fill values when studies were trimmed. Overall, there was no publication bias recorded in this study, but indirect or unknown bias may always exist.

Discussion

Beauveria bassiana has gained special attention in the last two decades due to its multifaceted capabilities, such as the ability to colonize plants endophytically (Resquin-Romero et al., 2016), protect plants from insect pests by causing white muscardine disease (Prasad and Syed, 2010; Dara et al., 2013) and protect plants from phytopathogens (Ownley et al., 2008). Various studies have provided evidence that endophytic Bb could be one an alternative control measure to minimize pesticide usage in agriculture (Lacava and Azevedo, 2014). More recently, several researchers have examined the colonization efficacy of endophytic Bb and the possibility of advancing its use. A large amount of data has accumulated on Bb as an endophyte and its colonization efficacy in plants, but most of the studies were non-quantitative (Hawkins et al., 1993). Therefore, we performed a quantitative analysis to validate and illustrate the findings, and draw conclusions from various scientific studies conducted over the past two decades on the ability of Bb to act as a plant endophyte (Augé et al., 2016).

Meta-analysis was used to assess the efficacy of plant colonization by endophytic Bb because authentic data is generated, and this method is considered a powerful statistical tool

to evaluate and validate research results accumulated over years. The best meta-analysis technique depicts study-to-study, qualitative and quantitative analysis, and enables determination of the most effective and influential treatments overall, as well as variations and comparisons with each treatment, among and between studies, or within individual studies (Chandrasekaran et al., 2014; Gurevitch and Hedges, 1999; DerSimonian and Laird,1986). Meta-analysis also assesses and estimates synthetization, abstraction, authentication, observation evidence, advancement, integration, inter-relation, interpretation, consistency, simplification, summarization, precision, and standardization of data, as well as assessment of results and systematic output, and pooled systematic findings (Russo, 2007; Gurevitch and Hedges,1999; Glass, 1976; Haidich, 2010).

Furthermore, meta-analysis qualifies the extent of different treatment performances and examines the capacity and potential effects of treatments across similar independent or individual studies (Russo, 2007; Gurevitch and Hedges, 1999; Glass, 1976; Haidich, 2010). Meta-analysis can also ensure that an integrated outcome of results from a large number of studies can provide an outcome that could be more desirable and feasible for future use, and can improve the precision of estimates of effective studies, and provide opportunity to evaluate or examine new hypotheses, and results are more valuable and powerful than any individual study analysis (Haidich 2010).

Meta-analysis allows results from different individual or independent studies to be cumulatively evaluated, which helps to detect even small effects easily, and compare the effect sizes of different variables (Rosenberg et al., 2004; Fisher, 2015.). Further, meta-analysis enables identification of sources of variation, and heterogeneity between and among individual

studies that could lead to more effective treatments or modifications of management (Haidich 2010). Moreover, by meta-analysis, a global image can be generated of significant outcomes or results and risks from large amounts of data (Ioannidis et al., 2005).

Our current meta-analysis study aims to answer frequently asked questions about what factors influence the efficacy of endophytic Bb plant colonization and its impact on plant health. More specifically, the aim was to review, qualitatively and quantitatively, the efficacy of endophytic Bb colonization in diverse plant species, as well as clarifying analytical procedures and protocols via statistical assessment with meta-analysis (Borenstein et al., 2009). By determining existing strengths and weakness of published experiments we aim to provide recommendations (sample size, treatment combinations, and procedures) to enhance plant colonization with Bb.

In recent years, use of meta-analysis in agriculture has increased; however, compared to meta-analysis usage in medical studies (Haidich, 2010), the approach is in an early stage in agriculture (Alston et al., 2000; White and Waddington, 2012; Philibert et al., 2012). Topics of meta-analysis studies in agricultural sciences have included diverse subject matter such as weather parameters that effect and influence crop yield (Thiam et al., 2001), crop disease severity (Rosenberg et al., 2004), plant species diversity (Bengtsson et al., 2005), environment factors that influence agronomic characters of plant species (Philibert et al. 2012), efficacy of biocontrol agents for management of plant pathogens (Chandrasekaran et al., 2016; Ojiambo and Scherm, 2006), biocontrol agent efficacy for control of insect pests (Stiling and Cornelissen, 2005 and Chaplin-Kramer et al., 2011), geographic regions for phytopathogens (Rosenberg et

al., 2004), environmental effects on organic farming (Lee et al., 2015) and agri-production (Clark and Tilman, 2017).

Meta-analysis studies have also been conducted on mycorrhizal fungi including colonization of plants (Maltz and Treseder, 2015), response to soil nutrients (Treseder, 2004), arbuscular mycorrhiza fungi colonization in plants and their influence on plant biomass (Veresoglou et al., 2012), effects on plant growth characters and its relationship to plant host species (Mayerhofer et al., 2013), host plant micro-symbiosis (Larimer et al., 2010; Karst et al., 2008), and influence on host plant biomass and plant association (Karst et al., 2008). Overall, there are very few meta-analysis studies on endophytic fungi reported (Mayerhofer et al., 2013), and there are no quantitative meta-analysis reviews on endophytic Bb colonization efficacy in plants reported to date. Hence, the current research could be a stepping stone for future researchers in exploring new strategies, and better understanding the capabilities of endophytic Bb plant colonization.

In the current meta-analysis, we provided quantitative evidence by pooling results of endophytic Bb colonization of plants that included 1,051 studies conducted over the last two decades. We discovered strong evidence that supports the capacity of *B. bassiana* as a plant endophyte. Our results support the early conclusions of Bing and Lewis (1991, 1992a, 1992b, 1993), who demonstrated Bb establishment as an endophyte in corn by artificial inoculation of Bb into the corn plant. Many studies that evaluate the endophytic colonization nature of Bb in plants by artificial introduction techniques have been conducted since the original discovery (Quesada-Moraga et al., 2009).

In addition, Bb can occur as a natural endophyte in several plant species (Brownbridge et al., 2012). Likewise Researchers have demonstrated that Bb can be established as an endophyte successfully in several plant species (Parsa et al., 2013) by artificial inoculation in tomato (Yerukala and Ownley, 2016; Resquin-Romero et al., 2016), corn (Russo et al., 2015; Ramirez-Rodriguez and Sanchez-Pena, 2016), oilseed rape (Vidal and Jaber, 2015; Lohse et al., 2015), faba bean (Vidal and Jaber 2015), melon, alfalfa (Resquin-Romero et al., 2016), sorghum (Mantzoukas et al., 2016; Reddy et al., 2009), pine (Lefort et al., 2016), wheat (Russo et al., 2015; Sanchez-Rodriguez et al., 2015), tobacco (Russo et al., 2015), cotton (Lopez and Sword, 2015), haricot beans or beans (Behie et al., 2015), opium poppy (Quesada-Moraga et al., 2014), white jute (Biswas et al., 2013), banana (Prabhavathi et al., 2013), jute (Biswas et al., 2012), pumpkin (Gurulingappa et al., 2010), tissue cultured banana (Akello et al., 2007), coffee crop (Posada et al., 2007), cocoa (Amin et al., 2014), cauliflower (Razinger et al., 2014), soybeans (Russo et al., 2015), onion (Muvea et al., 2014), rice (Jia et al., 2013), squash (Jaber and Salem 2014), grapevine (Jaber, 2015), pumpkin (Gurulingappa et al., 2010), artichoke (Guesmi Jouini et al., 2014), strawberry (Dara et al., 2013), date palm (Gomez-Vidal et al., 2006 and 2009) and cassava (Greenfield et al., 2016). However, debates have been raised over application procedures and protocols to use with endophytic Bb. As this study revealed, there are many variations across and between published studies.

The process of colonization by endophytic Bb in plants may be influenced by several multitrophic interactions that occur in nature (Rodriguez, 2016). Hence in our study, we validated several influential variables that interact with the endophytic Bb fungus colonization process, symbiosis, or mechanism.

According to our meta-analysis, Bb isolated from native or indigenous species performed better in plant colonization. In addition, native Bb isolates were most studied or researched for Bb endophytic studies compared to commercially available Bb isolates. Our results are in accordance with Sery et al. (2016), who evaluated the arbuscular mycorrhizal fungi (AMF) capability in cassava for plant growth promotion and stress tolerance in the field conditions. They discovered that the native AMF application significantly improved crop yield as well as tolerance to biotic and abiotic stress. This suggests that native isolates can lead to the plant growth promotion and enhance tolerance to biotic and abiotic stresses. Estrada et al. (2013), also reported that native arbuscular mycorrhizal fungi colonization in plants improves plant tolerance to physiological stresses.

When native strains of Bb were used, there was more effective suppression of triatomine bugs *Triatoma infestans* (Klug) (Lecuona et al., 2001), found that. Lockwood (1993) preferred native indigenous isolates for microbial control in consideration of environmental and ecosystem safety. Local native entomopathogenic Bb caused high insect mortality of Mediterranean fruit fly (*Ceratitis capitata*) (77%) (Qazzaz et al., 2015). Endophyte colonization efficacy could also depend on the source of isolation from the same or different host species (Mayerhofer et al., 2013) or by geographical region. Hart and Reader (2002) suggested that AM fungi have diverse colonization strategies and colonization pattern may differ taxonomically. In some cases, fungi tend to preferentiality colonize specific crop species, e.g., the AM fungus *Glomus intraradices* showed preference for colonization of potato (*Solanum tuberosum* L.) roots (Cesaro et al., 2008).

In this meta-analysis, percent plant colonization was greatest when a foliar spray or a topical application of Bb was administered to the whole plant. This could be attributed to less competition with other microbes when applied to foliage, compared to when applied to soil or roots where there is likely more competition with other microbes already in soil for nutrients or survival.

Ojiambo and Scherm (2006) suggested that the application method for a microbe has a significant impact how it colonizes plants. Application procedures and protocols for inoculation by Bb have not been standardized and have rarely been compared. As this study revealed, there are many variations across and between published studies. In this meta-analysis, percent plant colonization was greatest when a foliar spray or a topical application of Bb was administered to the whole plant. This could be attributed to less competition with other microbes when applied to foliage, compared to when applied to soil or roots where there is likely more competition with other microbes already in soil for nutrients or survival.

Furthermore, the most studied application method for Bb was foliar spray on leaves. These results support the findings of Russo et al. (2015), who demonstrated that when Bb was artificially inoculated on various crops (tobacco, wheat, corn, and soybean), foliar or topical spray of leaves produced the highest colonization rates with Bb, compared to seed inoculation and root immersion methods. Similar results were obtained with topical Bb application in maize (Cherry et al., 2004). Resquin-Romero et al., 2016 suggested that when Bb was directly sprayed on plant tissue it caused temporary establishment of the fungus that led to systemic and non-systemic colonization of the crop, thereby improving the overall Bb treatment efficacy. Posada

et al., 2007 inoculated Bb by foliar spray, stem injection, or soil drench in coffee plants and found that Bb recovery was greater with the direct injection method.

Additionally, differences in efficacy between different application methods likely depend on the time applied, incubation period, and crop species. Further, the time needed for colonization differs with crop species (Russo et al., 2015). Endophytic Bb colonization also depends on the inoculation method and fungal isolate applied. Generally, to determine endophytic colonization, microbes are isolated from plant samples or plant tissues distant from the place of plant inoculation (El-Deeb et al., 2012), which shows that the endophytic microbe can move from one part of the plant to another (Posada et al., 2007).

Other application methods that also show significant Bb colonization or post recovery of Bb when inoculated for endophytic establishment, include injection of Bb inoculum into the plant (El-Deeb et al., 2012; Posada et al., 2007; Cherry et al., 2004), root (Rodriguez, 2016), dip plants in Bb conidial suspension (Akello et al., 2007), and Bb seed dressing or seed coating (Rodríguez, 2016, Ownley et al., 2008; Cherry et al., 2004). Posada et al., 2007 inoculated Bb by foliar spray, stem injection, or soil drench in coffee plants and found that Bb recovery was greater with the direct injection method.

The mechanism of Bb transmission to plants is primarily horizontal, which was the most studied mechanism and had the highest plant colonization rate in the meta-analysis. It has been suggested that Bb is a facultative endophyte that may be more frequently horizontally transmitted than other microbes, and could inhabit simultaneously both internal and external plant parts and tissues (Santamaria and Bayman, 2005). Therefore, for detection or establishment of endophyte association with plants, colonization by Bb at different plant life

stages and with plant lifestyles must be considered (Unterseher et al., 2013; McKinnon et al., 2017). Endophytic Bb can also occur as a natural endophyte in some plant species and can be transmitted to various generations of offspring through seed production (Quesada-Moraga et al, 2014).

Moderator analysis revealed that Bb colonization in leaves was effective and most studied. Our results are in accordance with Biswas et al. (2012), who demonstrated that when Bb was artificially applied to jute, higher colonization rates were found in leaves (56%) compared to stems (13%) and capsules (42%). Similarly, when Bb was inoculated by foliar spray, seed immersion and root immersion into tobacco, corn, wheat and soybean at 7, 14, 21 and 28 days, the highest colonization rates differed by plant species and inoculation method. Colonization was 100% in tobacco seedling treated by foliar spray at 7 days, 40% in wheat leaves treated by leaf foliar spray at 14 days, 7.8% in corn seedling treated at 7 days by leaf foliar spray, and 24% in leaves of soybean seedlings treated by leaf foliar spray at 7 days (Russo et al., 2015).

Among different types of plants, faba bean, members of the plant family Brassicaceae, eudicots, annual plants, forb/herb/vine, plants with fibrous or taproots, horticultural crops, industrial crops, oilseed crops, and pulse crops had higher rates of Bb colonization. Most research on Bb plant colonization was done with the following factors: maize, members of the plant family Poaceae, eudicots, especially angiosperms, perennial, herb, fibrous root, horticulture crops, edibles, fruits, and food crops. Our data output confirms that Bb exhibits a wide range of colonization strategies that differ by plant species. It has been suggested that each plant species has a specific association with endophytic fungi (Cesaro et al., 2008).

Endophytic fungi may have a wide host range and their colonization patterns may differ based on the specific host plant tissues they colonize (Sridhar, 2012). Endophytic colonization is affected by endophyte species, how they respond to plants, plant host specificity (Mayerhofer et al., 2013), plant host preferentiality (Klironomos, 2003) and habitat preference (Christensen, 1981; 1989, Christensen et al., 2008; Sieber and Grünig, 2006).

Some investigations have shown that different plant host species respond differently to endophyte inoculations (Mayerhofer et al., 2013). The plant response to inoculation or endophytic colonization may be positive, negative, or neutral (Mayerhofer et al., 2013). In gymnosperms, spruce (*Picea*) showed a negative response to endophytic colonization (Sieber and Grünig 2006; Grünig et al., 2008b; Mandyam et al., 2010), while angiosperms (monocots), especially sedges and grasses more often showed positive response to endophytic colonization (Sieber and Grünig 2006; Grünig et al., 2008b; Mandyam et al., 2010).

Furthermore, plant host-endophyte interactions are also influenced by environmental conditions (Sieber and Grünig, 2006). The endophyte-host plant relation may change over time to neutral or mutualistic or beneficial or pathogenic (Sieber and Grünig, 2006) based on host or prevailing environmental conditions. Sometimes the endophyte-plant relationship may switch from mutualistic to pathogenic behavior in plants, if conditions (abiotic or biotic) are unfavorable (Schulz et al., 1999; Sieber and Grünig, 2006; Grünig et al., 2008b; Mandyam et al., 2010; Kloepper et al., 2013; Hardoim et al., 2015). Furthermore, Schulz et al. (1999) hypothesized that plant-host-endophyte interactions develop into a mutualist endophyte-host relationship when the association leads to balanced antagonism. When their relationship is

imbalanced, it may lead to pathogen-host association and finally cause disease (Mayerhofer et al., 2013).

Moreover, habitat heterogeneities also cause endophyte ecological isolation, and that may lead to development of cryptic species (Sieber and Grünig, 2006). Further, within species diversity patterns of endophytes are likely influenced by several other abiotic and biotic factors; however, supportive evidence for specific reasons is lacking.

After endophyte inoculation, an incubation period follows before an assessment of endophyte presence. Both plant age at inoculation and incubation period were based on time or plant phenology in the studies examined. Plant age at 'leaf-stage', was the most effective age for application of Bb that resulted in high colonization rates, while inoculation of plants at 28-days were most studied or used for Bb colonization studies. However, plant age in time does not always correspond to the same development stages for all crops, making comparisons of plant age for effective Bb colonization across diverse plants difficult, but comparisons can be made for individual crops.

An incubation period of 120-140 days resulted in a high rate of plant colonization, but the most studied incubation period was 1-20 days. We assume that selecting the ideal plant age and the incubation period after Bb application for a crop are crucial for higher colonization rates with Bb. Incubation time plays a significant role in recovery of the fungus from inoculated plants, and the effect may differ among plant species, plant growth habits, and plant life spans. There was significant variation and diversity observed in the selection of plant age and incubation periods for colonization studies included in this meta-analysis. Our results are in accordance with Biswas et al. (2012) who demonstrated that percent Bb colonization differed

by plant age in jute. Higher colonization rates were reported in 60-day-old plants (43%) compared to plants evaluated at 30-days (24%) and 120 days (35%). Further, incubation times and the fungal recovery procedure may vary among studies (Schulz and Boyle, 2005; McKinnon et al., 2017).

Environmental conditions ranged from laboratory, to protected environments to open fields. High rates of Bb colonization were found in studies conducted in growth chambers compared to other environments. Most studies were conducted under controlled conditions, especially greenhouses. In general, many studies have shown that microbes perform better under controlled environment conditions, but fail to perform under field conditions (Wei et al., 2011). Poor field performance of microbes could be attributed to several abiotic factors including temperature, relative humidity, season of application, and many other co-related abiotic factors (Wei et al., 2011). There could also be a change in colonization capacity, inoculum delivery or application method used in the field, as well as more intense competition from other microbes in the rhizosphere (Lugtenberg et al., 2001) and phyllosphere of field plants.

The fact that more effective research findings are reported from controlled conditions such as growth chamber or greenhouses are mainly attributed to the feasibility of manipulating the environment, according to microorganism (Paulitz and Belanger, 2001). The host plant-endophyte relationship is also significantly influenced by experimental conditions (Mayerhofer et al., 2013). Furthermore, plant-endophyte interaction studies are most commonly performed in controlled conditions, optimized for growth of host plants, while many variables interact in field conditions (Hardoim et al., 2015). Additionally, the endophytic nature in plants may shift

or change according to the prevailing conditions, i.e., a mutualistic endophyte in a healthy plant may change when that same plant is grown under stressful or less favorable conditions (Hardoim et al., 2015).

In conclusion, a deeper understanding of host plant-endophyte fungus interaction is needed to successfully conduct endophyte experiments. Generally, most researchers prefer to experiment with or investigate endophytes that are easy to cultivate and manage, which could lead to bias in their understanding of the plant-host endophytic interactions, so it is essential to expand and explore knowledge on the ecology of plant endophyte research (Hardoim et al., 2015).

In the studies included in this meta-analysis, recovery of Bb from the plant was confirmed mostly by culture-dependent methods that included different types of microscopy. All studies had data for colonization, but not all studies provided confirmation evidence that the endophyte observed was Bb. Microscopic observation of Bb intercellular hyphal growth in leaf mid-rib cross-sections had high colonization efficacy, while most studies confirmed Bb colonization by microscopic observation of conidia and by colony morphology in culture.

Generally, endophytes are detected or confirmed by isolation from plant materials either by cultivation on selective growth media (culture dependent) followed by microscopy (Hyde and Soytong, 2008) or by molecular confirmation of endophyte DNA from plant tissues with PCR (culture-independent). Cultivation and confirmation of specific endophytes can be challenging because of contamination caused by resident endophytes or epiphytic microbes, even with selective media (Hyde and Soytong, 2008).

Further, endophyte detection methods may differ for each experiment, which could lead to bias towards the methodology used (Sieber, 2002; Sieber and Grünig, 2006). Adoption of specific protocols for isolation, such as surface sterilization, are necessary to eliminate microbes from external surfaces. Further, each protocol must be optimized for the specific plant material used (Schulz and Boyle 2005; McKinnon et al., 2017).

In this analysis, most studies used culture dependent microscopic confirmation of Bb. Microscopic methods play a key role in detection of endophyte location, and microbe movement or pathway inside the plant can be visualized. Transmission (TEM) and scanning electron microscopy (SEM), fluorescent microscopy, and confocal microscopy have enabled visual tracking of microbes in plants more feasible. Wagner and Lewis (2000), first exhibited visualization of Bb penetration inside plant leaf tissues of maize by light and electron microscopy and discovered that 3% of conidia germinated on the leaf surface and less than 1% of conidia penetrated the leaf via stomata. Several researchers documented visualization of Bb penetration inside plant tissues via stomata or wounds (Quesada-Moraga et al., 2006; Wagner and Lewis, 2000). Hyphal structures have been observed between parenchyma cells and in vascular bundles such as xylem vessels (Wagner and Lewis 2000; Quesada-Moraga et al. 2006). Bb hyphae have been reported intercellularly in date palm leaves (Gomez-Vidal et al., 2006) and opium poppy (Quesada-Moraga et al., 2006). Appressorium-like structures were not reported in any Bb plant infections, unlike insect infections with Bb, where it is more prominent (Gomez-Vidal et al., 2006).

Successful endophytic colonization with Bb can be influenced by several factors, including amount of inoculum applied, plant species, type of plant tissue inoculated, plant

selected for study, method of inoculation or application, or visualization method for Bb detection or confirmation in plant tissue (Gomez-Vidal et al., 2006). Further, identification of endophytes with microscopy can be challenging (McKinnon et al., 2017) due to other resident endophytes. Use green fluorescent protein technique and microscopy may allow better distinction of Bb from other resident endophytes in the plant tissue (McKinnon et al., 2017).

Herein, we critically analyzed the methodologies used for detection or confirmation of endophytic Bb in published data. Most researchers confirmed Bb in the plant by culture-dependent methods; however, some studies did use a culture-independent method, i.e., PCR. In our analysis, molecular-confirmation was most effective and resulted in higher colonization rates than culture-dependent methods. For PCR, the primer pair ITS1/ITS4 was most effective with high colonization rates. Primer pair EF349/EF1685R had the highest number of studies but colonization rates were low.

Molecular detection (culture-independent method) based on PCR is aimed at identification of a specific endophyte (Vidal and Jaber, 2015; Samuels et al., 2006). Care must be taken to ensure specificity and sensitivity of the methodology used to exclude unnecessary or non-target DNA from plants or other microbes (Vidal and Jaber 2015; McKinnon et al., 2017). Another major challenge is low endophytic DNA quantity (McKinnon et al., 2017). Landa et al. (2013), evaluated a two-step nested PCR protocol to test the sensitivity of Bb isolate EABb 04/01-Tip in opium poppy and developed primers for rDNA internal transcribed spacer (ITS) region specific to the Bb fungus. Use of two sets of primers allows for greater specificity and enhances endophyte detection even with low quantities of DNA (Tellenbach et al., 2010). However, sometimes greater sensitivity can lead to more false positives by detection of DNA

from contaminant microbes (Nocker et al., 2007). For effective detection results, care must be taken with surface sterilization of plant samples to reduce error in molecular confirmation studies (Nocker et al., 2007).

Even if a surface sterilization protocol is adopted, care should be taken to ensure that the method is appropriate for the experimental type (natural or artificial inoculation) and plant tissue. However, a culture-dependent method may still be needed for validation or confirmation of the endophyte by cultivation on growth medium or microscopic observation (Unterseher et al., 2013). Combining culture-dependent and -independent methods should provide a better understanding of plant-endophytic interactions, and reduce errors.

The most effective incubation period for observing Bb growth from plant tissues was 15 days after inoculation and the most studied incubation period was 20 days. The most effective (high rate of plant colonization) Bb spore concentration was 1×10^{12} conidia/ml, while the most effective amount applied was a 4 ml suspension of conidia. However, a third of the studies did not report the amount of Bb applied. There was extensive variation between and across the studies in data related to application method, concentration, amount of Bb used in experiments, incubation period, and plant age. There are no standard protocols for Bb application, which gives freedom to researchers to choose the application methodology they prefer, but could lead to different interpretation of the results by different groups, even if they studied the same organism on same crop species.

In this research, the most effective studies, those with high rates of Bb colonization in plants were reported in reports that were published during 2007-2011, but more articles were published in 2012-2016. The differences in numbers could depend on editorial board changes,

journal selected for submission, journal acceptance rates, quality of data submitted, and whether the work was significant. Similar trends and changes likely occur with the data on journals that published these reports.

In this analysis, there is inconsistency between and across the studies conducted, as well as high heterogeneity. This result is supported by a meta-analysis of the efficacy of biocontrol agents in management of plant diseases, which showed greater inconsistencies in data compared to other types of studies (Ojiambo and Scherm, 2006). The inconsistencies in data could be due to wide usage of a single strain of microbe, or indiscriminate use of a microorganism. Ojiambo and Scherm (2006) suggested that wider application of a microorganism, without proper screening or without knowing whether the microbe is a good match for that host ecosystem, is problematic. Chandrasekaran et al. (2014) illustrated that inconsistent endophytic efficacy results could be due to differential colonization abilities of the study microorganism or prevailing competition with another microbe.

In addition, Bb is influenced by several biotic and abiotic factors including host species (insect or plant), host abundance, Bb application, delivery and timing, rate, and target region, as well as moisture, temperate, season, rainfall, and soil factors (Kikankie et al., 2010). These factors could negatively influence Bb colonization and lead to poor plant colonization results (Bugeme et al., 2008; Kessler et al., 2003; Inyang et al., 2000).

The reasons for high heterogeneity in our studies include random error (Haidich, 2010). Differences in methods or outcome assessments suggest that the studies are not all estimating the same effect, but it does not necessarily indicate that the true effect varies. Heterogeneity associated solely with diversity in methodology indicates that studies suffer from different

degrees of bias. Aspects of experimental design may also affect the result of experiments (Haidich, 2010).

Additionally, care should be taken while analyzing meta-analysis because of certain constraints including retrieval of incomplete data or reporting of a study, which has publication bias (PB) in the data set; conflicting results of independent studies; and multiple small studies (Haidich, 2010). Determination of PB is a very important key in meta-analysis. However, PB was not found in the current research. The literature illustrates that larger studies (lower standard error) are more precise and cluster near the mean estimate or point estimate. Smaller studies tend to be more variable, less precise and are scattered on both sides of the mean point estimate.

In this meta-analysis, all studies were scattered symmetrically, indicating no evidence of publication bias. If PB exists in the dataset, the funnel plot is expected to be asymmetric. Asymmetry in funnel plots could be due not only to PB, but also to heterogeneity among studies, including differences in controls, exposure of subjects to confounders or effect modifiers, or differences in methodology between studies (Haidich, 2010), treatment or protocol failures, and missing studies or data. Moreover, journal editors and reviewers need to be aware of the existing bias towards publication of positive findings and ensure that papers with negative, non-significant or failed studies be considered for publication if they meet quality guidelines (Haidich, 2010).

In this paper, we present an historical overview of research on Bb as an endophyte, leading to the current understanding of this plant-fungus interaction. Our goals are to improve our understanding the current concepts and principles about endophytes, and to reveal new

directions for research on microbial endophytes based on our analysis of influential factors. It is apparent that more studies are needed on endophyte ecology, which will lead to a better understanding of their potential in agriculture, and further, we emphasize that there is a need for stringent, standardized methodologies or protocols for future evaluation of experimental endophytic Bb - host plant interactions, by considering the pitfalls mentioned above.

Advances in technology, including next-generation sequencing (Knief, 2014) and metagenome sequencing (Sessitsch et al., 2012) has made it more feasible for isolation, detection, and characterization of endophytic microbes. Furthermore, metabolic, and genetic sources linked to the host plant and endophyte interactions can be exploited and better understood (Knief, 2014; Sessitsch et al., 2012; Hardoim et al., 2015). Additionally, improvements in cultivation methods has extended paths to explore even uncultured microbes associated with plants (Nunes da Rocha et al., 2010; Nunes da Rocha et al., 2013).

Furthermore, detection of endophyte location in different plant parts or tissues has become more convenient with development of powerful image processing methods, including fluorescence in situ hybridization (Compant et al., 2011) and confocal laser scanning microscopy (Cardinale, 2014), which has enabled detection of microbes in plant tissues with high resolution (Landa et al., 2013; Behie et al., 2015). These techniques could provide a clearer understanding of endophytic-host plant relations (Bragina et al., 2012; Compant et al., 2011; Cardinale, 2014, Hardoim et al., 2015).

In summary, the present investigation confirmed that endophytic Bb has a strong colonization impact across a diverse plant kingdom. Additionally, the role of various influential variables and the effect on endophytic Bb colonization efficacy in plants was illustrated. This

knowledge can be used to improve our understanding of the colonization process in plants. This study provided a generalized overview of endophytic Bb colonization research on plants and provides evidence for our current understanding of this plant-microbe interaction. Results of this study provide theoretical expectations of endophytic Bb colonization capability in plants, and is a step toward quantitative analysis, better recommendations for future guidance, proper use of statistical and design models, improved strategies, and reliable conclusions for publishing more improved quality data.

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Appendix 1

All tables and figures for Chapter 1 are in Appendix 1.

Table 1. Moderators that influenced colonization of plants by endophytic Beauveria bassiana.

Category	Moderator	Factor
Endophytic fungus: *Beauveria bassiana* (Bb)	1. Specific Bb Isolate	ATP05 (6), WA (24), ARSEF 252 (6), ATCC 74040 (52), ATP01 (6), ATP02 (8), ATP03 (2), ATP04 (2), B12 (6), B13 (6), B16 (6), Bb CS16-1 (8), Bb G1LU3 (12), Bb IC-5486 (8), Bb LPSc 1067 (3), Bb03032 (2), Bb-04 (27), Bb04a (36), Bb101 (2), Bb1022 (2), Bb1025 (2), Bb11-98 (6), Bb135 (2), Bb14 (24), Bb1555 (2), Bb19 (24), Bb23 (24), Bb45 (24), Bb5a (24), Bb64 (2), Bb7 (24), Beauveriplant@WP (8), Bovetrópico@WP (6), CIAT 359 (8), CIAT 405 (6), EABb 01/33-Su (36), EABb 04/01-Tip (82), F647 (30), F668 (60), G41 (93), GHA (120), IGE3 (1), IGE4 (1), IGE5 (1), IGE6 (1), ITCC 4688 (24), ITCC 6063 (1), ITCC4512 (1), ITCC4563 (1) , ITCC4644 (1), ITCC4705 (1), ITCC4796 (12), ITCC4925 (1), ITCC5408 (1), ITCC5562 (1), Micosis@WP (6), NK (96), S204 (24), S4SU1 (1), SFBb1 (45), Overall (1051).
	2. General isolate type	Commercial (192), Native (859), Overall (1051).
	3. General source of Bb	Insect (583), Compost (1.00), Fruit (8), NK-not known (157), Plant (110), Soil (192), Overall (1051).
	4. Specific source of endophytic Bb	Pine shoot moth (2), Okra (1), Black vine weevil adult (2), Boll weevil cadaver (52), Bollworm (25), Maize stalk borer (77), Carrot rhizosphere soil (24), Cashew root and stem borer (24), Chrysomelid beetle (120), Click beetle (10), Codling moth larva (2), Coffee berry borer (32), Coffee berries (10), Compost (1), Dead leaf (Unidentified) (2), Elephant weevil (30), European spruce bark beetle adult (2), Flea beetle (11), Insect (unidentified) (27), Legume pod borer (24), Maize and sorghum stem tissues (1), Mature pine trees (90), Monocots (12), NK-not known (157), Oil palm insect (1), Orthoptera: Tettigoniidae (3), Paddy insect (1), Pine seedlings (4), Poppy stem gall wasp (71), Soil (3), Soil from banana plantations (165), Sorghum chafer beetle (10), Sugarcane borer (1), Opium stem borer (11), Traditional olive orchard (36), Unidentified curculionid beetle (6), White grub (1), Overall (1051).
	5. Specific insect source of endophytic Bb	Pine shoot moth (2), Black vine weevil adult (2), Boll weevil cadaver (52), Cotton bollworm (25), Maize stalk borer (77), Carrot rhizosphere soil (1), Cashew root and stem borer (24), Chrysomelid beetle (120), Click beetle (10), Codling moth larva (2), Coffee berry borer (32), Elephant weevil (30), European spruce bark beetle adult (2), Flea beetle (11), Insect (unidentified) (27), Legume pod borer (24), NK-not known (505), Oil palm insect (1), Orthoptera: Tettigoniidae (3), Paddy insect (1), Poppy stem gall wasp (71), Sorghum chafer beetle (10), Sugarcane borer (1), Opium stem borer (11), Unidentified curculionid beetle (6), White grub (1), Overall (1051).
	6. Method of application for endophytic Bb	Foliar (397), Natural (16), Plant (4), Plant whorl (8), Root and rhizome dip (37), Rhizome injection (43), Root dip (105), Root drenching (114), Seed (196), Soil (82), Solid substratum (37), Stem injection (12), Overall (1051).
	7. Mode of Bb transmission	Horizontal (1035), Natural (12), Vertical (4), Overall (1051).
	8. Mode of endophyte presence	Endophyte-artificially tested (1035), Natural endophyte (16), Overall (1051).
	9. Concentration of Bb applied	1×10^8 spores/ml (549), $5x10^7$ conidia/ml (90), $1x10^7$ spores/ml (48), $1x10^9$ conidia/ml (46), $1.5x10^{10}$ conidia/ml (43), $1x10^{11}$ spores/ml (34), $1.5x10^7$ conidia/ml (33), 1×10^{10} conidia/ml (30), $1x10^6$ spores/ml (19), naturally-occurring endophyte (16), 50 g rice + Bb $1.5x10^9$ spores/g (16), $1.5x10^8$ conidia/ml (12), $1x10^{12}$ spores/ml (12), $1.5x10^5$ conidia/ml (12), $1.5x10^6$ conidia/ml (12), $2.3x10^7$ CFU/ml-3% inoculum (5), 2.2×10^9 conidia/g (8), $2.3x10^7$ CFU-1% inoculum (8), 5g Rice + Bb $1x10^{12}$ conidia/g (6), 4.1x107 conidia/ml (6), fungal plugs (1 cm^2) inoculated into soil (6), 100 g wheat + $1x10^7$ conidia/ml-5 g inoculum/pot (6), $5x10^9$ conidia/ml (6), $6.3x10^7$cfu/seed (5), $7.5x10^8$ conidia/ml (4), 5000 conidia/mm^2 10 liters/min spray deposition (3), $1:7x10^6$ conidia/ml (3), $2.3x10^7$ CFU-5% (3), $2:1.4x10^7$ conidia/ml (3), $1.4x10^7$ conidia/ml (3), 5.9×10^4 ($4.4\text{-}7.3\times10^4$) conidia/ml (2), desirable amount (1) and $5.5x10^8$ conidia/ml (1), Overall (1051).

"Table 1. Continued"

Category	Moderator	Factor
Endophytic fungus: *Beauveria bassiana*	10. Amount of Bb applied	0.4 g inoculum/plant (8), 100 ml conidial or spore suspension (58), 100 µl conidial or spore suspension (16), 10 ml conidial or spore suspension (37), 15 ml conidial or spore suspension (24), 1 g inoculum/ml (9), 1 ml conidial or spore suspension (21), 20 ml conidial or spore suspension (2), 200 ml conidial or spore suspension (90), 25 ml conidial or spore suspension (90), 2 ml conidial or spore suspension (25), 3 ml conidial or spore suspension (37), 300 liters/ha conidial or spore suspension (3), 300 ml conidial or spore suspension (107), 4 ml conidial or spore suspension (28), 5 g inoculum/plant (18), 5 ml conidial or spore suspension (144), Desirable Amount (1), NK-not known (333), Overall (1051).
	11. Colonization range (%) of Bb	0-10 (375), 10-20 (125), 20-30 (97), 30-40 (93), 40-50 (92), 50-60 (115), 60-70 (59), 70-80 (38), 80-90 (28), 90-100 (29), Overall (1051).
	12. Plant part colonized by Bb	Green capsule (1), Hypocotyl (4), Lateral shoot sucker (1), Leaves (432), Petiole (31), Plant (27), Pseudostem (61), Rhizome (61), Root (213), Seed (4), Seedling (7), Soil (18), Squares-cotton (1), Stem (172), Sterile needle (18), Overall (1,051).
Plant factors	13. Plant species	Alfalfa (36), Banana (48), Banana - tissue culture (141), *Calico aster* (4), Cassava (34), Coffee (16), Common bean (50), Cotton (27), Faba bean (15), Grapevine (41), Jute (11), Maize (163), Melon (36), Oilseed rape (20), Opium poppy (24), Pine (94), Pumpkin (4), Purple coneflower (1), Sorghum (55), Strawberry (90), Sugarcane (36), Sweet pepper (24), Tall goldenrod (4), Tomato (51), Wheat (17), White jute (9), Overall (1051).
	14. Plant family	Asteraceae (9), Brassicaceae (20), Cucurbitaceae (40), Euphorbiaceae (34), Fabaceae (101), Malvaceae (47), Musaceae (189), Papaveraceae (24), Pinaceae (94), Poaceae (271), Rosaceae (90), Rubiaceae (16), Solanaceae (75), Vitaceae (41), Overall (1051).
	15. Plant taxa - group	Angiosperms - Eudicots (497), Angiosperms - Monocot (460), Gymnosperms - Monterey pine (94), Overall (1051).
	16. Plant life span	Annual (404), Perennial (647), Overall (1051).
	17. Plant growth habit	Forb or herb (90), Forb/herb/vine (65), Graminoid (235), Herb (381), Shrubs (69), Tree (94), True grasses (36), Vine/Creepers (81), Overall (1051).
	18. Root system	Adventitious roots (34), Fibrous and rhizomatous roots (8), Fibrous or taproots (51), Fibrous roots (426), Rhizome (189), Taproots (343), Overall (1051).
	19. Crop distribution	Agronomic crop (366), Horticultural crop (685), Overall (1051)
	20. Horticultural classification	Edibles (821), Forage crop (36), Industrial crop (91), Ornamental (103), Overall (1051).
	21. Horticultural sub-classifica-tion based on use	Beverage crops (16), Drugs and medicinals (24), Fiber crop (47), Field and grain crops (36), Forage crop (36), Fruits (320), Grain crops (235), Landscape plant (98), Oilseeds (20), Perennial garden and pocket prairie (4), Temperate gardens and medicinal use (1), Vegetables (214), Overall (1051).
	22. Agricultural crop classification	Beverage crops (16), Cash crops (67), Food crops (435), Forage crops (36), Grain crops (235), Nonfood crops (127), Pulse crops (65), Sugar and sweetener crops (36), Tuberous crops (34), Overall (1051).
	23. Plant age at inoculation	10 days (3), 10 months (1), 112 days (1), 120 days (5), 120-140 days (8), 13 years (8), 14 days (6), 15 days (2), 15-30 days old seedling (144), 17 days (6), 2 years (1), 20 days (1), 21 days (38), 22 days (2), 28 days (165), 30 days (5), 34 days (1), 35 days (17), 42 days (16), 48 days (2), 49 days (17), 50 days (3), 60 days (60), 7 days (30), 9 months (1), 90 days (3), 9-10 months old (34), Fourth leaf stage (6), Leaves (28), NK-not known (102), Primary shoot stage- sugarcane (36), Seed (98), Seedling (61), Three true leaf stage (24), True leaf stage (108), Whorl-stage V7 growth stage (8), Overall (1051).

"Table 1. Continued"

Category	Moderator	Factor
Plant factors	24. Plant part treated with Bb	Root and Rhizome (37), Leaf whorl (6), Leaves (233), Natural (12), Plant (30), Plant base (90), Plant whorl (8), Primary shoot (12), Rhizome (43) Root (129), Seed (196), Seedling (148), Soil (107), Overall, (1051).
Experimental factors	25. Country where research was conducted	Argentina (3), Australia (30), Canada (14), China (18), Colombia (40), Cuba (4), Denmark (2), Germany (87), Greece (4), India (236), Jordan (40), Kenya (12), Malawi (36), Mexico (2), New Zealand (94), Spain (148), Uganda (141), USA (140), Overall (1051).
	26. Experimental conditions	Field (65), Glasshouse (168), Greenhouse (547), Growth chamber (76), Laboratory (6), Screen house (189), Overall (1051).
	27. Incubation period after plant inoculation	10 months (1), 100-120 days (47), 1-20 days (374), 120-140 days (13), 2 years (1), 20-40 days (249), 220-240 days (9), 260-280 days (30), 40-60 days (169), 5-6 true leaves (24), 60-80 days (74), 80-100 days (31), 9 months (1), 90-100 days (6), Collar stage (2), First and fifth leaf stage (4), NK (12), Seedling stage (1), Third true leaf stage (3), Overall (1051).
	28. Bb growth observed in culture after plating	Daily observation recorded (108), Every other day (7), 4 days (16), 7 days (30), 10 days (85), 11 days (1), 12 days (115), 10-14 days (90), 14 days (187), 15 days (24), 20 days (200), 21 days (44), 28 days (73), 30 days (40), 35 days (8), 45 days (5), NK-not known (18), Overall (1051).
	29. Confirmation method for endophytic *Bb*	Microscopic+molecular (344), Microscopic (692), Molecular (15), Overall (1051).
	30. Plant tissue used for confirmation of endophytic Bb	Conidia and colony morphology (1030), Leaf and root samples (6), Intercellular hyphal growth observed in each mid-rib leaf cross-section (6), Hypocotyl, Leaf epidermal cells and vascular bundles (5), Overall (1051)
	31. PCR primers for molecular confirmation of Bb	ITS5/ITS4 (12), SCAR primers (9), 5'CGGCGGACTCGCCCCAGCCCG 3'/ 3'CCGCGTCGGGGTTCCGGTGCG 5' (12), Bt (12), EF349/EF1685R (90), EF52F 5'-3'/EF481R 5'-3' (4), ITS1/ITS4 (15), NK-not known (896), RD1-F (1), Overall (1051)
	32. Publication years	2002-2006 (29), 2007-2011 (236), 2012-2016 (667), 2017-2018 (119), Overall (1051).
	33. Journal	*Entomological Science* (5), *Indian Journal of Microbiology* (144), *Science and Technology* (2), *AMB Express* (1), *Asia-Pacific Entomology* (24), *Biocontrol* (74), *Biological Control* (256), *Crop Protection* (12), *Current Science* (28), *Entomologia Experimentalis et Applicate* (13), *Environmental Entomology* (9), *Fungal Ecology* (50), *Insect Science* (10), *Journal of Berry Research* (90), *Journal of Invertebrate Pathology* (184), *Jove-Journal of Visualized Experiments* (6), *Microbial Ecology* (2), *Microbial Pathogenesis* (18), *Mycopathologia* (5), *Mycoscience* (16), *New Zealand Plant Protection* (4), *Phytoparasitica* (9), *PloS ONE* (22), *Scientia Horticulturae* (16), *Southwestern Entomologist* (2), *Symbiosis* (1), *Uttar Pradesh Journal of Zoology* (48), Overall (1051).

*Values in parenthesis are number of studies (n).

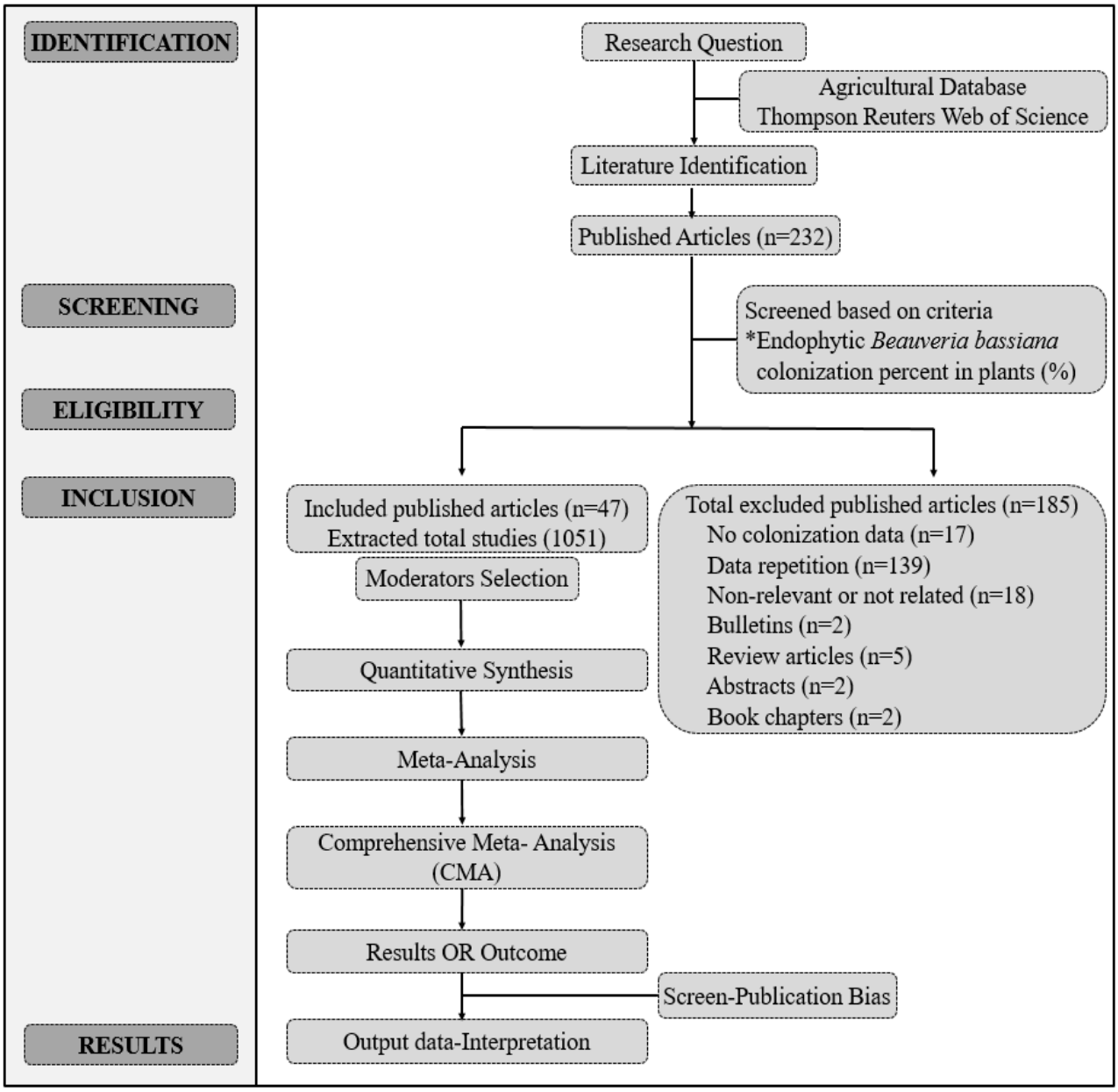
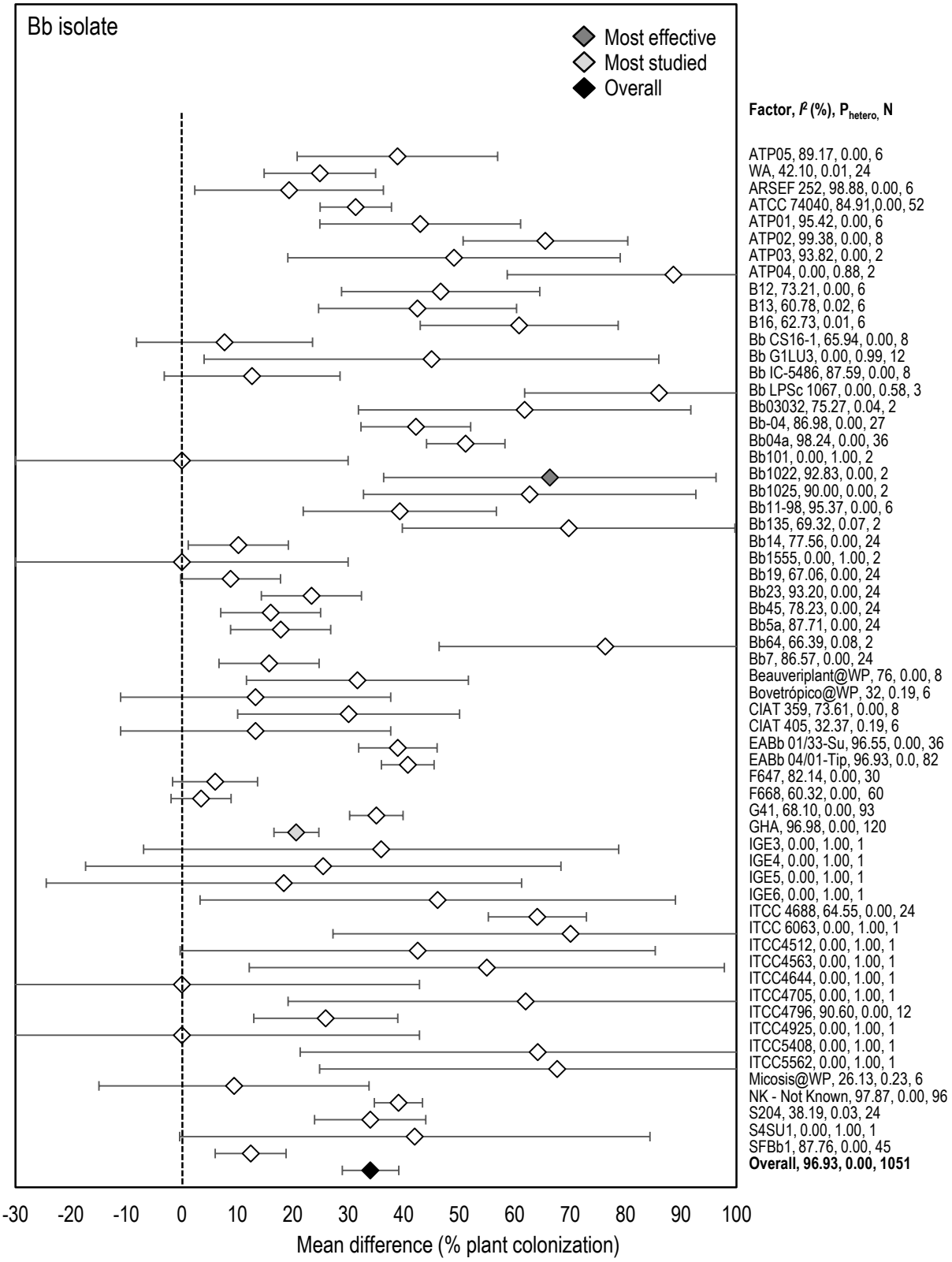


Figure 1.1. Schematic representation of meta-analysis procedure followed in the study.

Figure 1.2. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific Bb isolate.

Moderator: Comparison among studies on specific Bb isolates used for colonization studies in plants for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (Bb isolate) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.


Bb isolate
Most effective
Most studied
Overall
Factor, I^2 (%), $P_{hetero,}$ N
ATP05, 89.17, 0.00, 6
WA, 42.10, 0.01, 24
ARSEF 252, 98.88, 0.00, 6
ATCC 74040, 84.91,0.00, 52
ATP01, 95.42, 0.00, 6
ATP02, 99.38, 0.00, 8
ATP03, 93.82, 0.00, 2
ATP04, 0.00, 0.88, 2
B12, 73.21, 0.00, 6
B13, 60.78, 0.02, 6
B16, 62.73, 0.01, 6
Bb CS16-1, 65.94, 0.00, 8
Bb G1LU3, 0.00, 0.99, 12
Bb IC-5486, 87.59, 0.00, 8
Bb LPSc 1067, 0.00, 0.58, 3
Bb03032, 75.27, 0.04, 2
Bb-04, 86.98, 0.00, 27
Bb04a, 98.24, 0.00, 36
Bb101, 0.00, 1.00, 2
Bb1022, 92.83, 0.00, 2
Bb1025, 90.00, 0.00, 2
Bb11-98, 95.37, 0.00, 6
Bb135, 69.32, 0.07, 2
Bb14, 77.56, 0.00, 24
Bb1555, 0.00, 1.00, 2
Bb19, 67.06, 0.00, 24
Bb23, 93.20, 0.00, 24
Bb45, 78.23, 0.00, 24
Bb5a, 87.71, 0.00, 24
Bb64, 66.39, 0.08, 2
Bb7, 86.57, 0.00, 24
Beauveriplant@WP, 76, 0.00, 8
Bovetrópico@WP, 32, 0.19, 6
CIAT 359, 73.61, 0.00, 8
CIAT 405, 32.37, 0.19, 6
EABb 01/33-Su, 96.55, 0.00, 36
EABb 04/01-Tip, 96.93, 0.0, 82
F647, 82.14, 0.00, 30
F668, 60.32, 0.00, 60
G41, 68.10, 0.00, 93
GHA, 96.98, 0.00, 120
IGE3, 0.00, 1.00, 1
IGE4, 0.00, 1.00, 1
IGE5, 0.00, 1.00, 1
IGE6, 0.00, 1.00, 1
ITCC 4688, 64.55, 0.00, 24
ITCC 6063, 0.00, 1.00, 1
ITCC4512, 0.00, 1.00, 1
ITCC4563, 0.00, 1.00, 1
ITCC4644, 0.00, 1.00, 1
ITCC4705, 0.00, 1.00, 1
ITCC4796, 90.60, 0.00, 12
ITCC4925, 0.00, 1.00, 1
ITCC5408, 0.00, 1.00, 1
ITCC5562, 0.00, 1.00, 1
Micosis@WP, 26.13, 0.23, 6
NK - Not Known, 97.87, 0.00, 96
S204, 38.19, 0.03, 24
S4SU1, 0.00, 1.00, 1
SFBb1, 87.76, 0.00, 45
Overall, 96.93, 0.00, 1051
-30
-20
-10
0
10
20
30
40
50
60
70
80
90
100
Mean difference (% plant colonization)

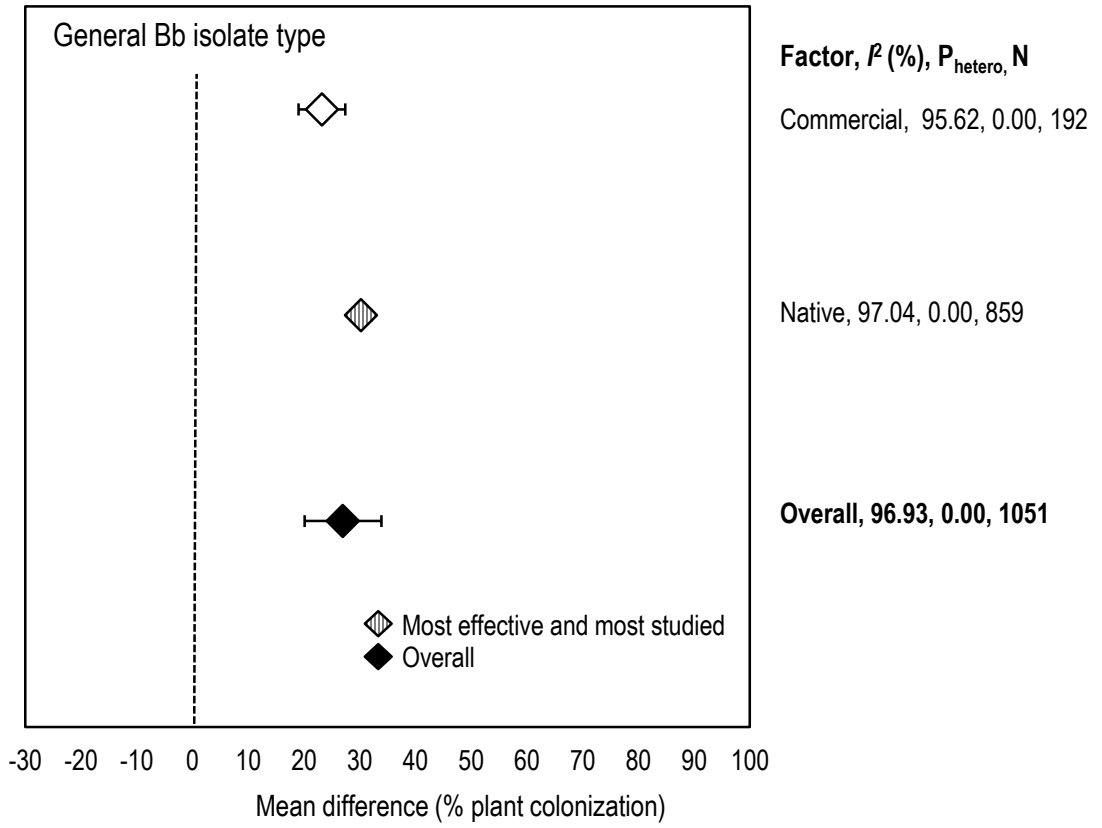


Figure 1.3. Mean difference and 95% confidence intervals for percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on general Bb isolate type.

Moderator: Comparison among studies on general isolate type of endophytic Bb isolate used for colonization of plants for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (general isolate type) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

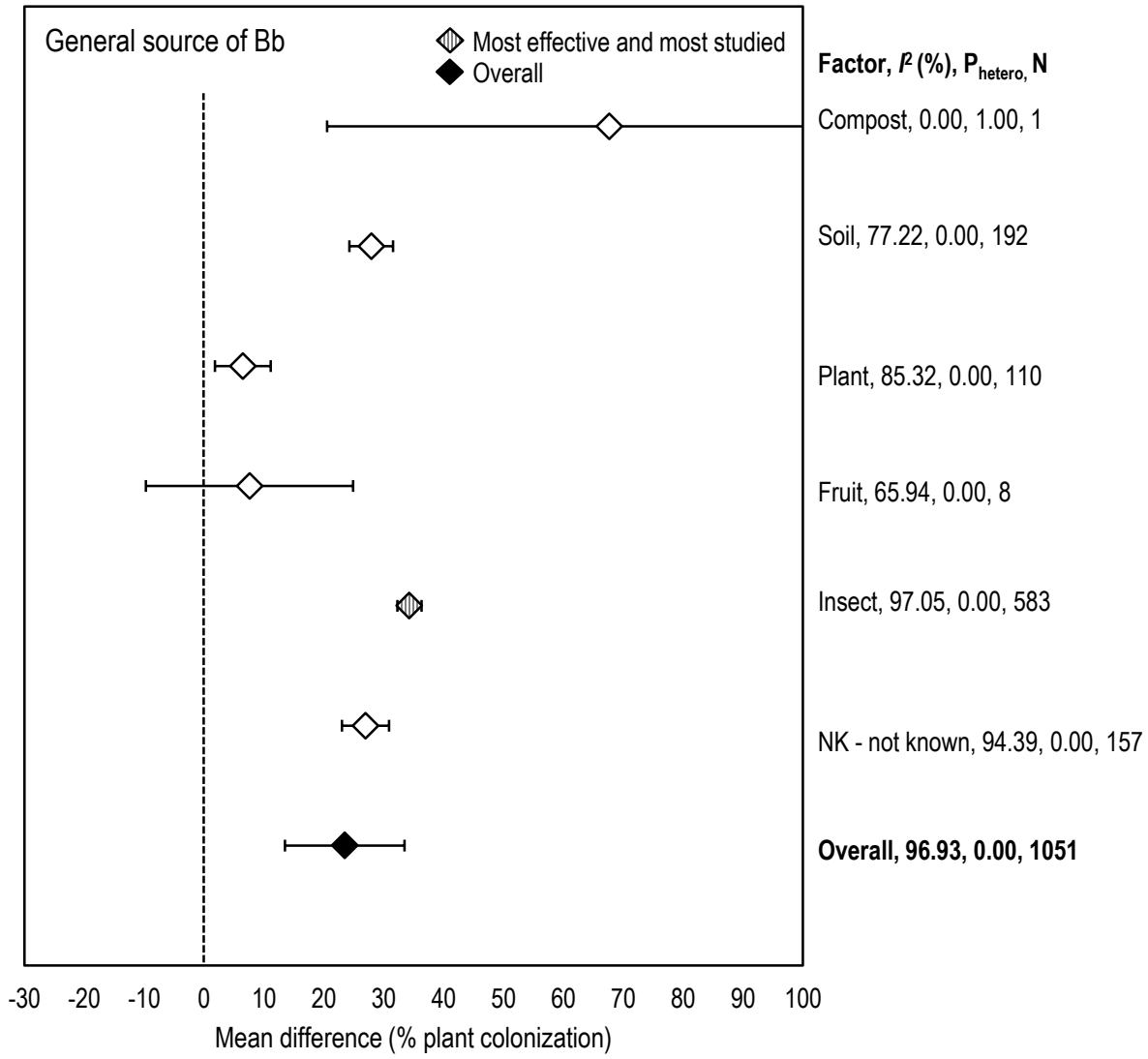
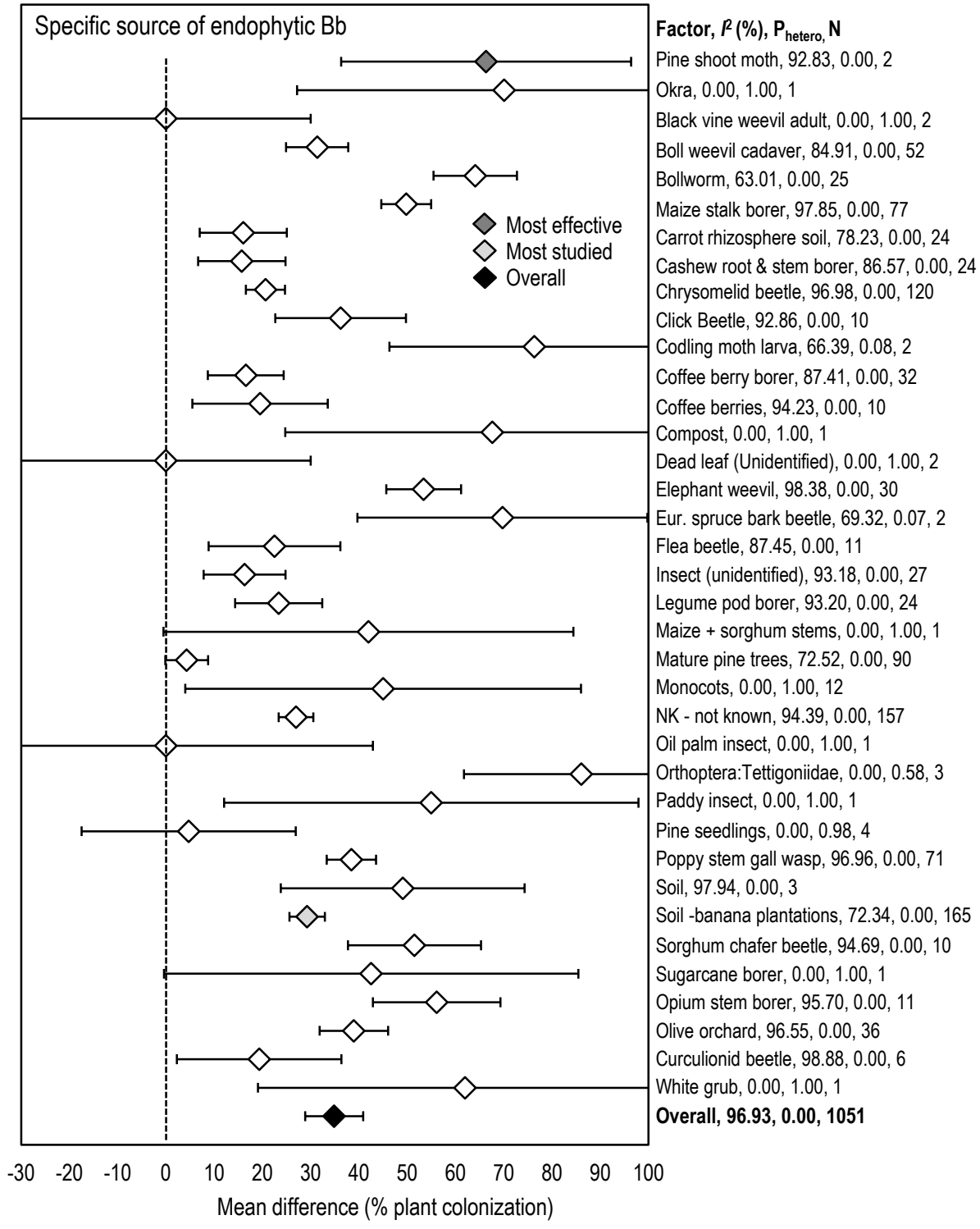


Figure 1.4. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on general source of Bb isolate.

Moderator: Comparison among studies on general source of Bb isolate used in plant colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (general source of Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

Figure 1.5. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific source of Bb isolate.

Moderator: Comparison among studies on specific source of endophytic Bb used in plant colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (specific source of Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.


Specific source of endophytic Bb
Most effective
Most studied
Overall
Factor, I^2 (%), P_{hetero}, N
Pine shoot moth, 92.83, 0.00, 2
Okra, 0.00, 1.00, 1
Black vine weevil adult, 0.00, 1.00, 2
Boll weevil cadaver, 84.91, 0.00, 52
Bollworm, 63.01, 0.00, 25
Maize stalk borer, 97.85, 0.00, 77
Carrot rhizosphere soil, 78.23, 0.00, 24
Cashew root & stem borer, 86.57, 0.00, 24
Chrysomelid beetle, 96.98, 0.00, 120
Click Beetle, 92.86, 0.00, 10
Codling moth larva, 66.39, 0.08, 2
Coffee berry borer, 87.41, 0.00, 32
Coffee berries, 94.23, 0.00, 10
Compost, 0.00, 1.00, 1
Dead leaf (Unidentified), 0.00, 1.00, 2
Elephant weevil, 98.38, 0.00, 30
Eur. spruce bark beetle, 69.32, 0.07, 2
Flea beetle, 87.45, 0.00, 11
Insect (unidentified), 93.18, 0.00, 27
Legume pod borer, 93.20, 0.00, 24
Maize + sorghum stems, 0.00, 1.00, 1
Mature pine trees, 72.52, 0.00, 90
Monocots, 0.00, 1.00, 12
NK - not known, 94.39, 0.00, 157
Oil palm insect, 0.00, 1.00, 1
Orthoptera:Tettigoniidae, 0.00, 0.58, 3
Paddy insect, 0.00, 1.00, 1
Pine seedlings, 0.00, 0.98, 4
Poppy stem gall wasp, 96.96, 0.00, 71
Soil, 97.94, 0.00, 3
Soil -banana plantations, 72.34, 0.00, 165
Sorghum chafer beetle, 94.69, 0.00, 10
Sugarcane borer, 0.00, 1.00, 1
Opium stem borer, 95.70, 0.00, 11
Olive orchard, 96.55, 0.00, 36
Curculionid beetle, 98.88, 0.00, 6
White grub, 0.00, 1.00, 1
Overall, 96.93, 0.00, 1051
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant colonization)

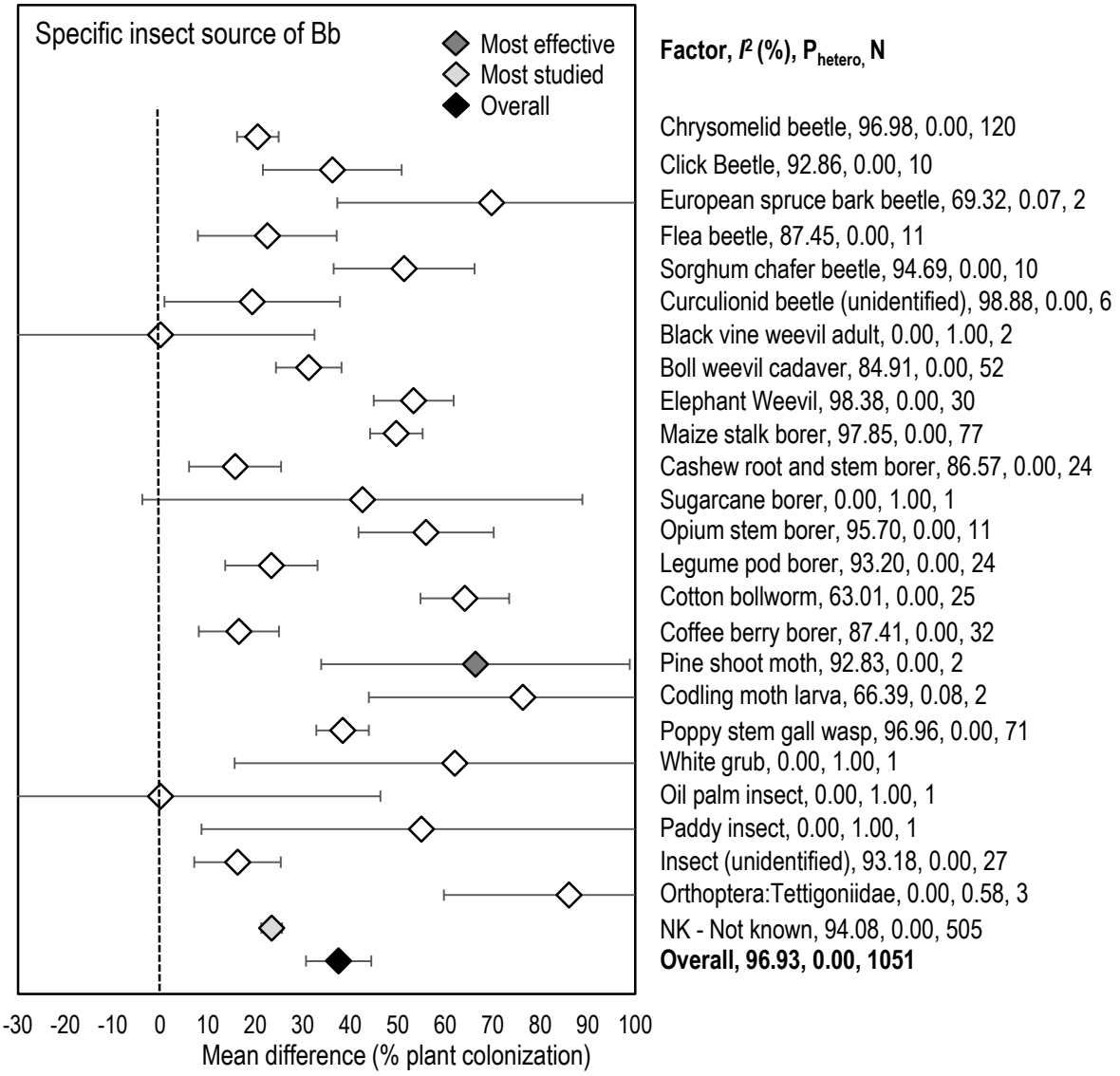


Figure 1.6. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific insect host source of Bb.

Moderator: Comparison among studies on specific insect host source of Bb isolate used in plant colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (insect host source of Bb) listed on the right, the numerical values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size when P>0.05, no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

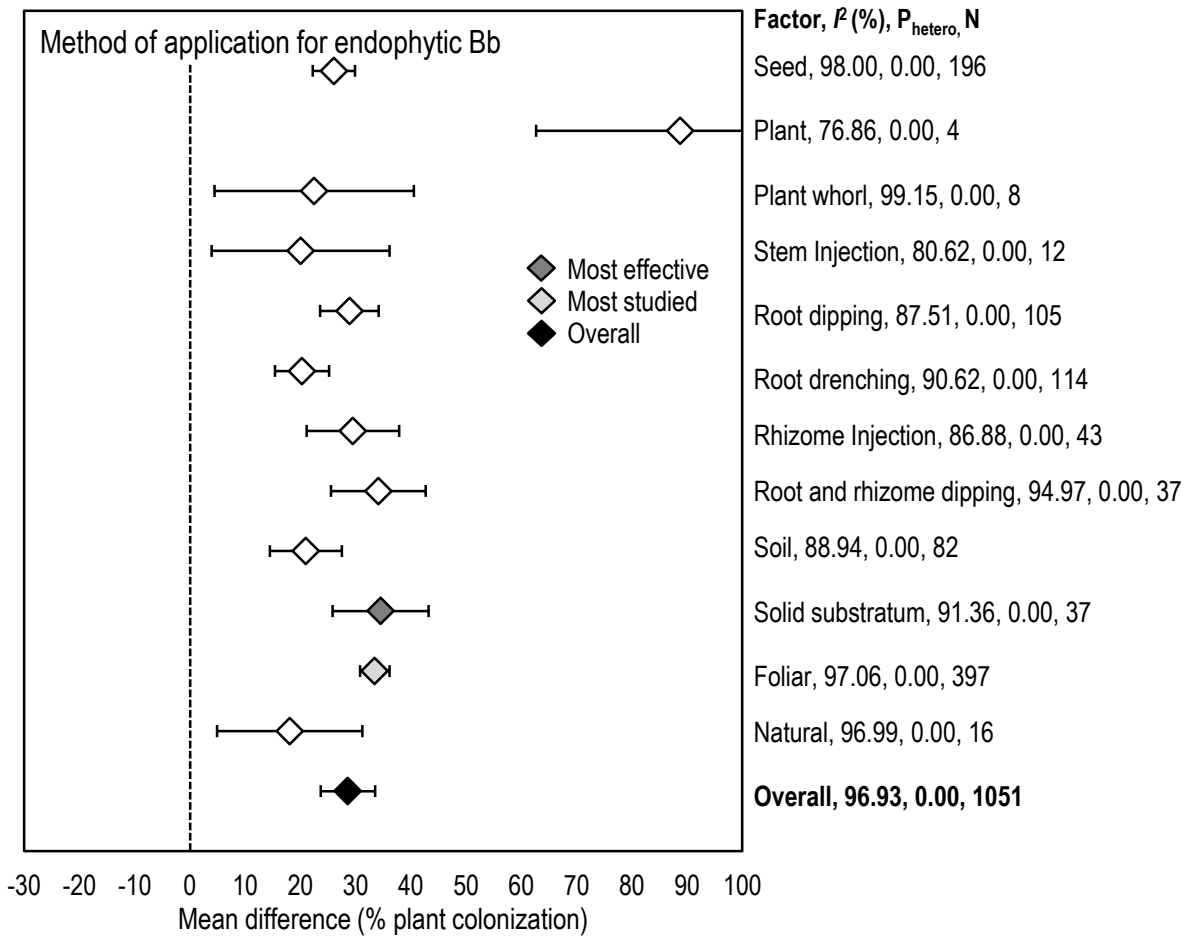


Figure 1.7. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on method of application for Bb.

Moderator: Comparison among studies on method of application for Bb used in plant colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (method of application of Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

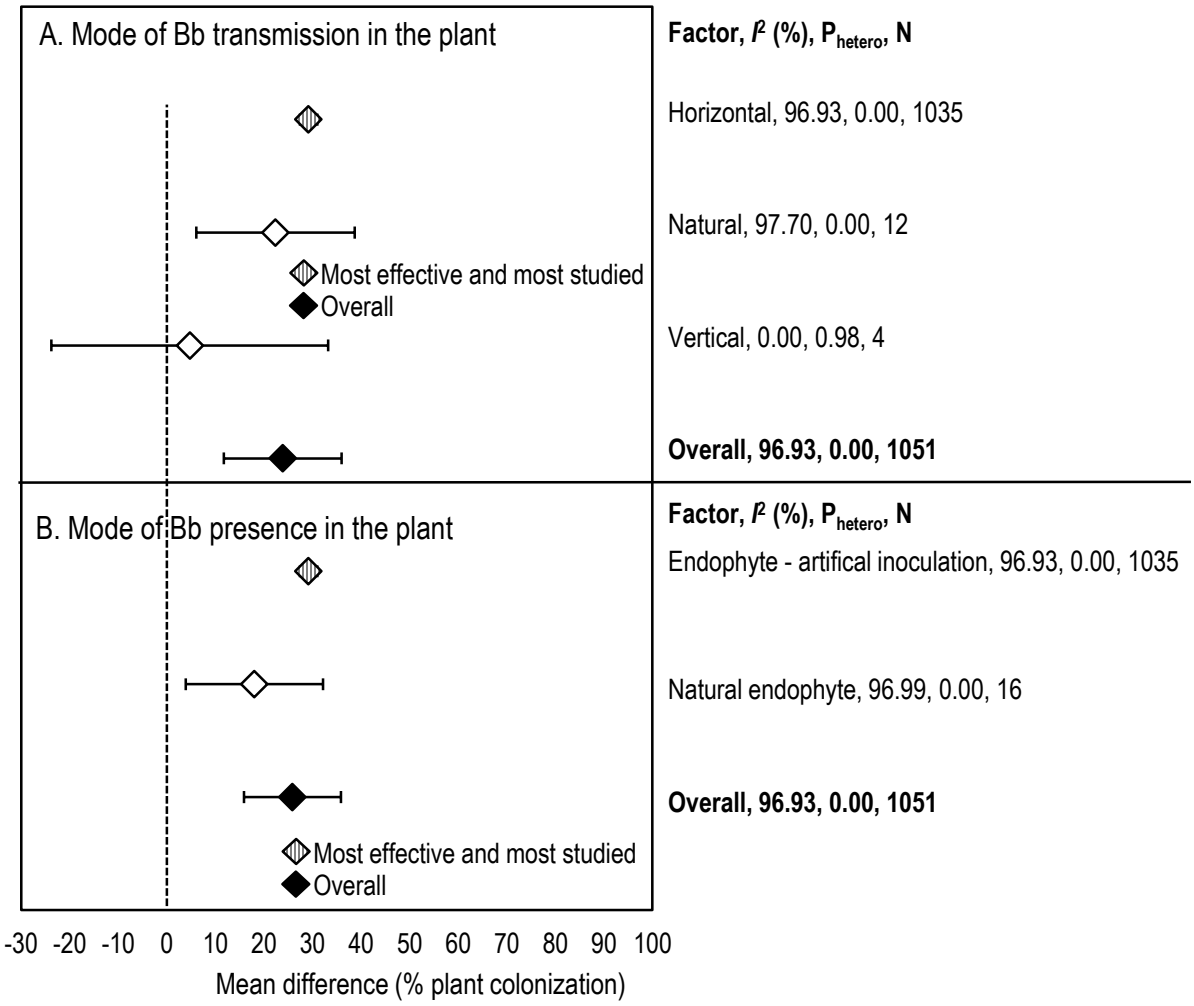
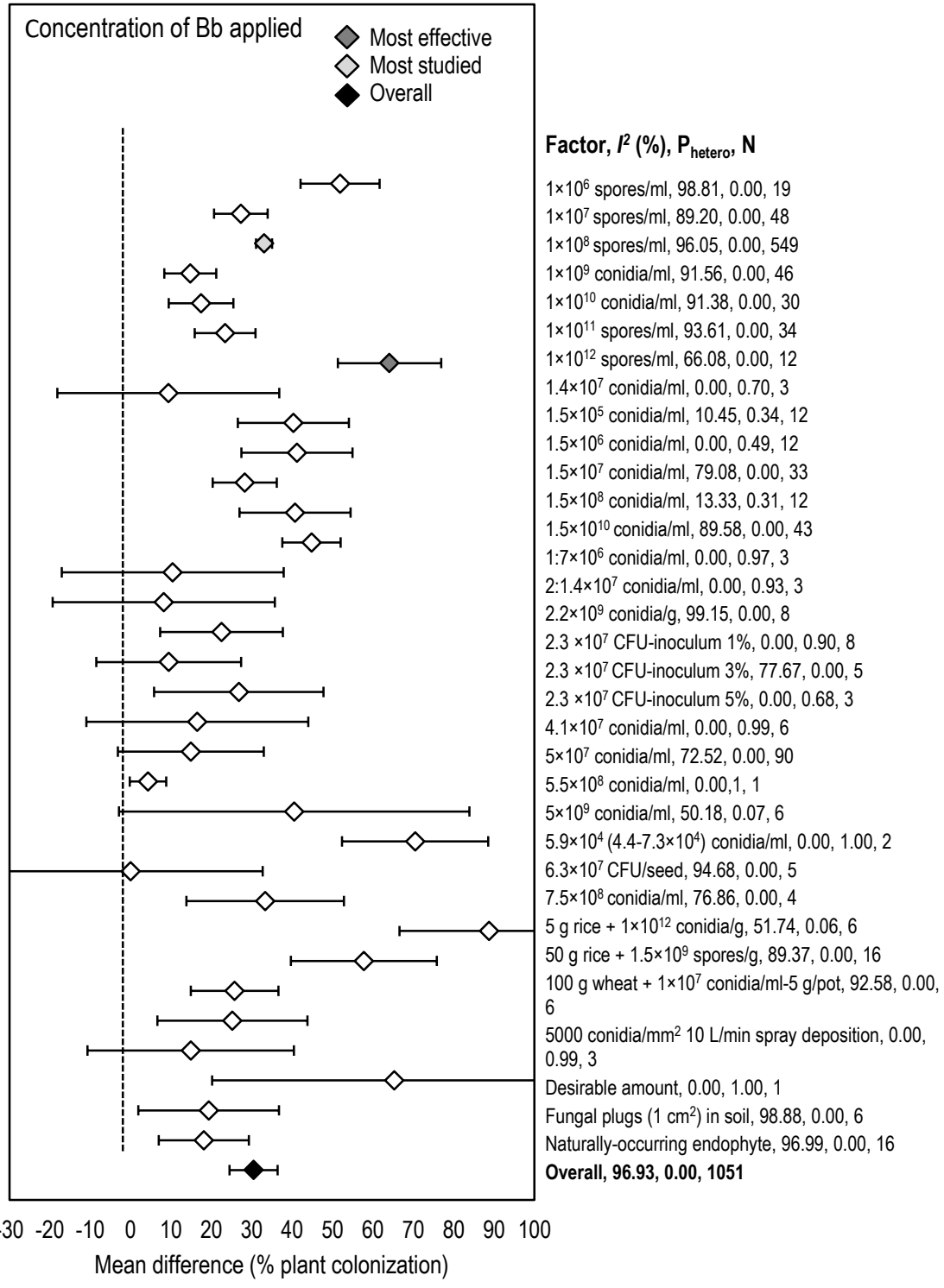


Figure 1.8. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on A) mode of Bb transmission in the plant, and B) mode of Bb presence in the plant.

Moderator: A) Comparison among studies on mode of transmission of Bb in the plant, and B) mode of Bb presence in the plant used in colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor [mode of Bb transmission in the plant (Fig. 1.8A) and mode of Bb presence in the plant (Fig. 1.8B)] listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

Figure 1.9. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on concentration of Bb applied.

Moderator: Comparison among studies on concentration of Bb applied in plant colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (concentration of Bb applied) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.


Concentration of Bb applied
Most effective
Most studied
Overall
Factor, I^2 (%), P_{hetero}, N
1×10^6 spores/ml, 98.81, 0.00, 19
1×10^7 spores/ml, 89.20, 0.00, 48
1×10^8 spores/ml, 96.05, 0.00, 549
1×10^9 conidia/ml, 91.56, 0.00, 46
1×10^10 conidia/ml, 91.38, 0.00, 30
1×10^11 spores/ml, 93.61, 0.00, 34
1×10^12 spores/ml, 66.08, 0.00, 12
1.4×10^7 conidia/ml, 0.00, 0.70, 3
1.5×10^5 conidia/ml, 10.45, 0.34, 12
1.5×10^6 conidia/ml, 0.00, 0.49, 12
1.5×10^7 conidia/ml, 79.08, 0.00, 33
1.5×10^8 conidia/ml, 13.33, 0.31, 12
1.5×10^10 conidia/ml, 89.58, 0.00, 43
1:7×10^6 conidia/ml, 0.00, 0.97, 3
2:1.4×10^7 conidia/ml, 0.00, 0.93, 3
2.2×10^9 conidia/g, 99.15, 0.00, 8
2.3 ×10^7 CFU-inoculum 1%, 0.00, 0.90, 8
2.3 ×10^7 CFU-inoculum 3%, 77.67, 0.00, 5
2.3 ×10^7 CFU-inoculum 5%, 0.00, 0.68, 3
4.1×10^7 conidia/ml, 0.00, 0.99, 6
5×10^7 conidia/ml, 72.52, 0.00, 90
5.5×10^8 conidia/ml, 0.00,1, 1
5×10^9 conidia/ml, 50.18, 0.07, 6
5.9×10^4 (4.4-7.3×10^4) conidia/ml, 0.00, 1.00, 2
6.3×10^7 CFU/seed, 94.68, 0.00, 5
7.5×10^8 conidia/ml, 76.86, 0.00, 4
5 g rice + 1×10^12 conidia/g, 51.74, 0.06, 6
50 g rice + 1.5×10^9 spores/g, 89.37, 0.00, 16
100 g wheat + 1×10^7 conidia/ml-5 g/pot, 92.58, 0.00, 6
5000 conidia/mm^2 10 L/min spray deposition, 0.00, 0.99, 3
Desirable amount, 0.00, 1.00, 1
Fungal plugs (1 cm^2) in soil, 98.88, 0.00, 6
Naturally-occurring endophyte, 96.99, 0.00, 16
Overall, 96.93, 0.00, 1051
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant colonization)

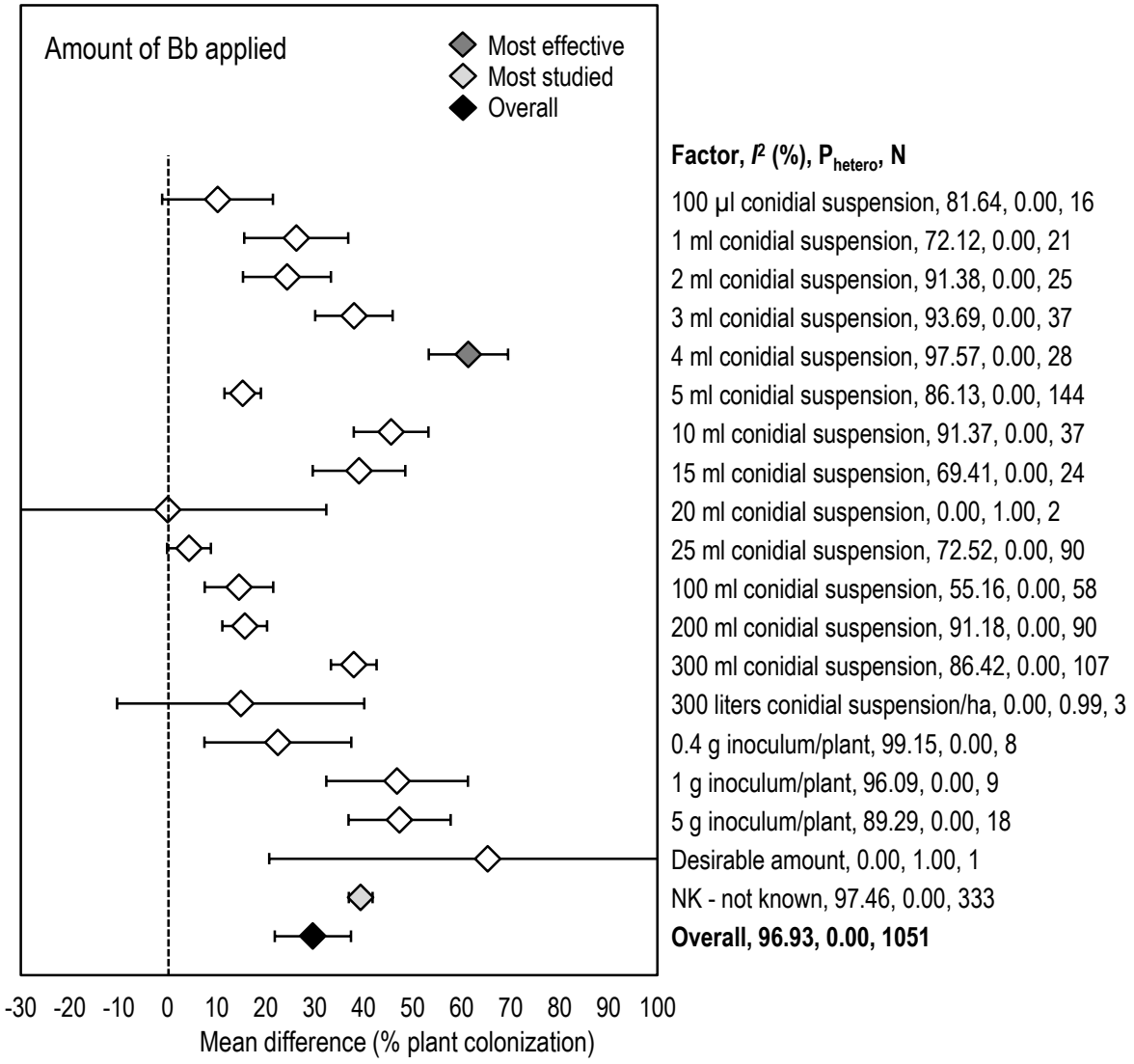


Figure 1.10. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on the amount of Bb applied.

Moderator: Comparison among studies on the amount of Bb applied in plant colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (amount of Bb applied) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

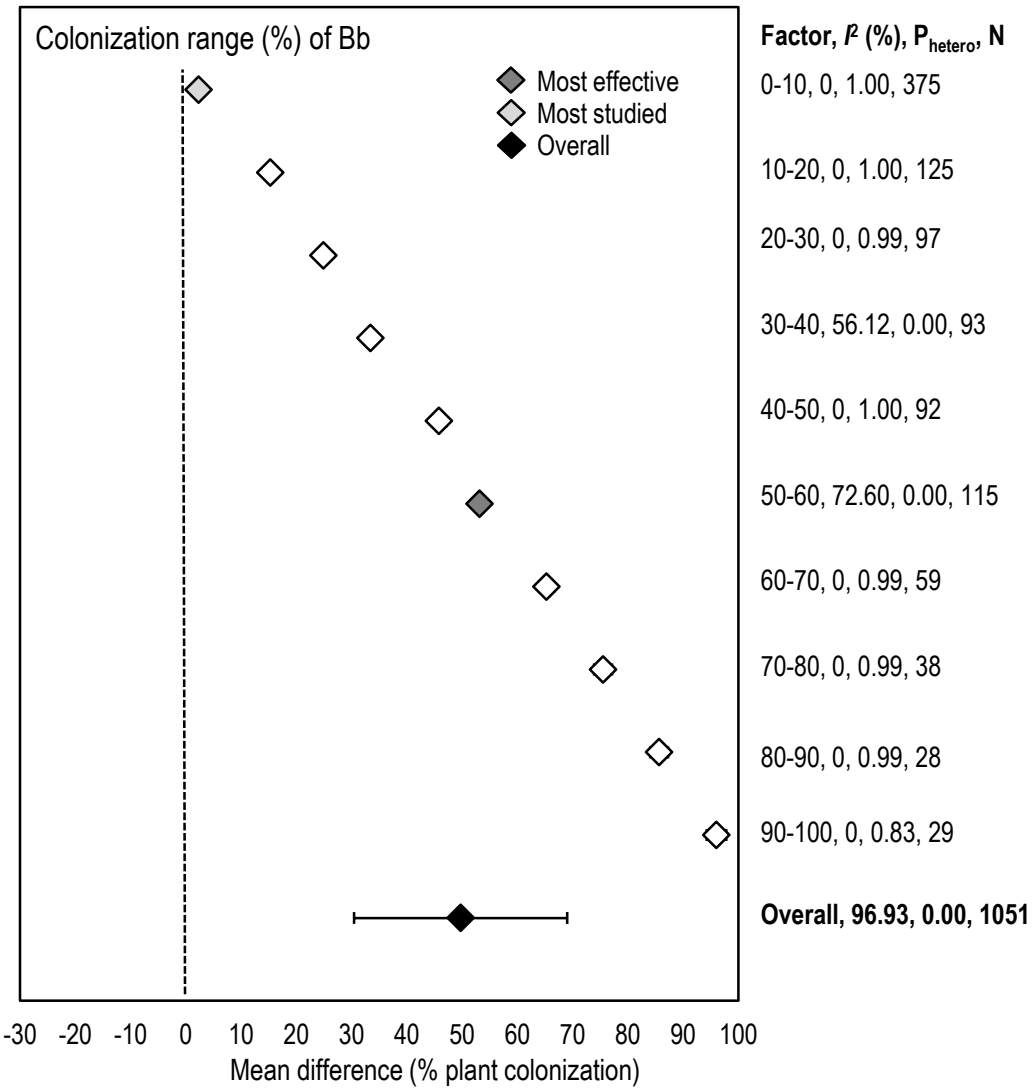


Figure 1.11. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific percent colonization range.

Moderator: Comparison among studies on colonization range of Bb reported in plant colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (percent colonization range of Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

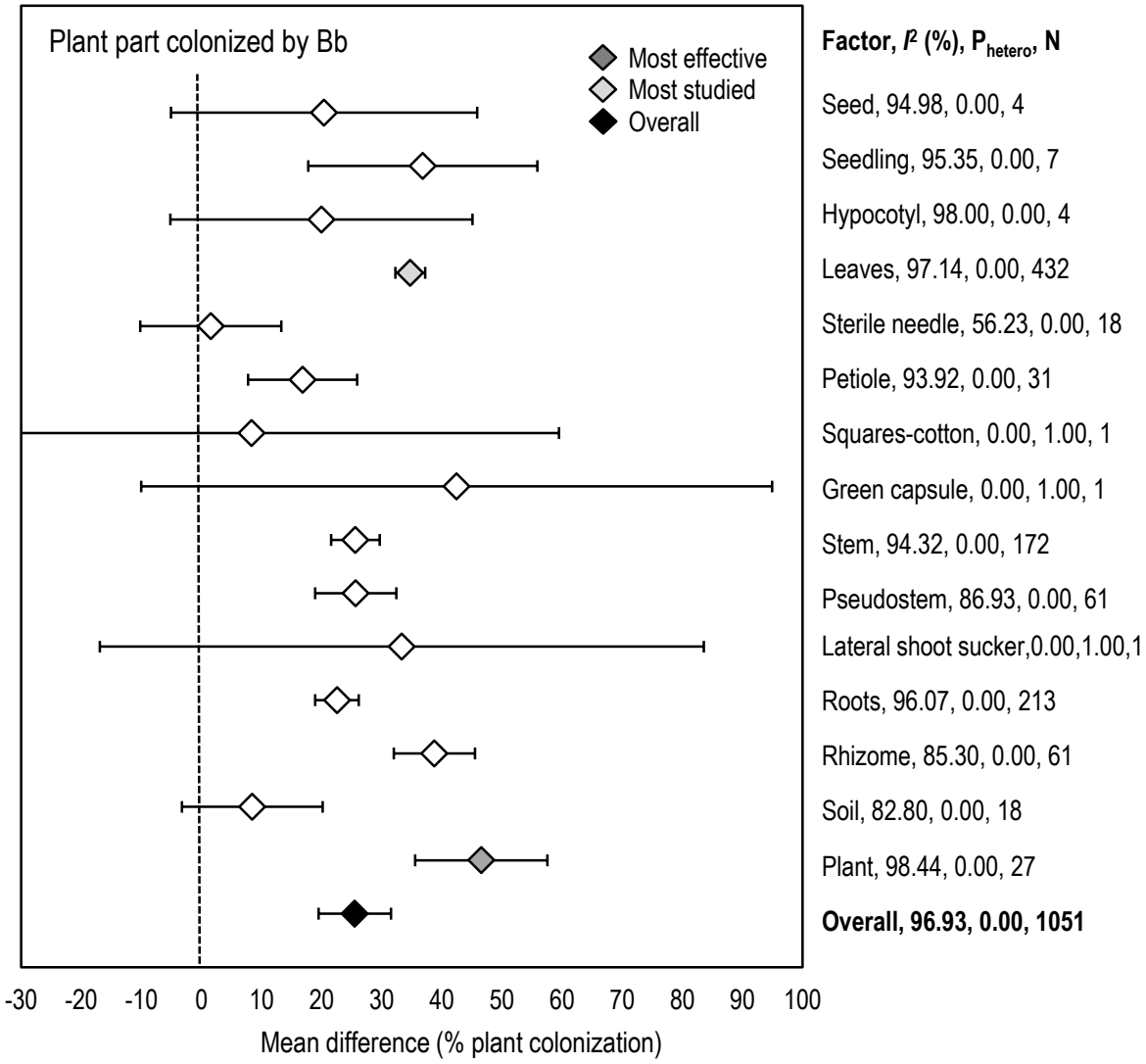


Figure 1.12. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific plant part colonized by Bb.

Moderator: Comparison among studies on the plant part colonized by Bb reported in colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (plant part colonized by Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

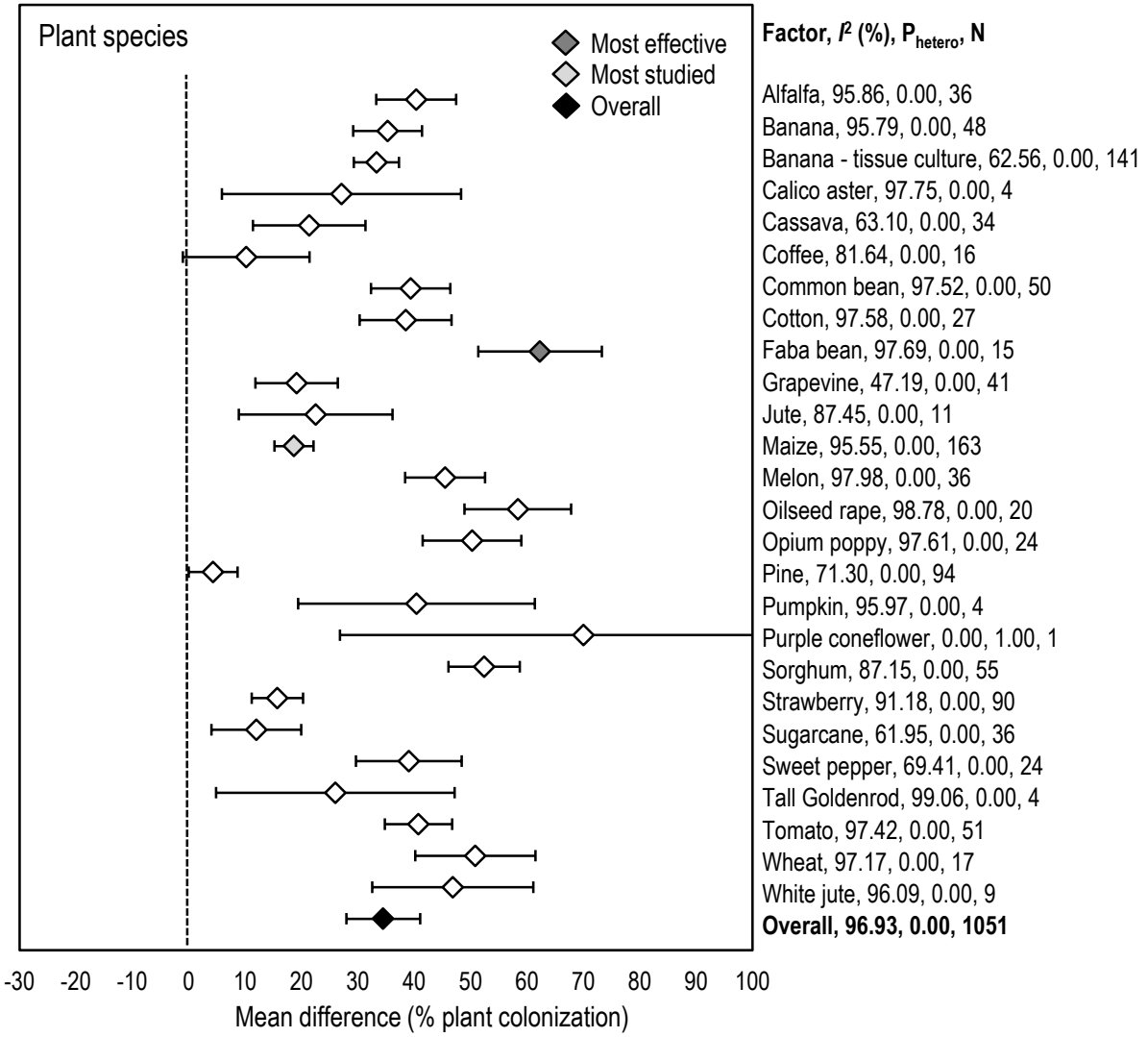


Figure 1.13. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific plant species.

Moderator: Comparison among studies on specific plant species used in plant colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (plant species) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

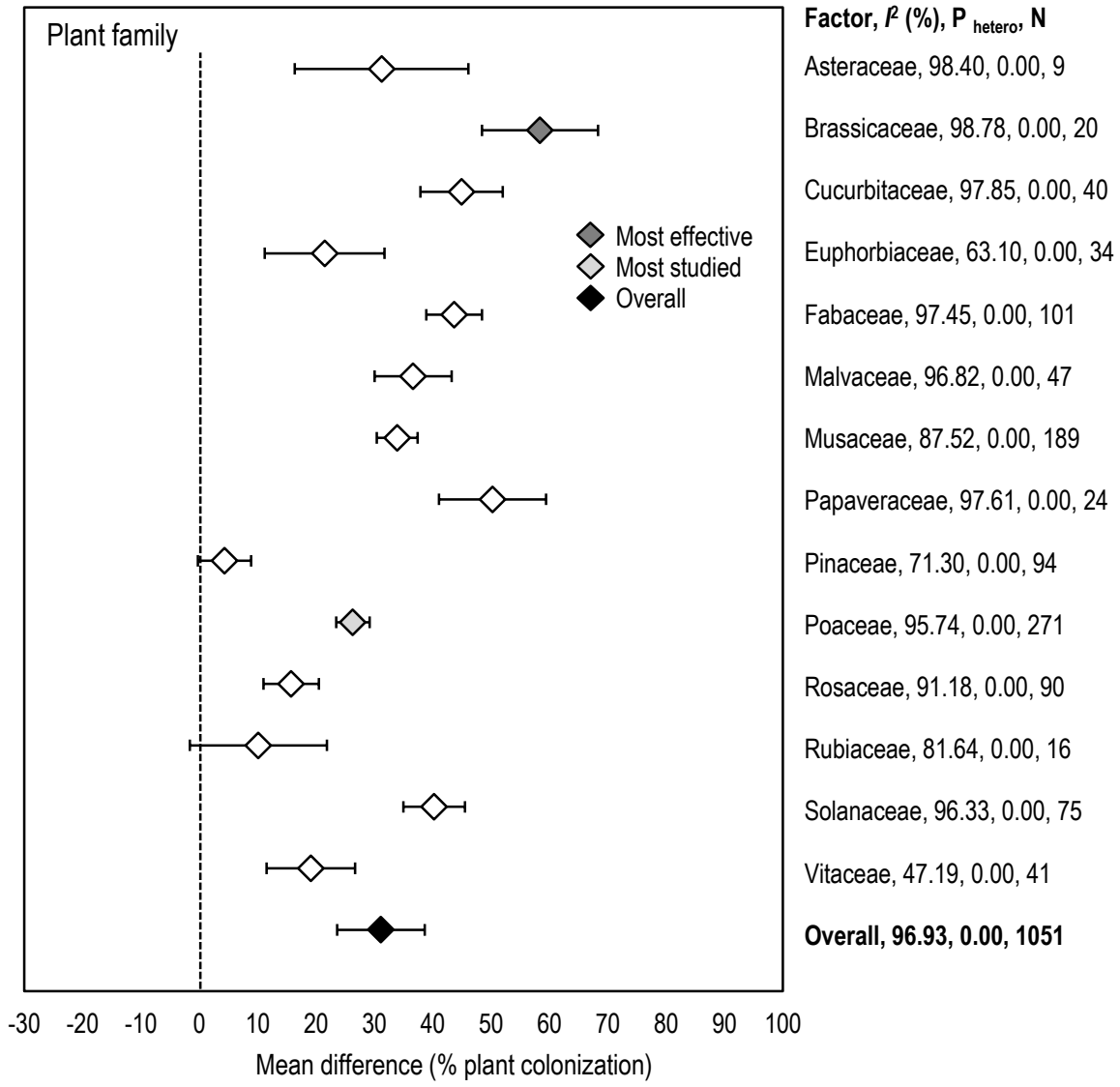


Figure 1.14. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific host plant family.

Moderator: Comparison among studies on plant host family used in colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (host plant family) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

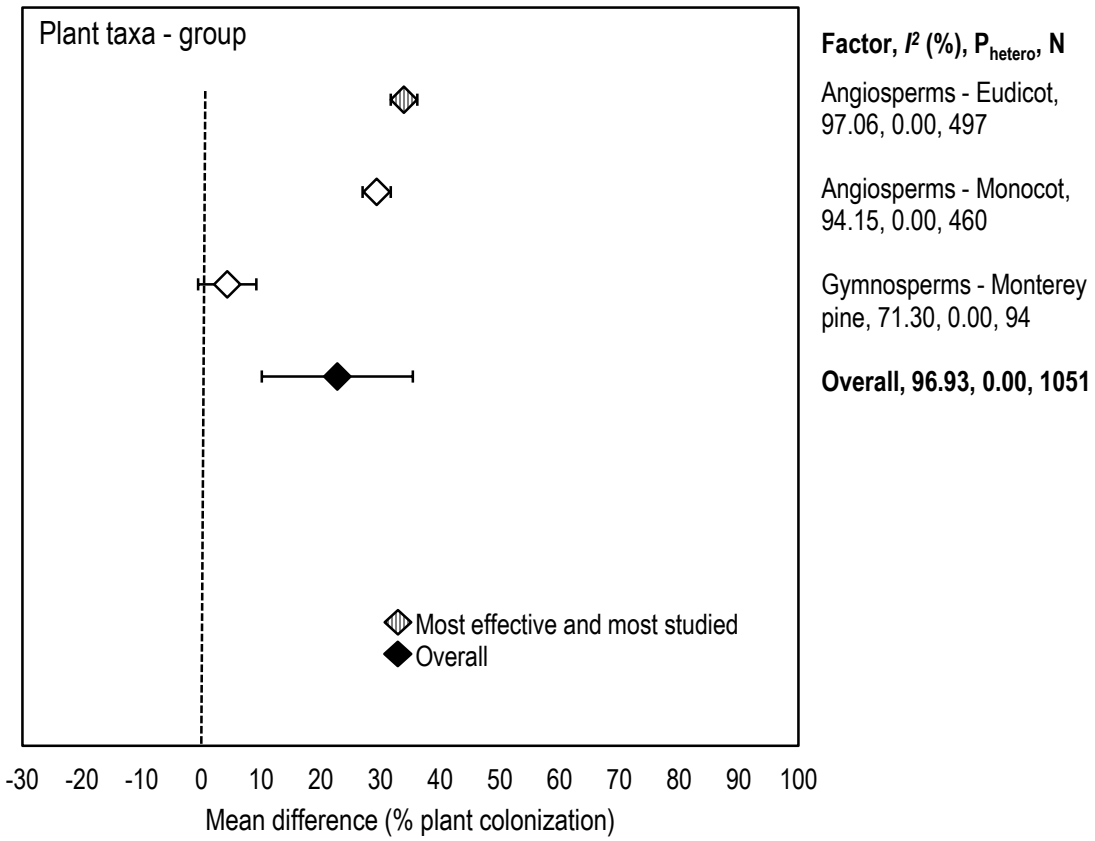


Figure 1.15. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific plant taxonomic group.

Moderator: Comparison among studies on plant taxonomic group used in colonization studies with Bb for each level of moderator. Values presented on the horizontal axis are mean differences in percent plant colonization (endophytic Bb treatment vs. control). For the factors (plant taxonomic group) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

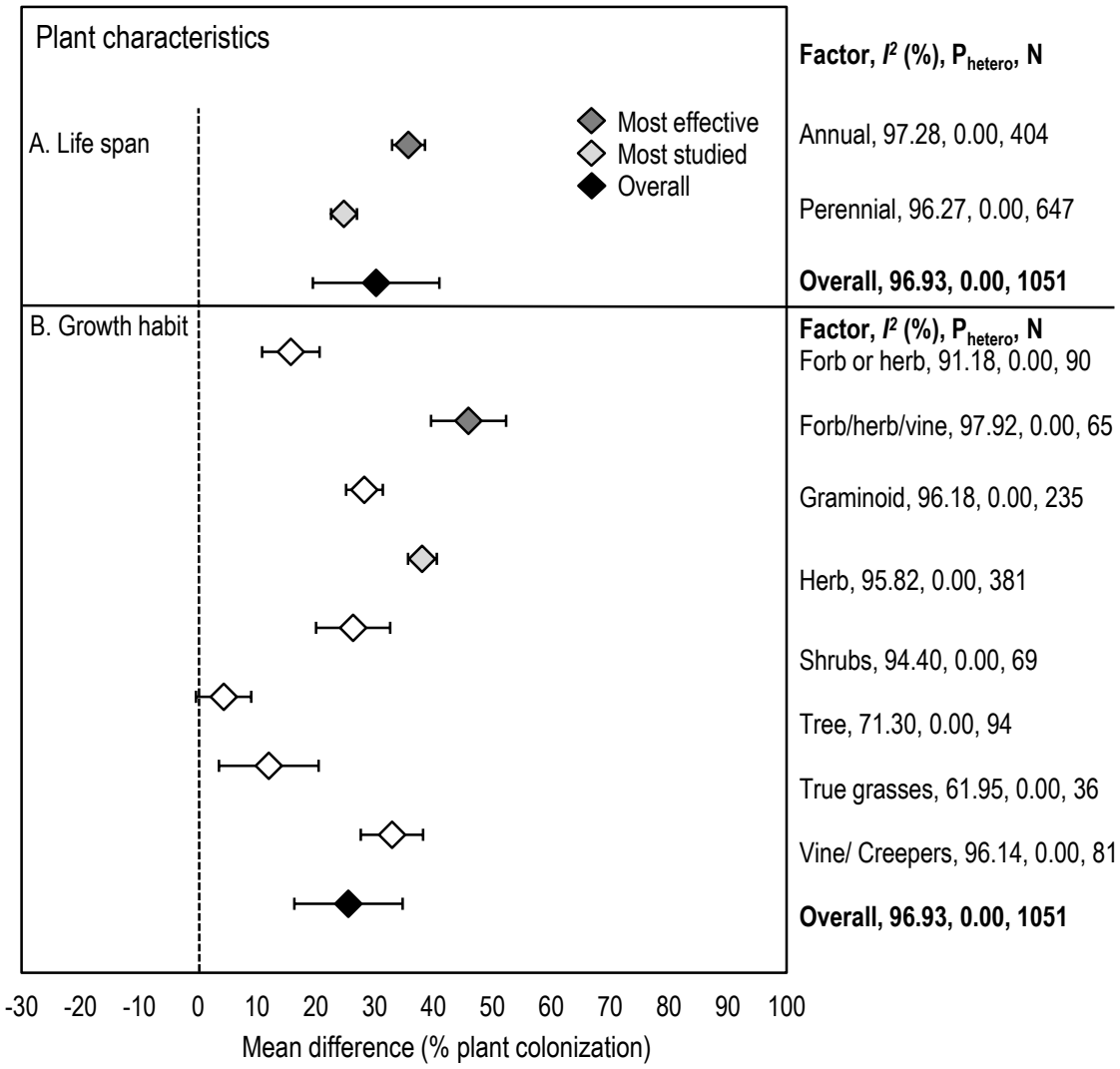


Figure 1.16. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific plant characteristics: (A) life span and (B) growth habit.

Moderator: Comparison among studies on plant characteristics, (A) life span and (B) growth habit, used in colonization studies with Bb for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For the factors (plant characteristics: (A) life span; (B) growth habit) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

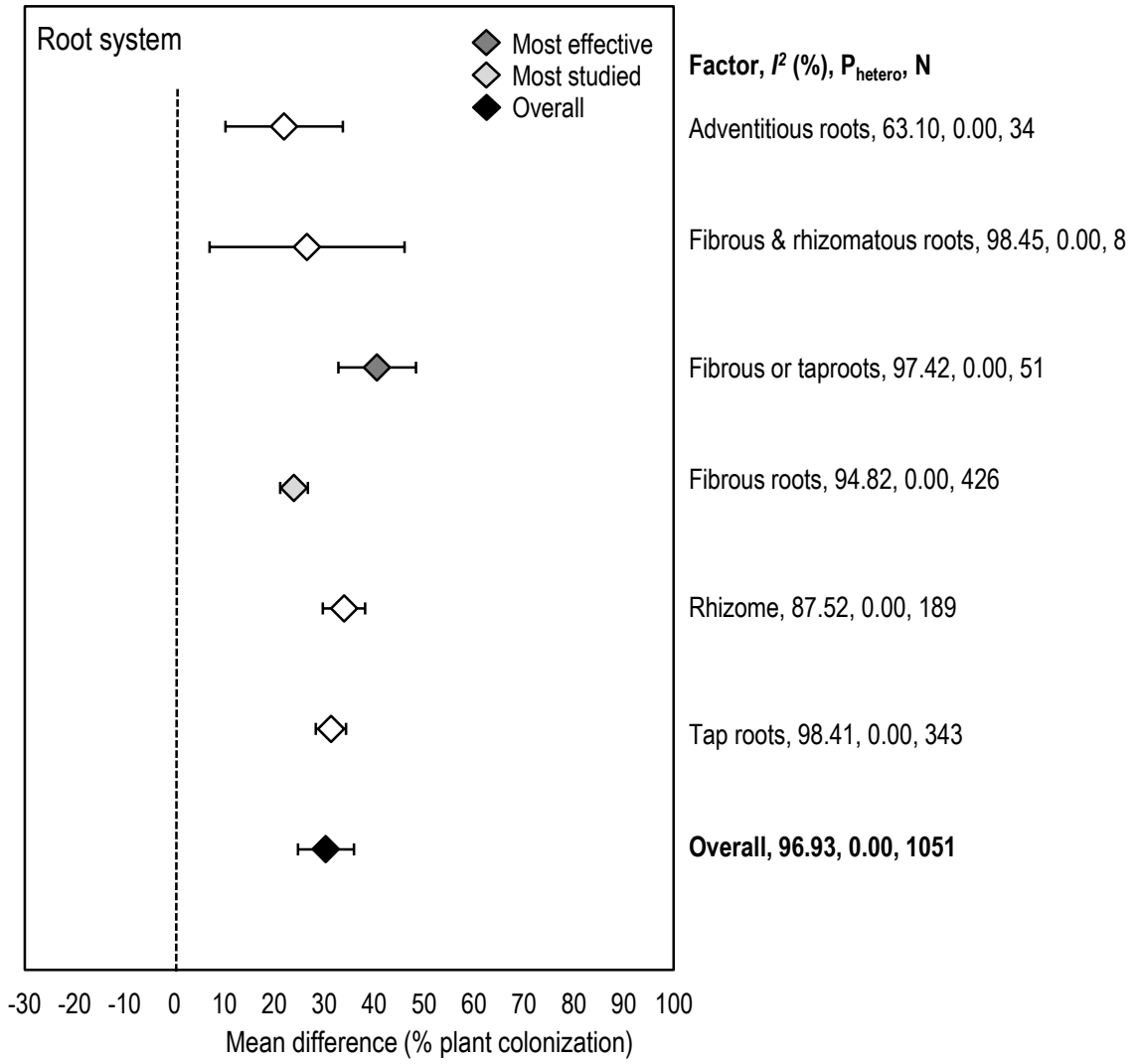


Figure 1.17. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific type of plant root system.

Moderator: Comparison among studies on specific type of root systems of plants used in colonization studies with Bb for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For the factors (type of plant root system) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

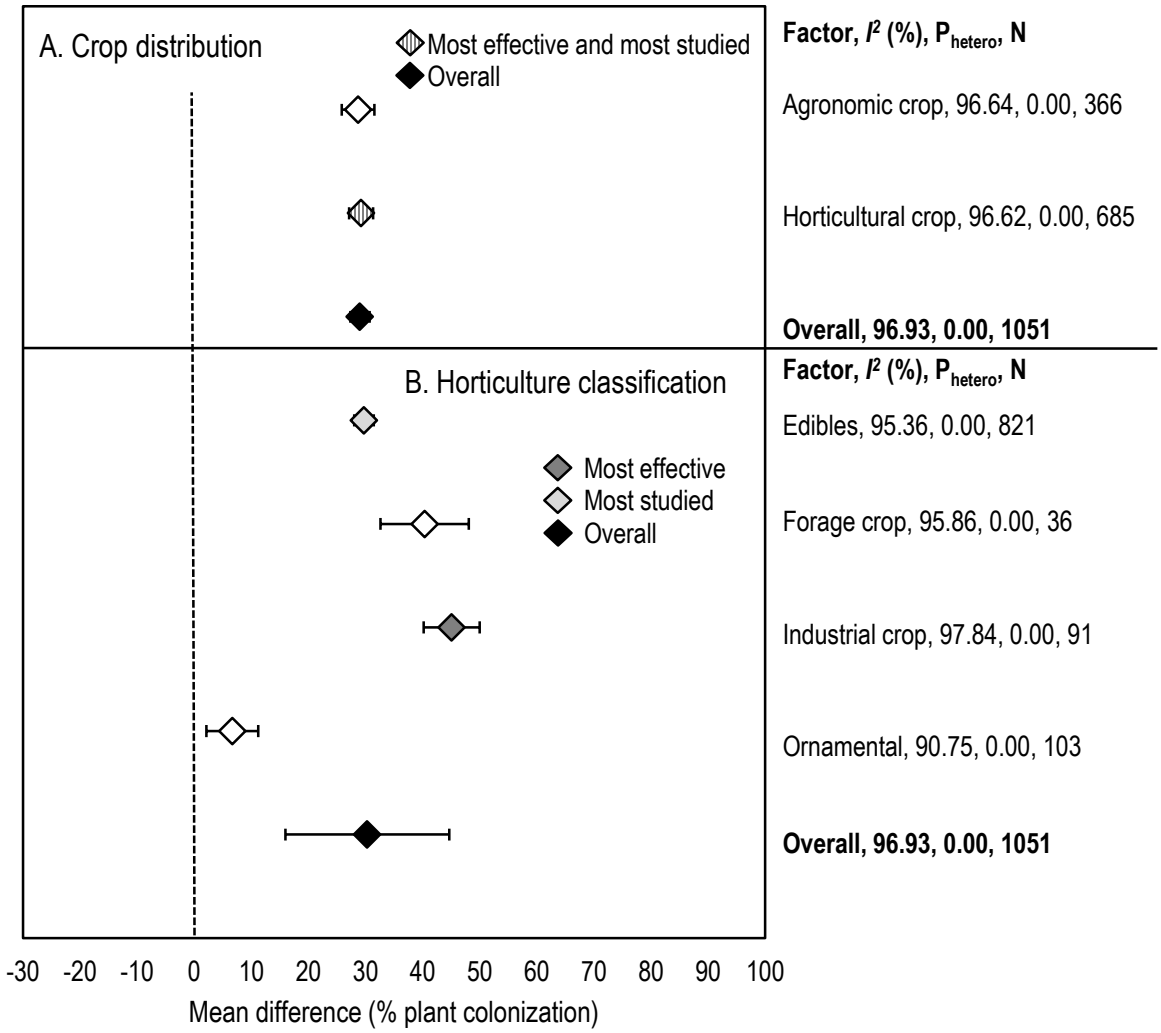


Figure 1.18. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific crop characteristics (A) crop distribution and (B) horticultural classification.

Moderator: Comparison among studies on specific crop characteristics (A) crop distribution and (B) horticultural classification used in colonization studies for each level of moderator. Values presented on the horizontal axis are percent mean difference in Bb plant colonization (endophytic Bb treatment vs. control). For the factors (A) crop distribution and (B) horticultural classification listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with I^2 = 0, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

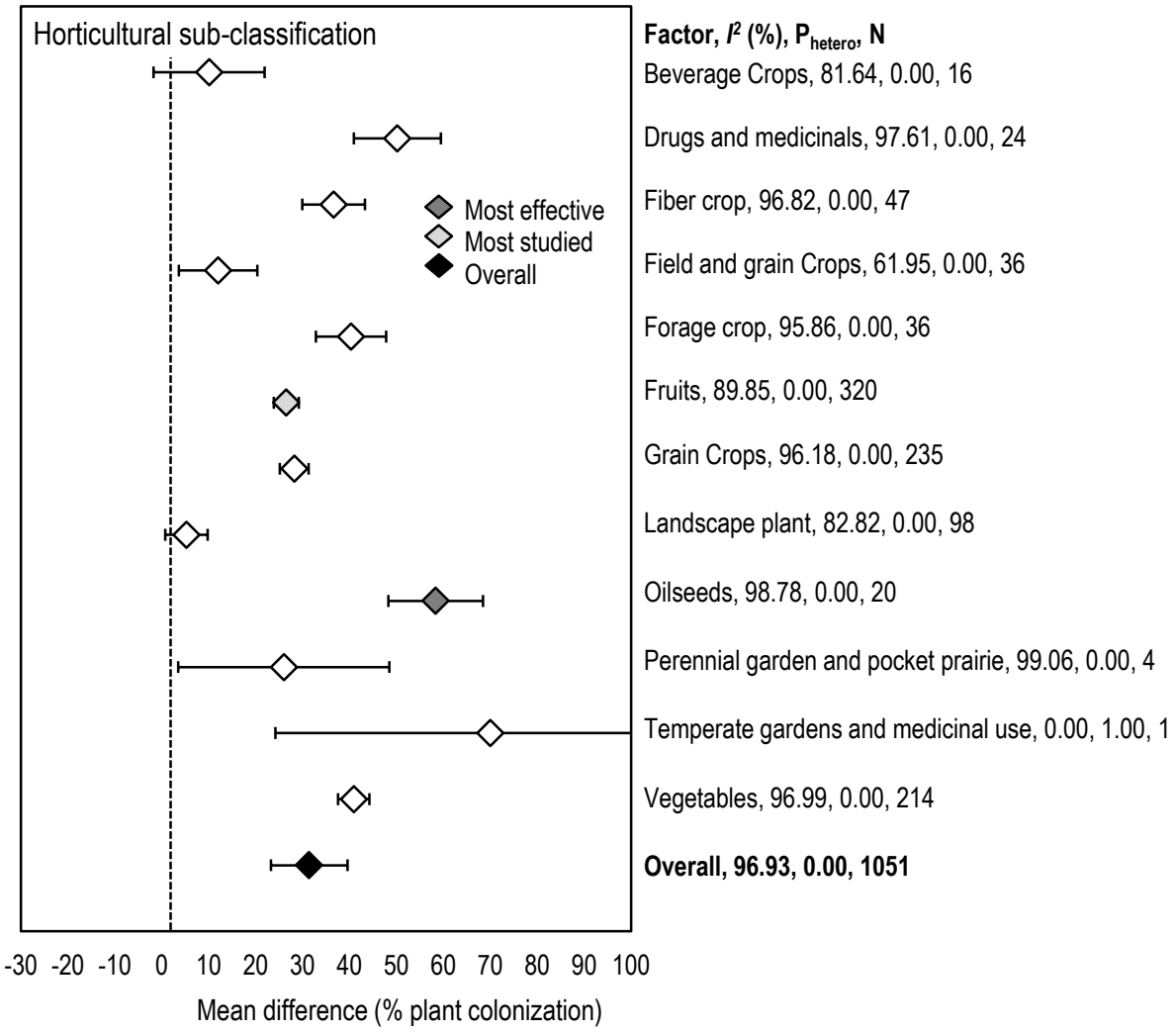


Figure 1.19. Mean difference and 95% confidence interval of percent plant colonization by endophytic B. bassiana (Bb) with meta-analysis based on specific horticultural sub-classification of the plant host.

Moderator: Comparison among studies on specific horticultural sub-classification of plants used in colonization studies by *B. bassiana* for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For factors (horticultural sub-classification) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

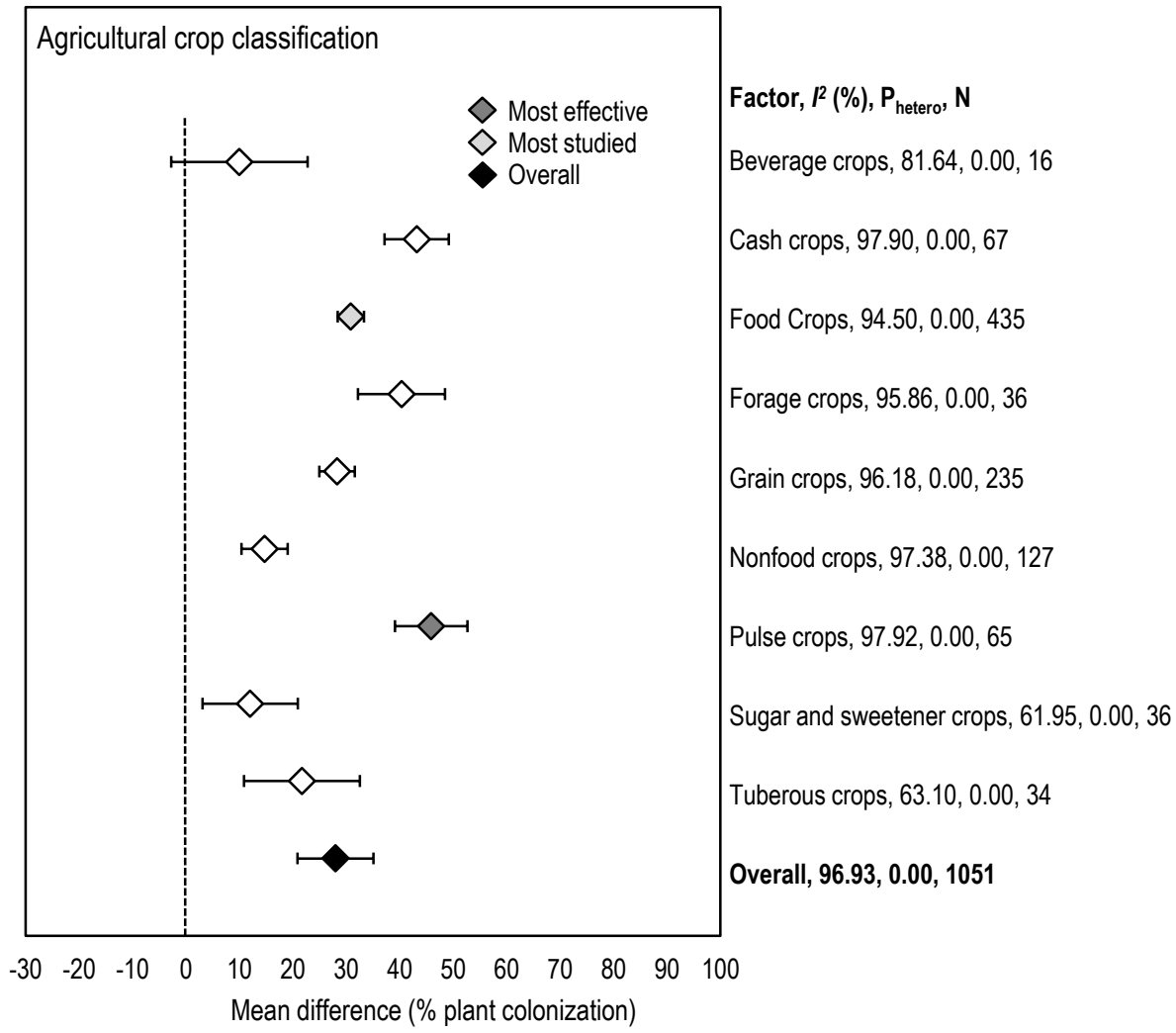
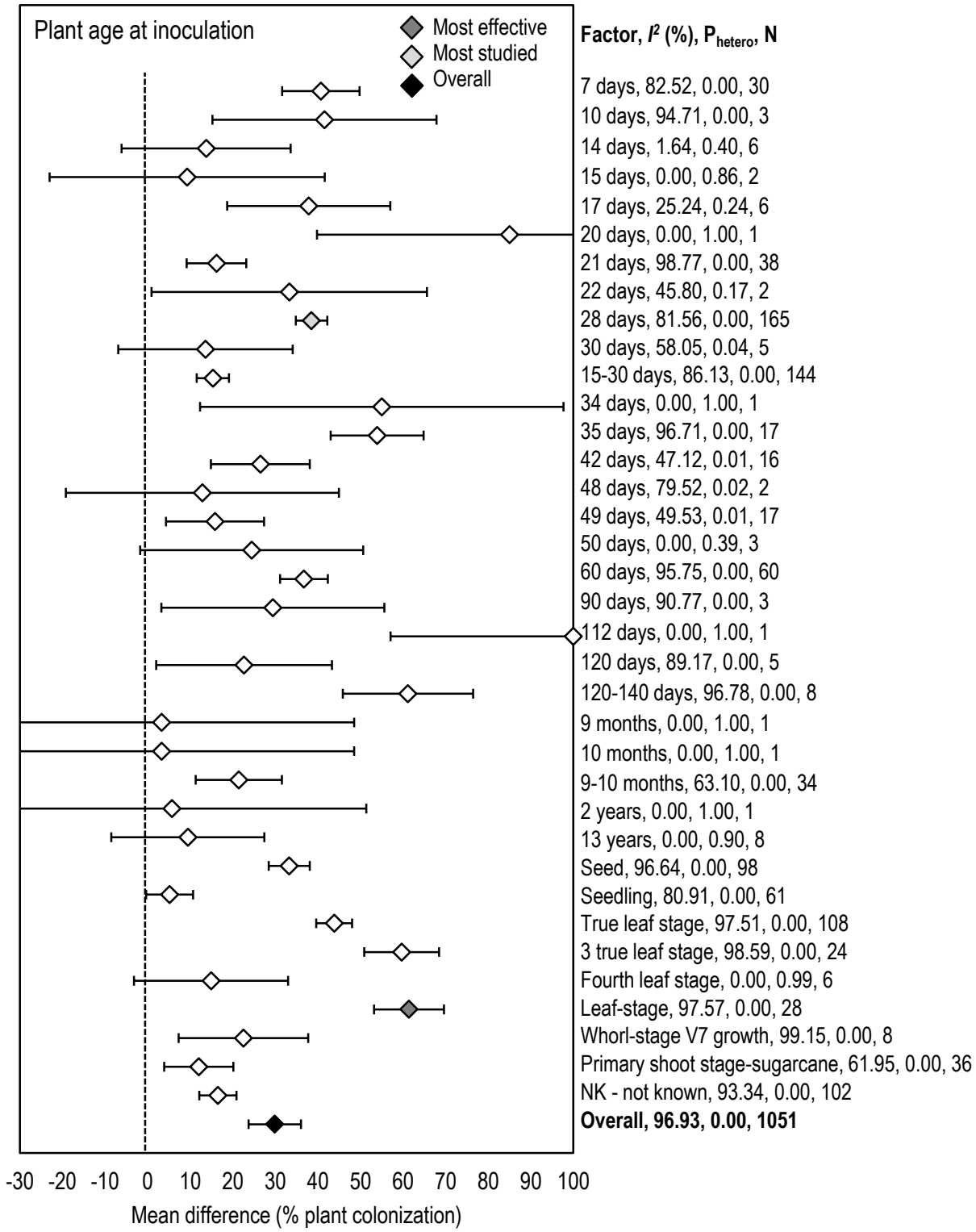


Figure 1.20. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific agricultural crop classification of the plant host.

Moderator: Comparison among studies on agricultural crop classification of the plant used in colonization studies with Bb for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For the factors (agricultural crop classification) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

Figure 1.21. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant age at inoculation with Bb.

Moderator: Comparison among studies on plant age at inoculation with Bb used in colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For the factors (plant age at inoculation) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.


Plant age at inoculation
Most effective
Most studied
Overall
Factor, I^2 (%), P_{hetero}, N
7 days, 82.52, 0.00, 30
10 days, 94.71, 0.00, 3
14 days, 1.64, 0.40, 6
15 days, 0.00, 0.86, 2
17 days, 25.24, 0.24, 6
20 days, 0.00, 1.00, 1
21 days, 98.77, 0.00, 38
22 days, 45.80, 0.17, 2
28 days, 81.56, 0.00, 165
30 days, 58.05, 0.04, 5
15-30 days, 86.13, 0.00, 144
34 days, 0.00, 1.00, 1
35 days, 96.71, 0.00, 17
42 days, 47.12, 0.01, 16
48 days, 79.52, 0.02, 2
49 days, 49.53, 0.01, 17
50 days, 0.00, 0.39, 3
60 days, 95.75, 0.00, 60
90 days, 90.77, 0.00, 3
112 days, 0.00, 1.00, 1
120 days, 89.17, 0.00, 5
120-140 days, 96.78, 0.00, 8
9 months, 0.00, 1.00, 1
10 months, 0.00, 1.00, 1
9-10 months, 63.10, 0.00, 34
2 years, 0.00, 1.00, 1
13 years, 0.00, 0.90, 8
Seed, 96.64, 0.00, 98
Seedling, 80.91, 0.00, 61
True leaf stage, 97.51, 0.00, 108
3 true leaf stage, 98.59, 0.00, 24
Fourth leaf stage, 0.00, 0.99, 6
Leaf-stage, 97.57, 0.00, 28
Whorl-stage V7 growth, 99.15, 0.00, 8
Primary shoot stage-sugarcane, 61.95, 0.00, 36
NK - not known, 93.34, 0.00, 102
Overall, 96.93, 0.00, 1051
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant colonization)

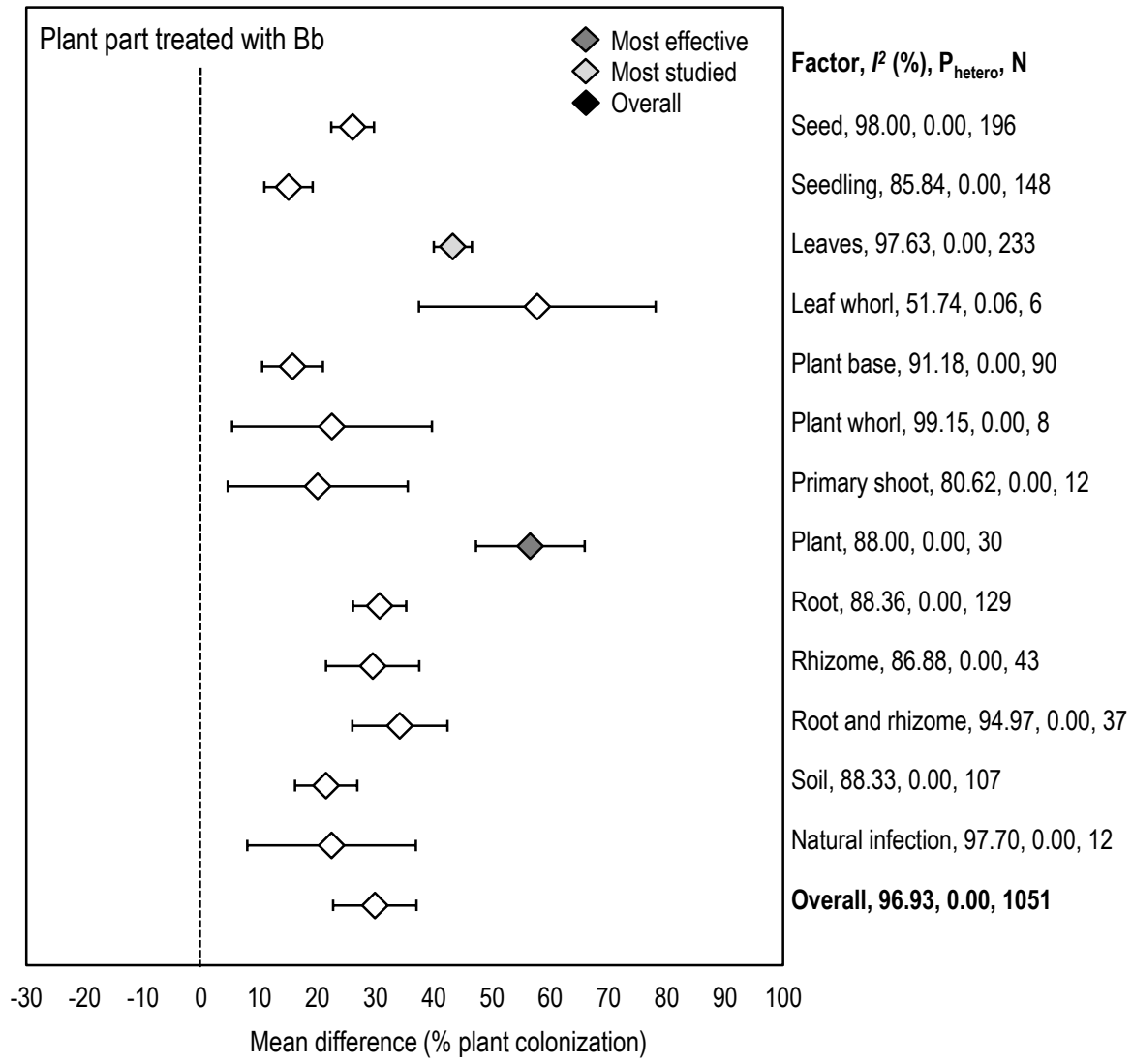


Figure 1.22. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific plant part treated with Bb.

Moderator: Comparison among studies on specific plant part treated with Bb used in colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For the factors (specific plant part treated with Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

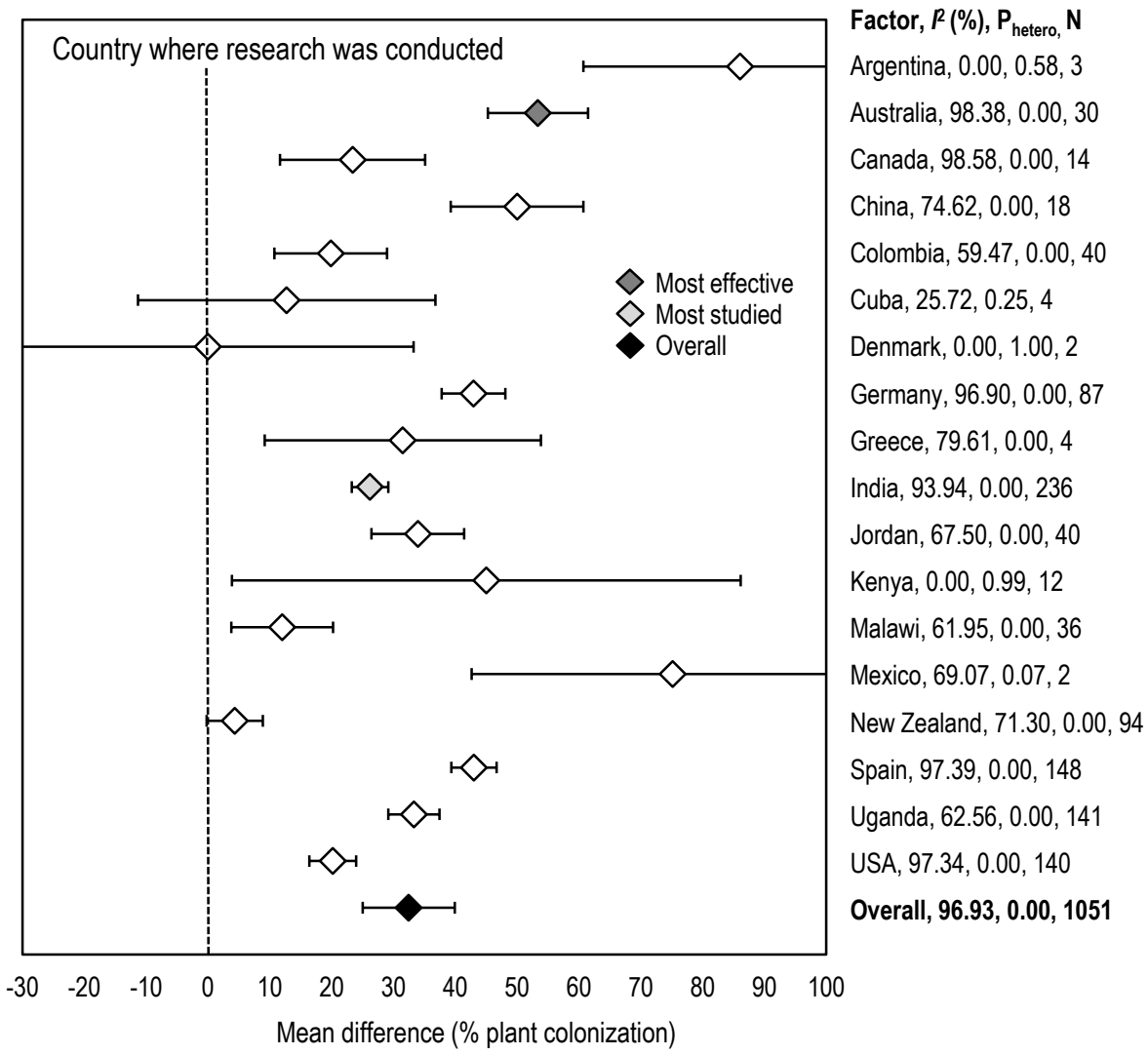


Figure 1.23. Mean difference and 95% confidence intervals for percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis on country where research was conducted.

Moderator: Comparison among studies where research on plant colonization by endophytic Bb was conducted for each level of moderator (country). Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For each factor (country where research occurred) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

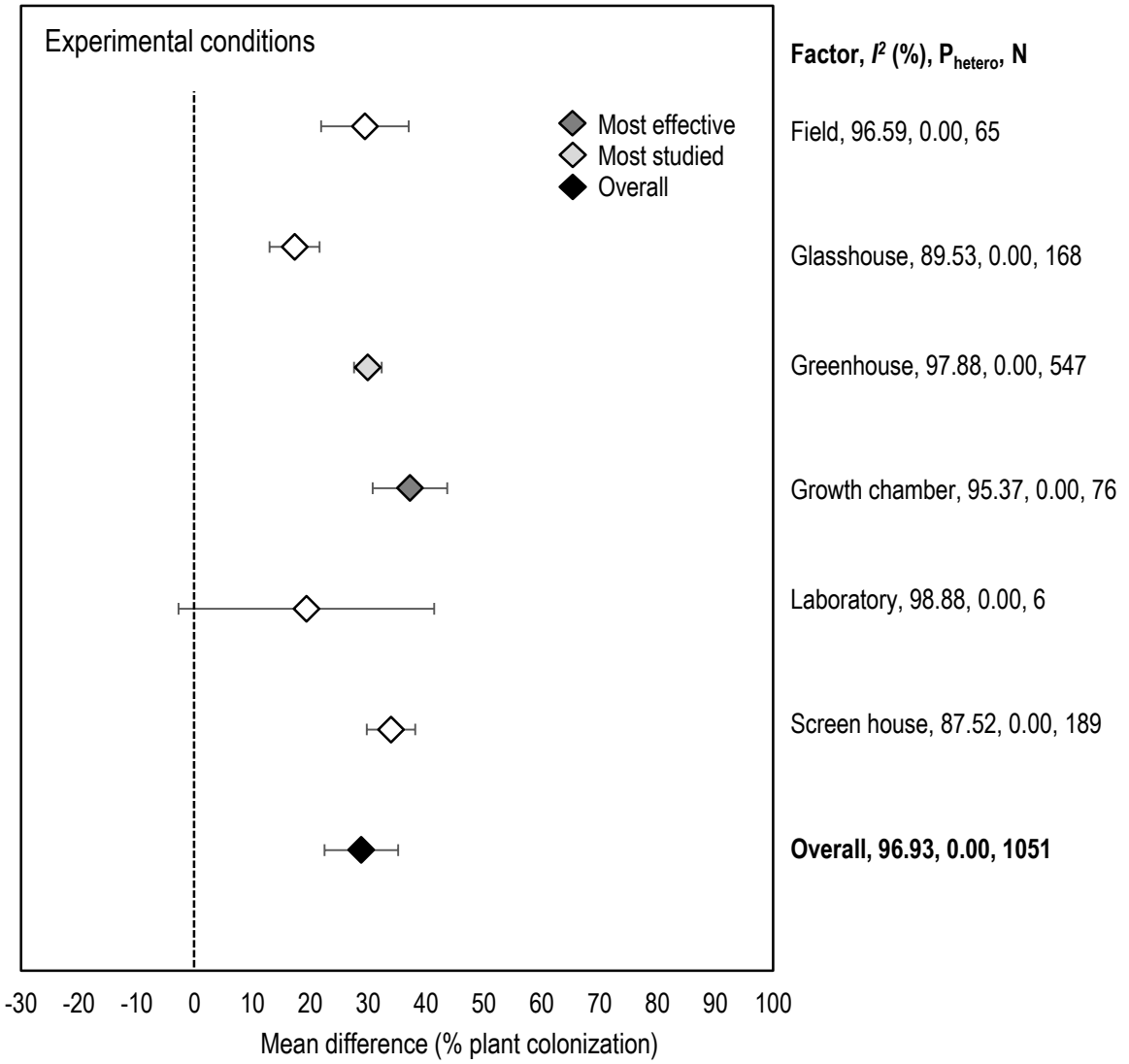


Figure 1.24. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific experimental conditions.

Moderator: Comparison among studies on specific experimental conditions used in plant colonization studies with endophytic Bb for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For factors (experimental conditions) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

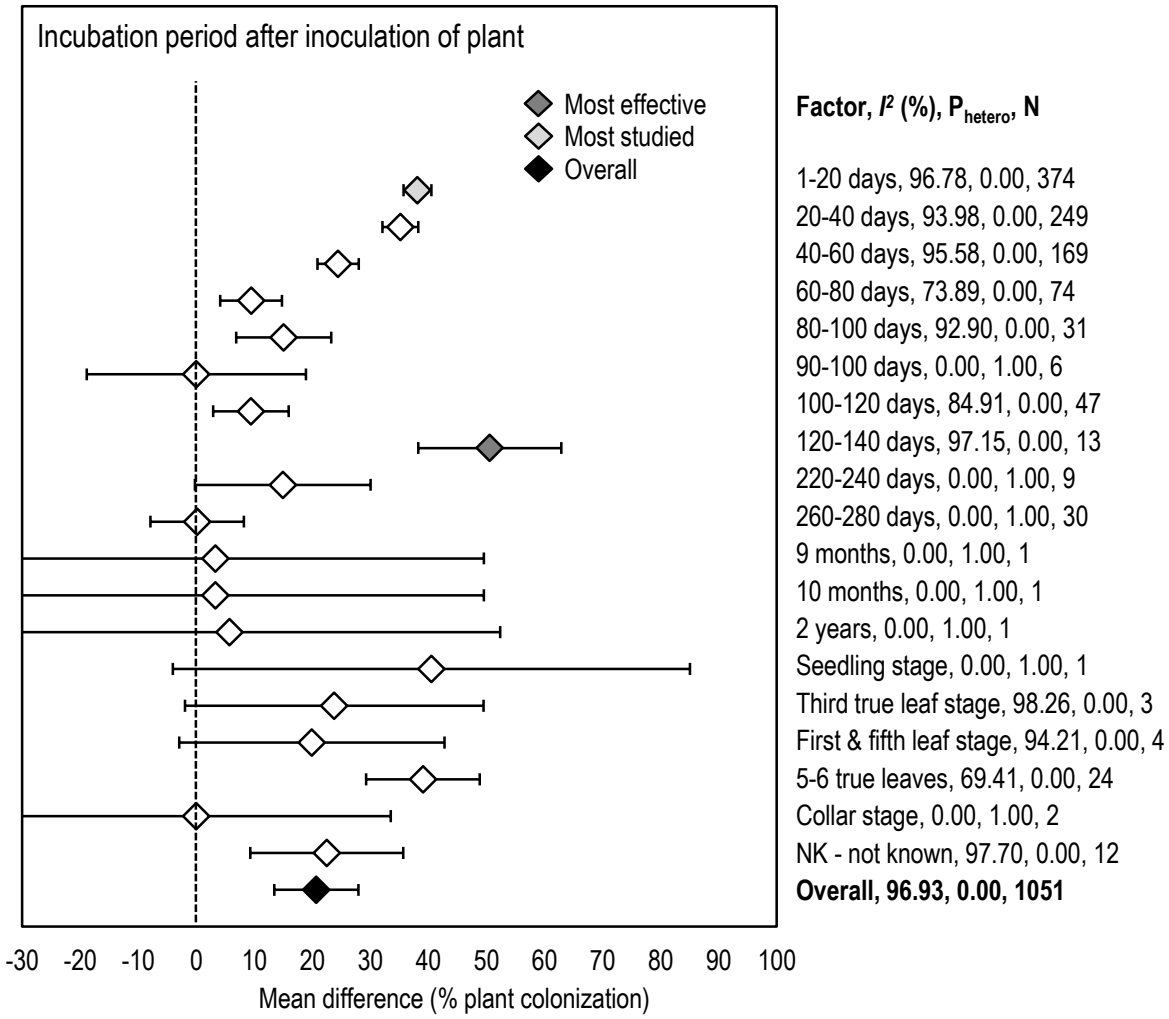


Figure 1.25. Mean difference and 95% confidence intervals of percent colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific incubation period following inoculation of the plant with Bb.

Moderator: Comparison among studies on specific incubation period following inoculation of the plant with Bb during plant colonization studies with Bb for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For the factors (incubation periods after application of Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

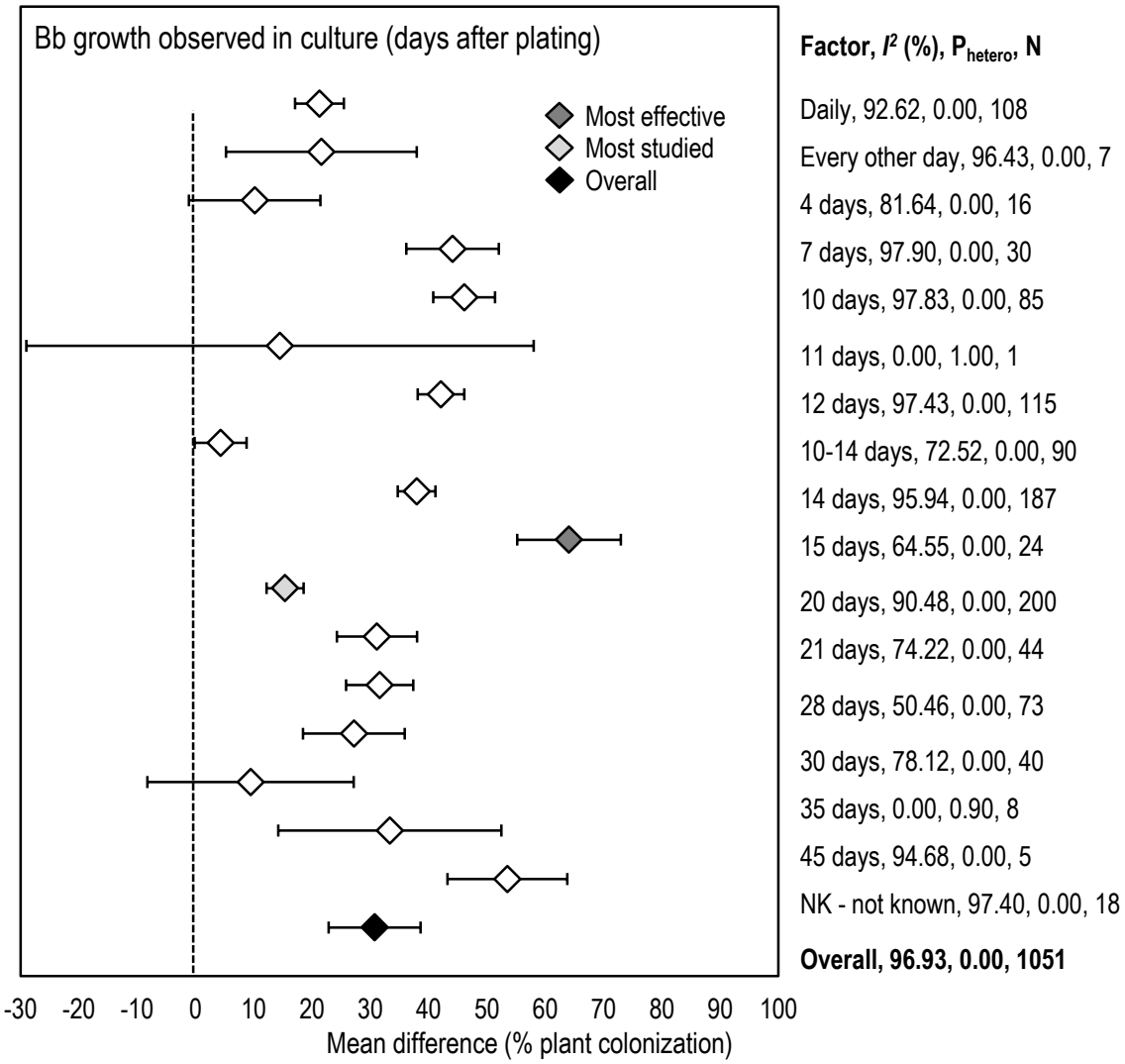


Figure 1.26. Mean difference and 95% confidence intervals of percent colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific culture period of incubation before fungal growth was observed).

Moderator: Comparison among studies on specific observation period of Bb isolate used in colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For the factors (culture periods) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

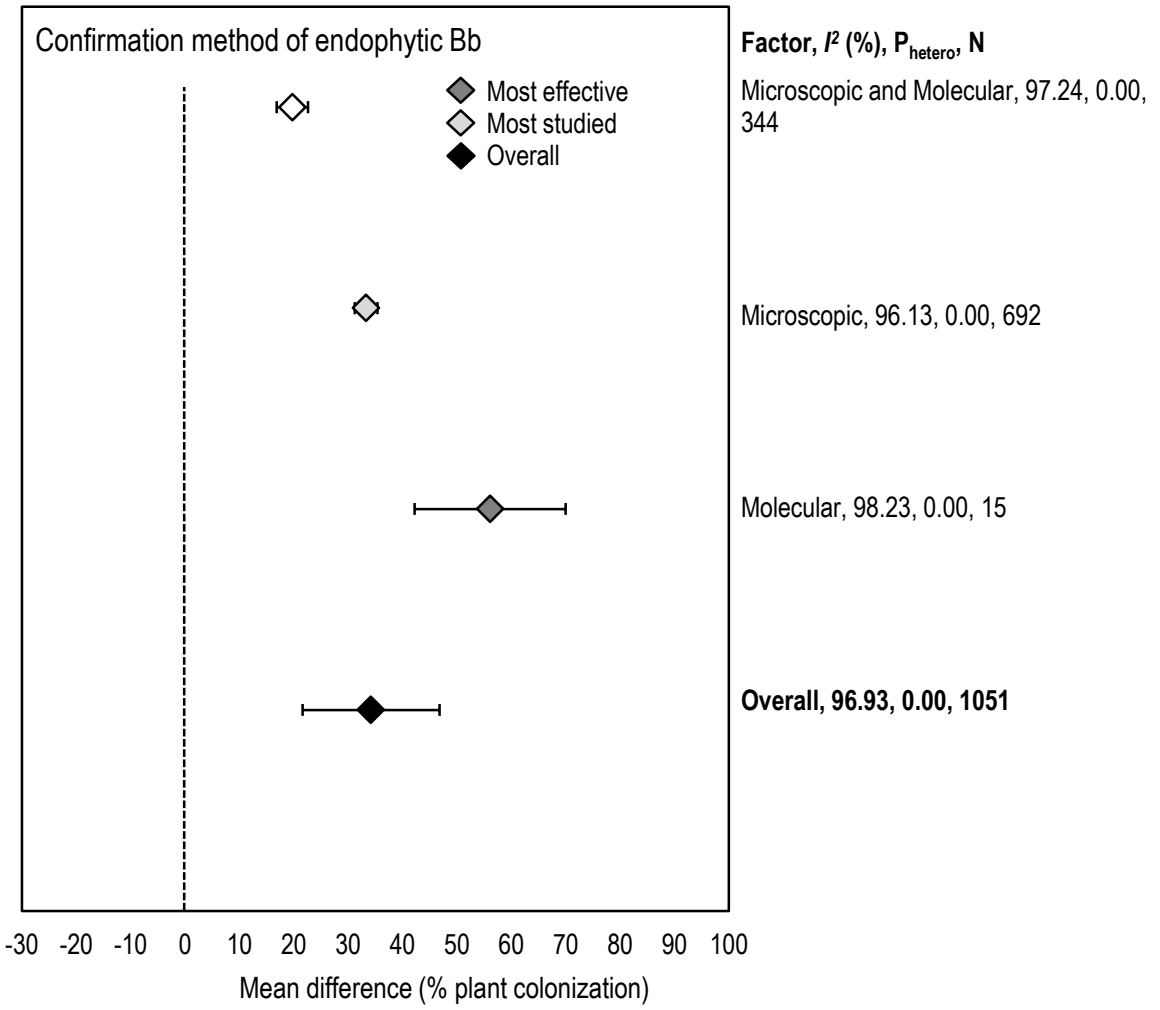


Figure 1.27. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific method of confirmation of endophytic Bb.

Moderator: Comparison among studies on specific method of confirmation of endophytic Bb in colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For factors (confirmation method for Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

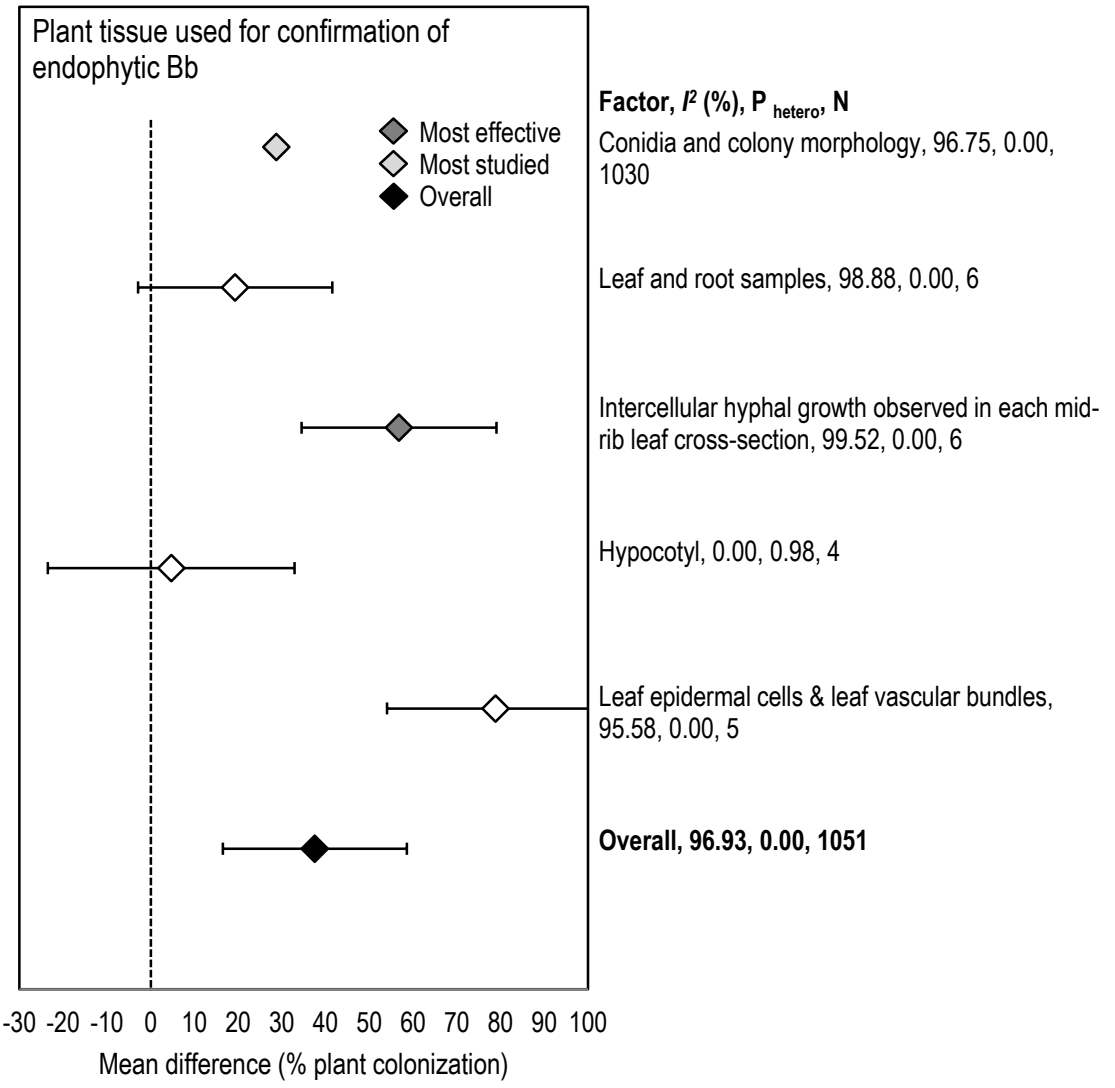


Figure 1.28. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific plant tissue used for confirmation of the presence of Bb.

Moderator: Comparison among studies on specific plant tissue used for confirmation of Bb in colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For factors (plant tissue used for confirmation of Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

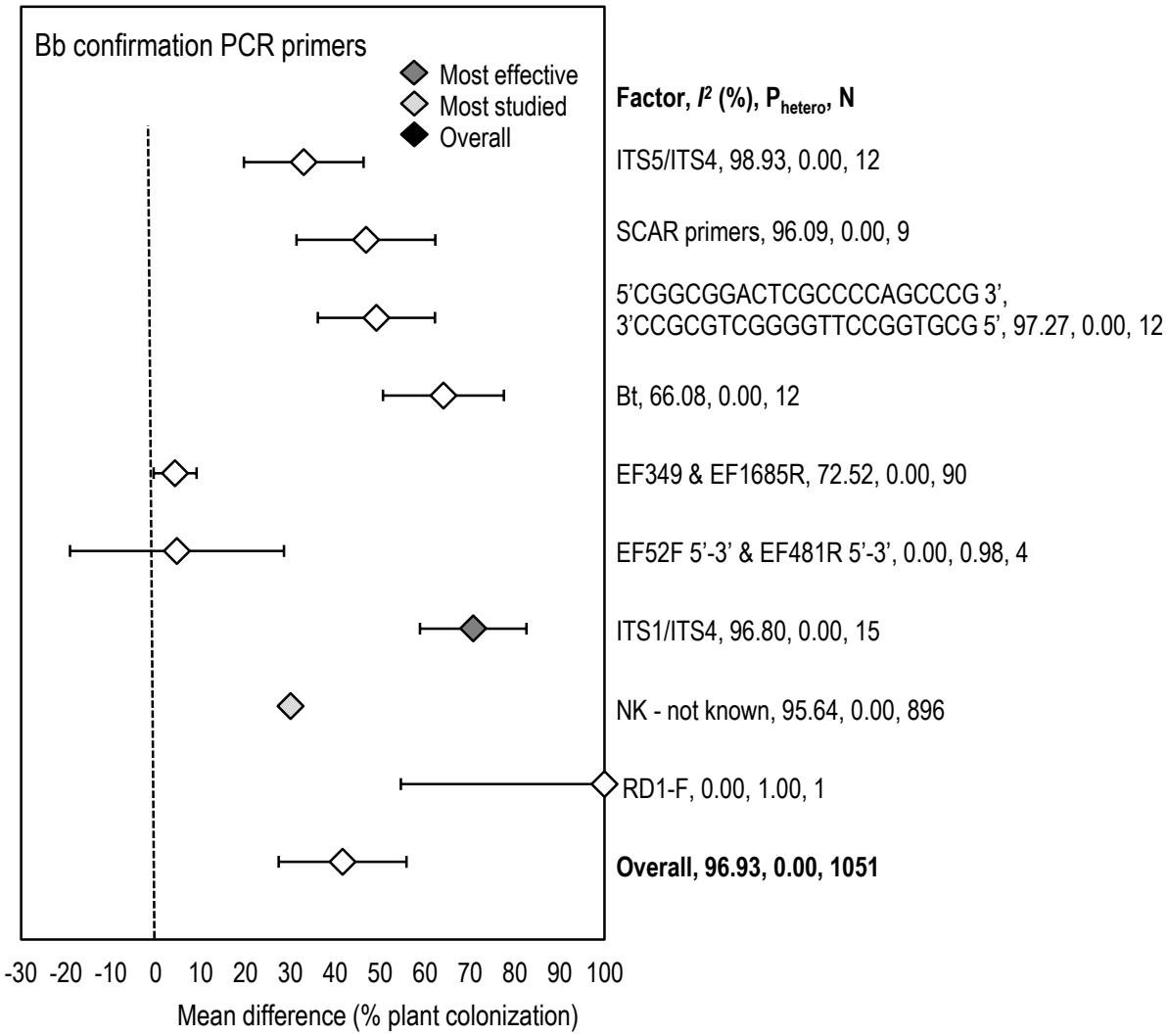


Figure 1.29. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific PCR primers used to confirm Bb.

Moderator: Comparison among studies on specific PCR primers used to confirm Bb in plant colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For the factors (PCR primers used to confirm Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

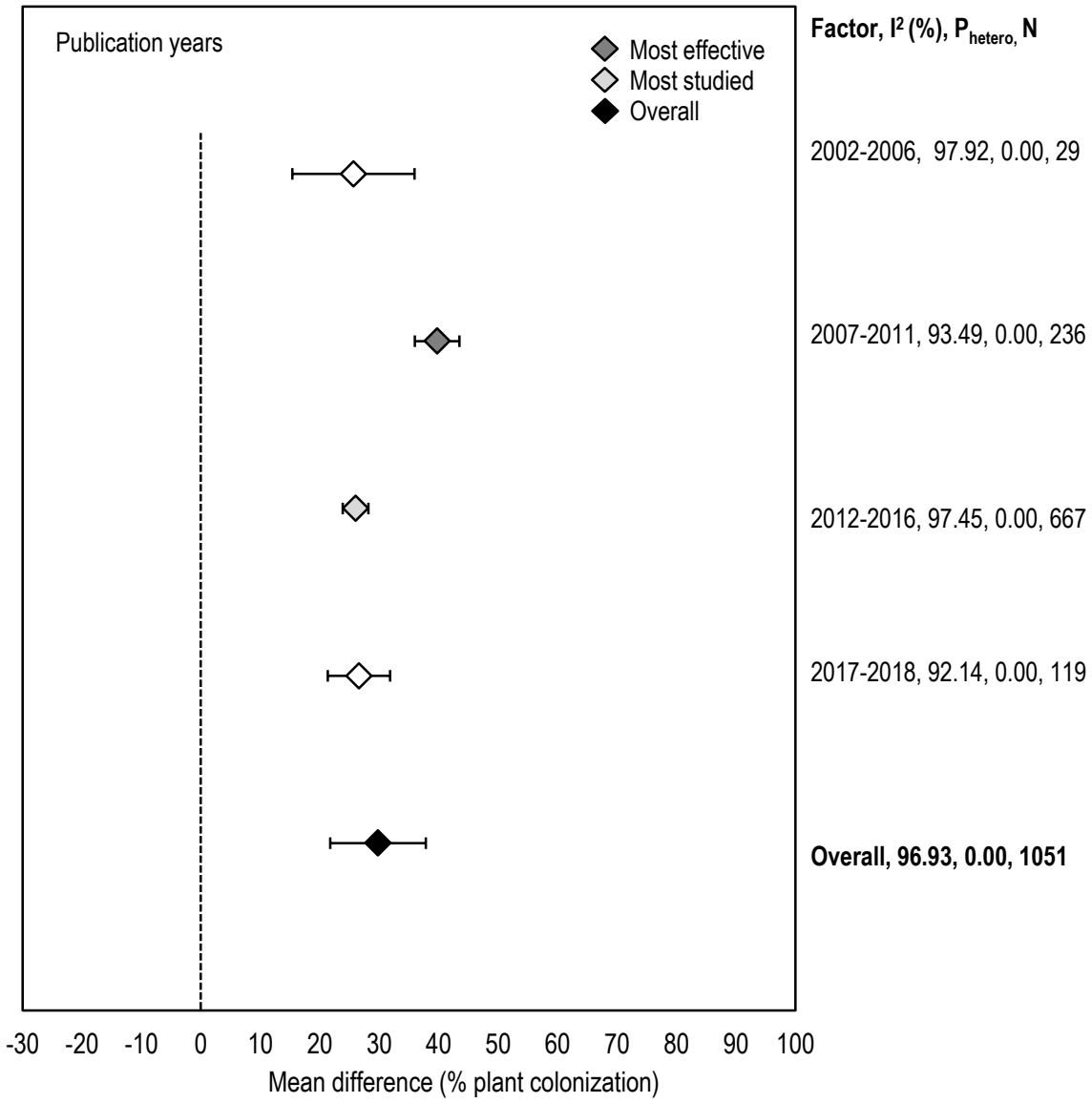
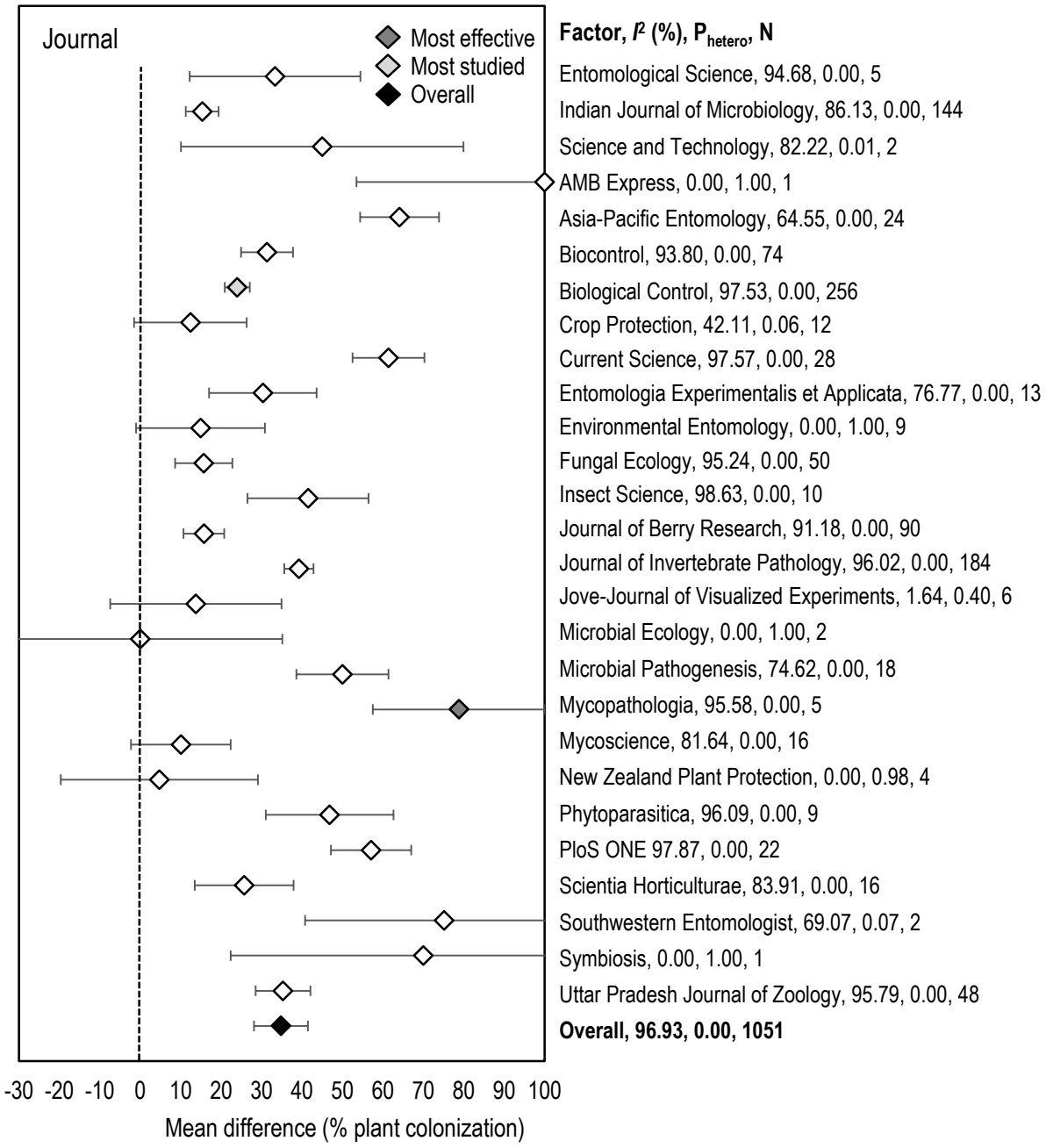


Figure 1.30. Mean difference and 95% confidence intervals of percent plant colonization by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific period of publication.

Moderator: Comparison among studies on specific period of publication used in colonization studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For the factors (publication year periods) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.

Figure 1.31. Mean difference and 95% confidence intervals of percent colonization by endophytic B. bassiana (Bb) of plant species analyzed with meta-analysis based on journal.

Moderator: Comparison among studies based on journal where the article on Bb plant colonization was published for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant colonization (endophytic Bb treatment vs. control). For the factors (journal) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb colonization effect.


Journal
Most effective
Most studied
Overall
Factor, I^2 (%), P_{hetero}, N
Entomological Science, 94.68, 0.00, 5
Indian Journal of Microbiology, 86.13, 0.00, 144
Science and Technology, 82.22, 0.01, 2
AMB Express, 0.00, 1.00, 1
Asia-Pacific Entomology, 64.55, 0.00, 24
Biocontrol, 93.80, 0.00, 74
Biological Control, 97.53, 0.00, 256
Crop Protection, 42.11, 0.06, 12
Current Science, 97.57, 0.00, 28
Entomologia Experimentalis et Applicata, 76.77, 0.00, 13
Environmental Entomology, 0.00, 1.00, 9
Fungal Ecology, 95.24, 0.00, 50
Insect Science, 98.63, 0.00, 10
Journal of Berry Research, 91.18, 0.00, 90
Journal of Invertebrate Pathology, 96.02, 0.00, 184
Jove-Journal of Visualized Experiments, 1.64, 0.40, 6
Microbial Ecology, 0.00, 1.00, 2
Microbial Pathogenesis, 74.62, 0.00, 18
Mycopathologia, 95.58, 0.00, 5
Mycoscience, 81.64, 0.00, 16
New Zealand Plant Protection, 0.00, 0.98, 4
Phytoparasitica, 96.09, 0.00, 9
PloS ONE 97.87, 0.00, 22
Scientia Horticulturae, 83.91, 0.00, 16
Southwestern Entomologist, 69.07, 0.07, 2
Symbiosis, 0.00, 1.00, 1
Uttar Pradesh Journal of Zoology, 95.79, 0.00, 48
Overall, 96.93, 0.00, 1051
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant colonization)

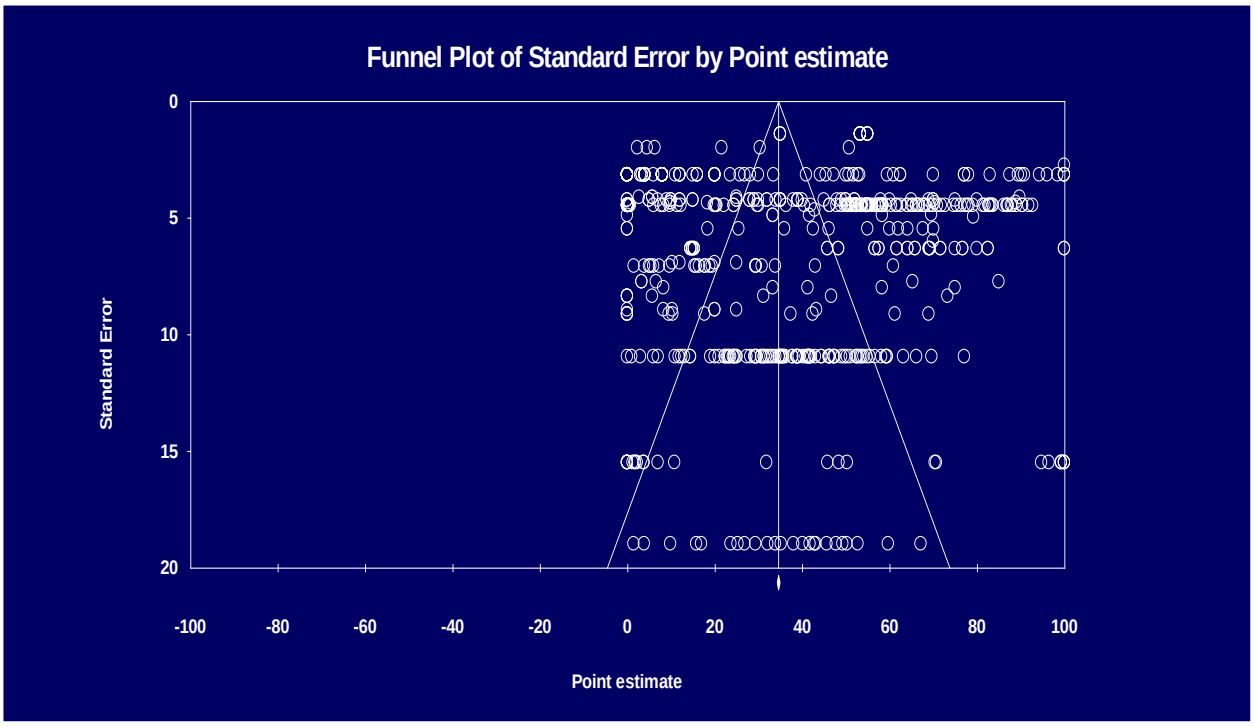


Figure 1.32. Publication bias (PB) represented as a funnel plot (Standard error by point estimate).

The horizontal axis shows the point estimate or mean difference (%) at 95% confidence intervals for percent plant colonization by endophytic Bb extracted from different research studies. The circles represent each individual study (total = 1,051). The point estimates are plotted against standard error of the mean (Y-axis).

CHAPTER 2

META-ANALYSIS ON THE EFFICACY OF ENDOPHYTIC INSECT PATHOGENIC FUNGUS *BEAUVERIA BASSIANA* AGAINST DIVERSE INSECT PESTS

Abstract

In the past ten years, it has become more widely accepted that the well-known insect-pathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Bb), can endophytically protect plants from insect pests. Although several studies on plant protection with endophytic Bb have been published, quantitative studies on the consistency, synthesis and validation of these reports are lacking. To fill these knowledge gaps, a meta-analysis was conducted on the efficacy of endophytic Bb against insect pests. Data on insect mortality caused by endophytic Bb, under different experimental conditions, was collected from 185 studies that included an endophytic Bb treatment and a non-Bb control. The data was analyzed as a random-effects model with Comprehensive Meta-Analysis (CMA) Version 3 software. Several influential factors that impacted the effect of endophytic Bb on insect mortality were determined and analyzed as moderators (32). Moderators were categorized as those that primarily influenced endophytic Bb (11), insect hosts (10), plant hosts (5) and other experimental factors (6). Overall, endophytic Bb had a significant effect on insect mortality, which averaged 39%. Heterogeneity across studies was significant ($P \leq 0.05$) and high ($I^2 = 98.46\%$), therefore the null hypothesis (studies share a common effect) was rejected, and it was concluded that the 185 collected studies do not share a common effect. Moderator analysis revealed the most significant treatments when endophytic Bb was used to control insect pests (based on percent mortality). For example, Bb isolate EABb 01/33-Su caused a high and significant rate of mortality at 61%. High mortality was also recorded for native isolate of Bb (44%) (versus commercial), endophytic Bb isolates from Ethiopia (69%), and isolates originally from soil (68%), and insects (36%). Foliar application of Bb to flower buds resulted in 74% insect mortality. A concentration of 1×10^5 Bb conidia/ml caused

62% mortality, and Bb applied at a rate of 0.25% w/v dried mycelial extract in water caused 66% mortality. In studies that reported the percent plant colonization by Bb, insect mortality was higher (32%) with 50 to 100% plant colonization. Regarding factors that affected insects, Bb was highly effective against spotted stem borer causing 73% insect mortality, oligophagous insects (68%), insects in the Order Thysanoptera (64%), and Family Crambidae (73%). Endophytic Bb caused 73% mortality of insects in the fourth instar larval stage, 41% mortality of insects with chewing mouthparts, 64% mortality of insects that caused damage to growing tips and flower buds, and 47% mortality of insects that fed on rhizomes and pseudostems. Insects that live above and below (mainly rhizome) ground had 47% mortality caused by endophytic Bb, while insects with plant destructive stages that included larva, nymphs and adults had 52% mortality. Moderators that mainly affected plants included plant taxonomy, plant age at inoculation, and the nature of Bb colonized plant materials fed to insects. Endophytic Bb caused high insect mortality in roses (74%), and insects that were treated directly with endophytic Bb (61%). Mortality of insects associated with eudicots treated with endophytic Bb was 33% and 61% for insects associated with Bb-treated members of the family Cucurbitaceae. Endophytic Bb caused 74% insect mortality when plants at the tight bud stage were treated. Other factors included country where the research on endophytic Bb was conducted (74% insect mortality for studies conducted in Iran), experimental conditions (62% mortality in controlled conditions in the laboratory), incubation period after treatment with Bb (70% mortality with an incubation period of 10 days), and the timeframe for observation of fungus outgrowth from infected insects (73% mortality when insects were observed daily). Studies that reported mycosis had 47% insect death. The most effective results with endophytic Bb and insect mortality were

publications in 2008-2011 (53%), however most articles were published in 2016-2019 with 117 studies. In this study, there was much variability in the sources and plants associated with endophytic Bb, as well as a wide variety of insect species and methodologies for application of Bb to control insect pests. Identification of influential factors across multiple studies may provide new information and better understanding of Bb and its durability in different host kingdoms. These findings may provide directions for future research related to endophytic plant protection and new or improved sustainable biocontrol technologies.

Introduction

More than 10,000 insect species are known to cause damage to food crops in the world (Singh, et al., 2014; Dhaliwal et al., 2010). In the past decade, global crop losses caused by insect pests, diseases, and weeds have increased enormously, despite development of modern technologies for pest control (Singh, et al., 2014). Pesticides are most commonly used to control insect pests, but they can cause environmental pollution and health hazards (Wilson and Tisdell, 2001; Asogwa and Dongo, 2009), pest resurgence (Dutcher, 2007), pest outbreaks (Singh, et al., 2014), and pest resistance problems (Tabashnik et al., 1990). In addition, pesticides may be harmful to beneficial insects and natural enemies of insect pests (Flexner, et al., 1986; Porrini et al., 2003; Theiling and Croft, 1988) and non-target organisms (Anderson, 1982). Pesticide usage can result in bioaccumulation, which has become a major threat to the ecosphere (Yadav, 2010) including aquatic life, and plant fauna and flora (Igbedioh, 1991; Cope, 1966). Additionally, pesticides are often not cost effective (Wilson and Tisdell, 2001). To minimize the potential negative impacts of conventional pesticides, there is an urgent need to develop alternative approaches.

Development of eco-friendly endophytic bioagent-based protection to control insect pests is an alternative to minimize conventional pesticide usage. Recently, it has been established that some of the well-known insect-pathogenic fungi that are commercially used in biological control of insect pests, can also become endophytes in certain plants (Vega et al., 2008). One such example is *Beauveria bassiana* (Balsamo) Vuillemin (Bb). As an endophyte, Bb can live inside plant tissue and establish a mutualistic long-lasting relationship with the host plant (Yan et al., 2015).

Beauveria bassiana is a ubiquitous, entomopathogenic fungus, which is classified in the order Hypocreales (Ascomycota), and causes white muscardine disease in insects. The fungus has been reported as an endophyte in various host plants (Bing and Lewis, 1991), including cassava (Greenfield et al., 2016), banana (Akello et al., 2008), corn (Wagner and Lewis, 2000), cotton (Lopez, et al., 2014), coffee (Vega et al., 2010), tomato (Resquin-Romero et al., 2016), white jute (Biswas et al., 2013), and squash (Jaber and Salem, 2014). Many studies have been conducted to prove the endophytic nature of Bb by artificial introduction of it into a plant and examining its subsequent efficacy against insect pests (Maketon et al., 2013). Furthermore, Bb has been reported to protect plants from both insect pests (Reddy et al., 2009) and plant diseases (Ownley et al. 2004).

Several studies have provided evidence that Bb, as an endophyte, can protect the host plant against insect pests (Rondot and Reineke, 2013), and provide season-long protection against tunneling insects (Arab and El-Deeb, 2012), such as stem borers (Cherry et al., 2004), and poppy stem gall wasp (Quesada-Moraga et al., 2009); sucking insects (Gurulingappa et al., 2011), such as mealybug, grape leafhopper (Rondot, and Reineke, 2018), and aphids (Dara,

2016); chewing insects (Akello et al., 2008), such as beetles (Kreutz et al., 2004) and grasshoppers (Pelizza et al., 2017); and others, including fire ants (Broome, 1976). These approaches offer exciting possibilities for development of sustainable plant protection strategies utilizing endophytic entomopathogens for management of insect pests. Additionally, endophytic Bb is considered safe, eco-friendly (Shanthakumar, 2013), and economical for plant insect management.

Despite many studies on Bb efficacy against insects, individual studies cannot provide the same comprehensive view as an overall comparison of many different studies. In addition, variability may exist among and between studies that evaluated the same organism with different methodologies and treatments. Variability in the efficacy of endophytic Bb against insect pests could lead to different interpretations, and ultimately different recommendations about treatments and application methods. In the present study, a meta-analysis was conducted on the effect of endophytic Bb on percent mortality of insect pests, to combine results from several independent studies conducted over several years and provide generalized information on influential factors to help guide future researchers.

Meta-analysis is a quantitative, statistical approach that combines results from many independently conducted studies (Gurevitch and Hedges, 1999). This approach enables the reviewer to understand the results of any study in the context of all other related studies. The purpose of meta-analysis is to determine whether the effect size of the treatment of interest is consistent across the body of data, and to quantify whether the variance varies from study to study (Borenstein et al.,2009). Recently, meta-analysis has been applied to agricultural studies to provide a comprehensive conclusion from a set of individual studies (Poeplau and Don,

2015). Moreover, meta-analysis has been most widely used in health management studies to draw a comprehensive conclusion from many experimental studies (Haidich, 2010; Nelson et al., 2015).

Despite the number of studies conducted on the efficacy of endophytic Bb against insect pests in recent years, there are still knowledge gaps in understanding the endophytic lifestyle of Bb, the natural association and interaction of Bb with insects, and the potential for both pest and disease control utilizing the diverse capabilities found in endophytic Bb isolates. Furthermore, additional research is needed for commercial development of endophytic Bb, including delivery effectiveness, application feasibility, refinement of application protocols, and cost-benefit analyses. In general, application and delivery systems of entomopathogenic fungi have been given limited attention (Shanthakumar, 2013). The current meta-analysis on endophytic Bb against insect pests was conducted to provide more understanding and knowledge of this fungus, its interactions or co-existence with pests, its ecological niches, and the mechanisms involved in suppression of insect populations.

Quantitative synthesis of the literature, specifically about endophytic Bb against insects, has not been reported. Thus, there is a timely need to summarize the overall efficacy of endophytic Bb on insect mortality, and the various influential factors underlying Bb efficacy against insects from many available studies to draw stronger conclusions for future success.

The specific objectives of this study include the following:

1. Conduct a quantitative meta-analysis to synthesize, determine, and estimate the overall summary effect size of endophytic Bb efficacy against insect pests;

2. Synthesis and determine the influence of various experimental factors on effect sizes by moderator analysis, and assess publication bias;

3. Investigate whether efficacy of endophytic Bb on insect pests has been more pronounced or consistent under experimental conditions over time;

4. Assess whether percent endophytic colonization is correlated to insect mortality; and

5. Combine results of multiple studies to contribute to overall recommendations for insect control with endophytic Bb.

The findings of this comprehensive quantitative review provide clarification on the efficacy of endophytic Bb against insect pests, and may help to develop more authentic recommendations for development and wide-scale adoption of effective, sustainable, eco-friendly strategies for control of insect pests.

Materials and Methods

Literature search and data extraction (Fig. 2.1)

The schematic representation of the meta-analysis procedure followed in the study is presented in Fig. 2.1. The literature was extracted in April 2019 from several online databases. Search terms were filtered to include "*Beauveria bassiana*" AND "endophyte" AND "insects" in the search engine of the agriculture database Thompson Reuters Web of Science, which recorded 84 results collected from the Web of Science Core Collection. Other similar terms evaluated included "*Beauveria bassiana*" AND "endophyte" AND "insect pests," which recorded 55 results; "*Beauveria bassiana*" AND "endophyte" AND "insect" AND "colonization" AND "plant" AND "crop," which provided 16 results; and "*Beauveria bassiana*" AND "endophyte" AND "colonization" AND "plant," which returned 58 results. A search with Google Scholar with

the search terms "*Beauveria bassiana*" AND "*endophyte*" AND "*insect pests*" recorded 44 results. Likewise, CABI's International Comprehensive Database for Agriculture and Veterinary Medicine recorded 29 results with the search terms "*Beauveria bassiana*" AND "endophyte" AND "insect pests." The Agriculture & Environmental Science Database (AESC), with search terms "*Beauveria bassiana*" AND "endophyte" AND "insect pests" recorded 80 results. The total number of published articles extracted was 366.

Two primary criteria were established for screening the articles collected: all studies must have insect mortality data and must have used endophytic Bb to reduce the insect population. In addition, articles that lacked data repetition, were not relevant, or were bulletins, review articles, abstracts, book chapters, theses, and U.S. Patent Applications were excluded (Fig. 2.1). Only 24 publications met the established criteria and were considered further. All other articles (342) were excluded. From the articles included in this meta-analysis, 185 effect sizes (185 studies) were extracted.

Data extraction

From each of the 24 articles, the focus was mainly on data related to the effect of endophytic entomopathogenic Bb on insect mortality (%) of different insect taxa. The treatment means, control mean, and sample sizes of treatment and controls were obtained, with respect to insect mortality. The sample size was taken as n = 2, if the least squares difference or standard errors were mentioned in the publication; if these were not mentioned in the article, we assumed that n = 1. If the data was presented in the form of graphs, WebPlotDigitizer software (Rohatgi, 2011) was used to extract mean values.

Moderator variables

Moderator variables are generally used to determine if any significant heterogeneity exists in the meta-analysis (Auge et al. 2016). In the 185 studies, several external and internal influencers were identified that impacted insect mortality by endophytic Bb. Moderators were categorized into four groups, based on whether they mainly influenced the fungus (11), insect host (10), plant host (5), or other experimental elements (6), for a total of 32 moderators (Table 2). Each moderator was analyzed separately to understand the variation in effect sizes. For each moderator, there were several subcategories or factors. Moderators that mainly affected endophytic Bb were: Bb isolate, native or commercial source of Bb isolate, general host source (insect, plant, soil) of Bb, specific host source of Bb, country where research was conducted, application method for Bb on plants or on insects, concentration of conidia applied, amount of Bb applied, and percent plant colonization range reported for Bb. For comparison purposes, percent plant colonization was divided into two ranges, 0-50% and 50-100%. Studies with Bb colonization <50% were included in the first range, while those with >50% plant colonization was included in the second range. Moderators that influenced insect pests were species, food preference, mouthparts, insect Order and Family, stage of development, feeding behavior, type of damage caused on the plant, insect habitat, and insect stage of development that causes plant damage. The third group of moderators, which mostly affected plants, included plant species, plant taxa – group, plant Family, age of plant used in experiments, and plant materials (or not) fed to insect pests. The fourth group of moderators included experimental conditions (laboratory, protected structure, field), incubation period after inoculation with Bb, time needed for observation of Bb growth in culture after plating treated insects, mechanisms

involved in insect mortality by Bb, publication years, and journals publishing the articles. All moderators are listed in Table 2. Moderator mean effect sizes were noted as significant if heterogeneity was significant ($P \leq 0.05$) and the 95% confidence intervals did not overlap with 0% or 100%, then it was assumed that there was a common among study variance (Borenstein et al., 2009; Saxton, A., personal communication, February 20, 2018).

Effect size and meta-analysis

The methodology of Borenstein et al. (2009) was used in this study. The extracted data was analyzed and computed using the statistical package Comprehensive Meta-Analysis (CMA) software (Version 3, Biostat, Englewood, NJ, USA; 2014). A random-effects model (REM) was selected for this meta-analysis instead of a fixed-model (FM) because in most published meta-analysis studies in agriculture, the true effect is assumed to vary across the studies, i.e., there is variability among studies in effect size and heterogeneity exists in the true effect size, therefore REM was considered the best fit model for this study. For fixed models, the same value or single true effect is considered across all studies, no variation exists between effect sizes, and there is homogeneity.

Effect size calculations

Data extracted from each article was analyzed and the effect sizes were calculated as the raw mean difference (MD) by formula (Borenstein et al. 2009).

$$\boldsymbol{MD = Xt - Xc},$$

where, Xt is the treatment mean (mean percent mortality of treated insects) and Xc is the control mean (mean percent mortality of control insects). Variance (V) of the data was calculated by extracting the samples sizes of the treatment and controls of each article. Non-

parametric variance was used to weight variance within studies of each individual study (Auge et al. 2014).

$$\boldsymbol{V} = (\boldsymbol{nt} + \boldsymbol{nc}) / (\boldsymbol{nt} \times \boldsymbol{nc}),$$

where V = Variance, nt = treatment sample size (number of treated insects) and nc = control sample size (number of insects used in the control).

Statistical analysis and heterogeneity analysis

The data was considered non-parametric because several publications did not report standard errors or standard deviations. The Q statistic (measure of weighted squared deviations) was used to determine the presence or absence of heterogeneity and I^2 was calculated to quantify heterogeneity. The I^2 value was calculated as the ratio of true variance (heterogeneity) to total variance (heterogeneity) across the studies (Higgins and Thompson, 2002). The total heterogeneity (Qt) is expected variation or within-study heterogeneity (Qw, i.e. sample error mean) and excess variation or between study variation as Qb, i.e. true heterogeneity in effect sizes among studies.

$$\boldsymbol{I^2} = \left[\frac{\boldsymbol{Qt} - \boldsymbol{df}}{\boldsymbol{Qt}}\right] \times \mathbf{100}\%,$$

where df = degrees of freedom and denotes the expected variation Qw; and (Qt - df) is the excess variation or true heterogeneity. If df exceeds Qt, I^2 is set to 0 (%). I^2 measures the percentage of total variation across studies, it may be 0 or negative (-) or positive (+). When I^2 is negative (-), true heterogeneity lies between 0 and 100%, while 0 (%) indicates no true heterogeneity, positive (+) values indicate true heterogeneity with larger variation across the studies with larger values reflecting a larger proportion of the observed variation due to true

heterogeneity. An I^2 > 75% is considered substantial heterogeneity (Haidich, 2010). When Q-test (P values) for heterogeneity were less than 0.1, homogeneity assumptions were considered invalid (Iacovelli et al., 2014). We assumed variation among-studies or moderators. The total summary effect, between studies variance (T_2), and actual variance (T) were also determined. The summary effect sizes were used to illustrate true heterogeneity. To determine the amount of variation between the studies and estimate the overall result of information from individual studies, forest plots were used (Lewis and Clarke, 2001). The P value was used to test significance of the hypothesis. If P>0.05, studies were non-significant and the null hypothesis was accepted, which states that all studies share a common effect. Lack of significance could be due to reluctance to publish small studies, or the studies could have negative results (Haidich 2010). If P<0.05, the studies were significant and the null hypothesis was rejected. No common effect was shared between studies and they were likely considered positive results with larger studies, and were more likely to have been published (Haidich 2010).

Publication bias

Publication Bias (PB) arises due to inclusion of published studies that are not representative of unpublished studies (Rothstein et al. 2005). This can result in a conclusion biased towards significance or positivity (Duval and Tweedie, 2000). Further, PB could be due to reluctance of researchers to publish results that were either non-significant or non-randomized or not interesting (Haidich 2010). PB always exists in a meta-analysis one way or another and cannot be eliminated from the dataset completely, but the problem can be minimized by determining the missing studies number based on symmetric assumptions by visualization of a funnel plot, which illustrates if the studies were dispersed symmetrically or asymmetrically. Funnel plots

were included in this study. In addition, missing values were estimated with the “trim and fill” approach (Duval and Tweedie, 2000), which provides an overall effect on the inferences in the meta-analysis due to PB, and demonstrates how the summary effect size would shift if apparent bias was removed (Shrestha et al. 2016). Several additional statistics, available through CMA analysis, were calculated to check for PB, including Classic Fail-safe N, Orwin Fail-safe N, Kendall's tau b, and the Egger regression intercept.

Results

Based on our meta-analysis, we can draw an inference that endophytic Bb has a significant impact on percent mortality of diverse insect pest species. The effect of endophytic entomopathogenic Bb on insect pests was determined by calculating the mean percent difference in insect mortality between treatments with endophytic Bb and controls without Bb across all studies. Overall, 185 effect sizes (185 studies) with 32 moderators were extracted and meta-analyzed with respect to insect mortality (%) caused by endophytic Bb infection. The overall summary effect size of insect pest mortality was 39% across all insect species studied. Most research studies included in this analysis reported 0 to 50% insect mortality caused by endophytic Bb, with 95% confidence that the true value across studies is expected to lie between 34.86 (Lower Confidence Interval) and 43.96 (Upper Confidence Interval).

Heterogeneity measures

Several statistics were calculated to assess heterogeneity between studies, including Q, T_2, T and I^2. In the current study, the overall P value for heterogeneity was <0.05, which indicates that there were significant differences between the studies, possibly due to high power and larger studies. Additionally, the Q statistic was greater than expected variance (degrees of

freedom), which is evidence that all the studies do not share a common effect and there is heterogeneity in the studies. Higher total dispersion of studies might be due high error within studies or to differences in the true effect from study to study. Clearly the P-value and Q statistic suggest that we can reject the null hypothesis (i.e. all studies share common effect sizes) and conclude that all 185 studies do not share a common true effect size. Variance between studies (T_2) (actual true effect) was 918.52, indicating real differences among studies. Actual variance (T) was 30.30 and the summary effect was 39.41. We expect that 95% of the true effects will fall in the range of 39.41 ± 30.30. I^2 plays a key role in quantification of heterogeneity (high or low), and a value of 98.46% was found among the studies, indicating that 98.46% of observed variance is real in the studies, which suggests inconsistency among these studies.

Endophytic Bb fungus factors

Specific Bb isolate (Fig. 2.2)

Pooling all the isolates used in various studies, EABb 01/33-Su was significant and caused the highest rate of insect mortality (61%). Other isolates with a high mortality rate and a significant effect included EABb 12/04-Rf (53%), B16 (52%), G41 (47%), EABb 07/06-Rf (46%), B.b.T-A (44%), NK-not known (32%), GHA (37%), and Local strain (60%). Many other isolates had higher or lower percent insect mortality, but values were insignificant based on no or low heterogeneity, large confidence intervals, or small number of studies. Of those studies with a significant effect, the isolate was not known (NK) in 45, followed by GHA (12), G41 (10), Local strain (6), B.b.T-A (2), B16 (2), EABb 07/06-Rf (2), and EABb 12/04-Rf (2). Overall, endophytic Bb caused 46% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

General Bb isolate type (Fig. 2.3)

When we compared the studies for general Bb isolate type, the most effective ones were native isolates (44%) followed by NK-not known (30%) and commercial isolates (29%). Most studied included native isolates (128) followed by NK-not known (36) and commercial (21). These groups had high heterogeneity among and between studies, with P<0.05 and were larger studies with large sample sizes and narrow CI. Overall endophytic Bb caused 35% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Country of origin of Bb (Fig. 2.4)

Endophytic Bb isolated from Ethiopia was highly effective causing 69% insect mortality. Others with significant effect included Australia (66%), Egypt (60%), Spain (53%), Italy (53%), China (52%), Uganda (47%), Germany (49%), Israel (44%), NK – Not known (34%), USA (28%). Of those with significant effects, the most studied included NK-not known with 62 studies, followed by Ethiopia (31), USA (21), and Uganda (10). All had high heterogeneity among and between studies with P<0.05. The CI varied for those with a significant effect, but larger studies with large sample sizes had narrower CI. Overall, endophytic Bb caused 40% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

General source of Bb (Fig. 2.5)

Endophytic Bb isolates reported in the studies had been isolated from a variety of substrates. Those isolated from soil were the most effective, causing 54% insect mortality with 60 studies. In contrast, isolates form insects were the most commonly studied (77) and caused 36% insect mortality. Both factors, as well as isolates from unknown sources (NK) had high heterogeneity among and between studies (P<0.05), with narrow CI and larger numbers of studies. Although

isolates from plants had significant heterogeneity (P<0.05), percent insect mortality was much lower with 12%. Overall, endophytic Bb caused 35% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Specific source of Bb (Fig. 2.6)

Endophytic Bb isolated from soil was highly effective causing 68% insect mortality. Others that caused a significantly high rate of insect mortality were isolated from elephant weevil (66%), maize stalk borer (65%), traditional olive orchard (61%), weevils (50%), red palm weevil (49%), soil from banana plantations (47%), NK - not known (32%), boll weevil (29%), and sorghum chafer beetle (27%). The most studied group were those from unknown sources (NK), with 39 studies, followed by soil (30) and boll weevil (21). These factors had high heterogeneity among and between studies (P<0.05), and larger studies had narrower CI. Overall, endophytic Bb caused 37% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Method of application for Bb on plants (Fig. 2.7)

The highest rate of insect mortality was recorded with a foliar spray of Bb on the flower buds of plants, with 74% insect mortality. Several application methods resulted in high rates of insect mortality, including NK -Not known (61%), banana plantlets dip (55%), foliar-leaf (35%), injection-plant (35%), foliar-plant (31%), root dip (27%), topical-leaf (25%), seed (21%), solid substrate (12%), and soil drench-double (9.1%). Among these, most studied isolates were from unknown sources (NK) with 61 studies, followed by topical-leaf (27), root dip (15), foliar-leaf (14), injection-plant (14), solid substrate (9), foliar-flower buds (9), foliar-plant (8), seed (7), and banana plantlets dip (4). These factors had high heterogeneity among and between studies

(I^2>50%, P<0.05). Larger studies with larger sample sizes had narrower CI. Overall, endophytic Bb caused 32% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Method of application for Bb on insects (Fig. 2.8)

The method of application of endophytic Bb on insect that had the highest insect mortality, with a significant effect, was foliar spray on flower buds (74%), followed by direct insect dip (63%), foliar-insect (59%), topical on insect body (56%), inoculated leaf disc (31%), and whole plant (19%). Of this group, the most studied was whole plant inoculation (67 studies), followed by inoculated leaf disc (44), direct insect dip (41), foliar-insect (10), topical on insect body (10), inoculated-flower buds (9). All had high heterogeneity among and between studies (P<0.05) with narrow CI, and larger studies with large sample sizes. Overall, endophytic Bb caused 50% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Concentration of Bb applied (Fig. 2.9)

Several concentrations of endophytic Bb were highly effective with high rates of insect mortality. Those with significant heterogeneity, smaller confidence intervals, and more than 40% insect mortality included 1 x 10^5 conidia/ml (62% insect mortality), 10^7 conidia/ml (58%), 0.5 x 10^7/g (57%), 1.5 × 10^{10} conidia/ml (55%), 1.06 x 10^{12} conidia/ml (51%), 1.5 x 10^7conidia/ml (50%), and 1 x 10^8/ml (46%). The most studied concentrations were 1 x 10^8 conidia/ml (93 studies), followed by 10^7 conidia/ml (12). Overall, endophytic Bb caused 34% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Amount of Bb applied (Fig. 2.10)

Several reported amounts of endophytic Bb used in applications caused more than 40% insect mortality and had high heterogeneity rates, including 0.25% w/v dried mycelial extract in water

with 66% mortality), amount of Bb applied not known (NK) (53% mortality), 300 ml (47%), 100 ml (47%), and 4 ml (41%). Most studied included NK-not known with 84 studies followed by 10 ml (21, 21% mortality), 4 ml (12), and 300 ml (10). Overall, endophytic Bb caused 30% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Plant colonization range of Bb (Fig. 2.11)

Most studies did not report plant colonization rate (NK – Not known, 111 studies, 46% insect mortality). Reports of plant colonization rate were binned into two groups, 0-50% and 50-100%. Insect mortality was higher (32%) in studies that reported plant colonization in the 50-100% range, and 26% in studies with plant colonization in the 0-50% range. There were 50 studies for the higher plant colonization range and 24 for the lower colonization range. All factors had high I^2 values (P<0.05), indicating that there was no common effect between and among studies. Overall, (185 studies, I^2 = 98.46%, P<0.05), insect mortality caused by endophytic Bb was 35%.

Bb mechanism against insects (Fig. 2.12)

For the 185 studies, mycosis was reported for 98 and accounted for 47% insect mortality. No mycosis was reported for 8 studies and mortality was 39%. NK- not known (29%). In many studies (79) there was no report of whether mycosis was observed, but insect mortality was reported (29%). All three factors had high heterogeneity among and between studies (P<0.05), with relatively narrow CI, and were larger studies with large sample sizes. Overall, endophytic Bb caused 38% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Insect factors

Insect pest species (Fig. 2.13)

Endophytic Bb was highly effective against the spotted stem borer causing 73% insect mortality. Others studies with significant heterogeneity (P<0.05) and high colonization rates were cotton aphid (66%), western flower thrips (64%), cotton leaf worm (62%), adult spider mite (52%), silverleaf whitefly (50%), red palm weevil (49%), banana weevil (47%), lygus bug (47%), cotton bollworm (38%), fall army worm (33%), and greenhouse whitefly (30%). The most studied pest species included the pseudostem weevil (27), spotted stem borer (26), red palm weevil (16), and the cotton bollworm (13). Heterogeneity was very low and non-significant, confidence intervals were large, the number of studies was small, and insect mortality for most was relatively low for studies with the African pink stalk borer, grapevine mealybug, green peach aphid, South American bollworm moth, vine mealybugs, grasshopper, and stalk borer. The latter two had higher mortality rates. Heterogeneity was significant, but study number was small and insect mortality varied greatly for aphids, diamondback moth, greenhouse whitefly, and stalk borer. Overall, endophytic Bb caused 39% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Insect Order (Fig. 2.14)

The most highly effective Bb treatment based on insect Order was Thysanoptera, with 64% insect mortality. Others with significant heterogeneity were Trombidiformes (52%), Lepidoptera (46%), Coleoptera (32%) and Hemiptera (26%). The most studied orders were Lepidoptera (78), Coleoptera (53), and Hemiptera (38). Orthoptera, with 3 studies, had non-significant

heterogeneity (P>0.05). Overall, endophytic Bb caused 44% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Insect Family (Fig. 2.15)

The most effective Bb treatment for insect Family included Crambidae with the highest insect mortality (73%), Thripidae (64%), Tetranychidae (52%), Miridae (47%), Aleyrodidae (40%), Noctuidae (33%), Curculionidae (32%), and Aphididae (21%). Most studied included Curculionidae with 53 studies, followed by Noctuidae (49), Crambidae (26), Aphididae (18), and Thripidae (11). All had high heterogeneity among and between studies with P<0.05. Low or no heterogeneity (P>0.05) was found for Plutellidae, Pseudococcidae, Tristiridae and Yponomeutidae, which generally had smaller study numbers and large CI. Overall, endophytic Bb caused 42% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Insect feeding preference (Fig. 2.16)

All feeding preference factors had high heterogeneity (P<0.05). The highest insect mortality was recorded for oligophagous insects (68%), followed by polyphagous (37%) and monophagous (24%). Polyphagous pests were studied the most (119), followed by monophagous (37) and polyphagous (29). Overall, endophytic Bb caused 43% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Insect mouthpart type (Fig. 2.17)

Insects were categorized based on chewing or piercing-sucking mouthparts. Endophytic Bb caused similar percent mortality in the two groups. Insects with chewing mouthparts had 41% mortality and were studied the most (134), while insects with piercing-sucking mouthparts had 35% mortality, with 51 studies. Both had high heterogeneity among and between studies

(P<0.05), narrow CI, and were larger studies with large sample sizes. Overall, endophytic Bb caused 39% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Insect pest feeding behavior (Fig. 2.18)

Pests were grouped as foliage feeders or those that fed on rhizomes or pseudostems. Endophytic Bb caused higher mortality for those that fed on rhizomes and pseudostems (47%), compared with foliage feeders (39%). However, foliage feeders were studied more (175) than rhizome and pseudostem feeders (10). Heterogeneity was high among and between studies (P<0.05) for both groups. Smaller CI were found for foliage feeders due to larger study numbers. Overall, endophytic Bb caused 39% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Insect pest damage type (Fig. 2.19)

Endophytic Bb was most effective on insects that damaged growing tips and flower buds (64% mortality). Heterogeneity among and between studies was high (P<0.05) and mortality was >40% for several other groups, including pests that were categorized as foliage feeder (60%), caused damage on foliage and buds (47%), caused defoliation (42%), and tunneling (41%). Most studied were those that resulted in tunneling (90 studies), followed by bore holes (21), and leaf and sooty molds (20). Non-significant (P>0.05) heterogeneity was noted for groups with small study numbers, such as pests that caused leaf skeletonization, damage to leaves and stems, and damage to stems only. Overall, endophytic Bb caused 43% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Insect stage (Fig. 2.20)

Endophytic Bb was most effective against the fourth instar larval stage with high insect mortality (73%), followed by the larval stage (60%), second instar larvae (49%), third instar

larvae (47%), and adult (31%). These groups had high heterogeneity among and between studies (P<0.05), narrower CI, and generally larger study numbers. The most studied insect stage was adult, with 63 studies, followed by third instar larvae (35), fourth instar larvae (31), first instar larvae (21), larval stage (11), and second instar larvae (9). Overall, endophytic Bb caused 34% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Insect pest destructive stage (Fig. 2.21)

Insect stages that caused plant destruction were divided into five factors. Endophytic Bb was highly effective on the following groups: larva, nymphs, and adults (52% mortality), larva (46%), adults and larva (35%), and nymphs and adults (28%). The most studied included larva (88 studies), adults and larva (54), nymphs and adults (40), and larva, nymphs, and adults (2). Heterogeneity was high among and between studies (P<0.05) for these groups. Although mortality was 74% for hoppers, nymphs, and adults (74%), there was no heterogeneity and only one study. Overall, endophytic Bb caused 39% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Insect habitat (Fig. 2.22)

Endophytic Bb was most effective on insects that lived above and below (mainly rhizome) ground, causing 47% insect death, followed by above ground insects (39%). However, above ground insects were most studied (175), with 10 studies for above and below ground pests. There was high heterogeneity among and between studies (P<0.05) for both groups. Overall, endophytic Bb caused 39% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Plant factors

Plant species (Fig. 2.23)

Endophytic Bb was highly effective and heterogeneity was significant among and between studies (P<0.05) for control of pests on rose (74% insect mortality), alfalfa (62%), NK-not known (61%), melon (61%), tomato (56%), bean (52%), faba bean (41%), strawberries (26%), and banana (24%). Of groups with higher insect mortality, the most studied included NK-not known (58 studies), banana (37), faba bean (12), and rose (9). Overall, endophytic Bb caused 39% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Plant taxa - group (Fig. 2.24)

Endophytic Bb caused the highest rate of insect mortality (61%) for studies where plant taxa were not reported (NK). Insect mortality was 33% for Angiosperm – Eudicots, 25% for Angiosperm – Monocots, and 5.0% for Gymnosperms - Monterey Pine. The most studied included eudicots (73 studies), NK-not known (58), monocots (53) and Monterey pine (1). Except for Monterey pine, (1 study), there was high heterogeneity among and between studies (P<0.05), with narrow CI, and larger studies with large sample sizes. Overall, endophytic Bb caused 37% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Plant family (Fig. 2.25)

Endophytic Bb caused >40% insect mortality in several studies with different plant families, including studies where plant family was not reported (NK-not known, 61%), which was also the most studied (58). Other plant family groups with high and significant heterogeneity (P<0.05) for insect mortality included Cucurbitaceae (61%), Rosaceae (53%), and Fabaceae (47%). Other families with larger study numbers were Musaceae (37, only 24% insect mortality), Fabaceae

(18), Brassicaceae (15, only 11% mortality), and Rosaceae (15). Overall, endophytic Bb caused 34% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Plant age/stage (Fig. 2.26)

Endophytic Bb was most effective (high and significant heterogeneity) in causing insect mortality when treated plants were in the tight bud stage (74% mortality), followed by true leaf stage (61%), NK - not known (57%), 3 months-old (57%), 2 months-old (55%), 2-leaf stage (52%), 1.5 month and 7 days-old (50%), leaf stage (41%), and 14 days-old (31%). The most studied was NK -not known, with 64 studies. Others factors with high study numbers were 56 days (83), 4 months-old (27), 35 days (14), leaf stage (12), and 14 days-old (10). Overall, endophytic Bb caused 36% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Plant materials fed to insects (Fig. 2.27)

Insect mortality varied depending on which (if any) plant materials were fed to insects. Mortality was greatest, with high and significant heterogeneity, when insects were fed Bb-treated flower buds (74%), followed by direct application on insects (61%), leaves (28%), pseudostems (20%), and whole plant (17%). The most studied was direct application of Bb to the insect body with 61 studies, followed by whole plant (39), leaf (34), pseudostem (33), and flower buds (9). Non-significant studies included pine needles, direct application on the insect + leaf, date palm pulp and stem, which had no or low heterogeneity, smaller study numbers, and larger CI compared to others. Overall endophytic Bb caused 46% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Experimental factors

Country where research was conducted (Fig. 2.28)

Research on insect control with endophytic Bb conducted in Iran was highly effective causing 74% insect mortality. Similarly, high insect mortality was reported from other countries, including Ethiopia (73%), Australia (66%), Egypt (60%), Mexico (54%), China (52%), Spain (49%), Uganda (47%), USA (37%), Germany (34%), Argentina (24%), and India (16%). These studies had high and significant heterogeneity. Among them, study numbers were greatest in India (27 studies), followed by Ethiopia (26), Germany (21), Spain (17), USA (12), Argentina (11), Mexico (11), Uganda (10), Iran (9), Egypt (6), Australia (4), and China (2). Non-significant (P>0.05) studies included Benin, Greece, New Zealand, and South Africa with no heterogeneity. Overall, endophytic Bb caused 42% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Experimental conditions (Fig. 2.29)

Endophytic Bb was highly effective and statistically significant when experiments were conducted under controlled conditions, i.e. laboratory (62% insect mortality), screen house (47%), greenhouse (38%), and growth chamber (22%). Studies in the field (5.1% insect mortality) and climatic chamber (2.9%) were non-significant with no or low heterogeneity and smaller study numbers. The most studied included greenhouse with 79 studies, followed by laboratory (47), growth chamber (30), field (11), screen house (10) and climatic chamber (8). All had high heterogeneity among and between studies (P<0.05) and were larger studies with large sample sizes. Non-significant factors, with no heterogeneity (P>0.05), included field and climatic chamber with larger CI compared to others. Overall, endophytic Bb caused 31% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Incubation period after inoculation with Bb (Fig. 2.30)

There were many factors for the incubation period (time) following inoculation with endophytic Bb. An incubation period of 10 days, was associated with the highest percent insect mortality (70%), and this was also the most studied period (35 studies). Other incubation periods that had significant (P<0.05) and high (I^2>50%) heterogeneity, and insect mortality > 30% included 12 days (64%), 18 days (64%), 15 days (57%), 11 days (52%), 3 days (50%), 45 days (43%), 5 days (43%), 24 days (41%), 4 days (40%), 30 days (33%), and 7 days (32%). Overall endophytic Bb caused 29% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Bb growth observed in culture (after plating insect) (Fig. 2.31)

Studies that reported daily observations of insects for fungal growth which had received Bb treatments, had the highest rate of insect mortality (73%). Other observation periods with high mortality and significant heterogeneity included 15 days (57%), 5 days (47%), 7-10 days (44%), 14 days (41%), 45 days (37%), NK-not known (29%), and 7 days (25%). The most studied was NK-not known (79 studies), followed by 7 days (34), daily observation (26), 7-10 days (12), 14 days (12), 5 days (8), 15 days (5), and 45 days (5). Non-significant studies (P>0.05) included 10 days and 30 days, which had low study numbers and large CI compared to others. Overall, endophytic Bb caused 47% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Publication years (Fig. 2.32)

The most highly effective endophytic Bb treatments were published in the period of 2008-2011 (53% insect mortality), followed by 2016-2019 (41%), 2012-2015 (39%), and 2004-2007 (5.1%). The most studied included 2016-2019 with 117 studies, followed by 2012-2015 (43), 2008-2011 (14), and 2004-2007 (11). Except for 2004-2007, these factors had high and significant

heterogeneity among and between studies (P<0.05), with relatively narrow CI, and larger study numbers. Overall, endophytic Bb caused 36% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Journal (Fig. 2.33)

Studies with the highest rate of reported insect mortality caused by endophytic Bb treatments were published in the *Egyptian Journal of Biological Pest Control* (77% insect mortality, 8 studies), while the most studies were published in the *International Journal of Plant Protection* (27 studies, 16% insect mortality). Heterogeneity was high and significant for these two journals and others. Overall, endophytic Bb caused 40% insect mortality (185 studies, I^2 = 98.46%, P<0.05).

Publication bias (PB) (Fig. 2.34)

Publication bias was determined using CMA analysis. Several tests were performed to determine whether there was evidence of PB in the dataset. Tests included Classic Fail-safe N, Orwin Fail-safe N, Kendall's tau b, and the Egger regression intercept. No evidence of PB was found. In addition, point estimates of studies were dispersed symmetrically in funnel plots (Fig. 2.34A-B). Duval and Tweedie's Trim and Fill test was applied, and there was little change in Trim and Fill values. Overall, we conclude that there is no publication bias noted in the study; however, indirect, or unknown bias may always exist.

Discussion

Biocontrol with endophytic fungi against insect pests has gained special attention in the past two decades, and may be one of the best alternatives to minimize pesticide usage in agriculture (Lacava and Azevedo, 2014). Among these endophytic fungi, the insect pathogenic bioagent Bb

has been reported as effective against several insect pests by causing white muscardine disease (Prasad and Syed, 2010).

Furthermore, it has been established that Bb is an endophyte in various plant species (Resquin-Romero et al., 2016), and can protect plants from insect pests (Dara et al., 2013) and plant pathogens (Ownley et al., 2008), while it lives as an endophyte. A large amount of data has accumulated over the last two decades related to the efficacy of endophytic Bb against insect pests. Meta-analysis is a powerful statistical tool, which is used to validate research output accumulated over years, enables study to study qualitative analysis, and determination of influential and effective treatments overall and between individual studies (Chandrasekaran et al., 2014; Biondi-Zoccai et al., 2006). Variations and comparisons with each treatment among and between individual studies can also be made (Gurevitch and Hedges, 1999). Therefore, we performed a meta-analysis to explain and validate findings, and illustrate conclusions drawn from various scientific studies conducted over time on endophytic Bb as a control for insect pests. Our aim was to determine efficacy of endophytic Bb on diverse insect pests, and assist researchers with sample size selection needed for a best output and cost effectiveness, as well as appropriate methodologies for future studies.

Meta-analyses of bioagent efficacy for reduction of pest populations was examined by Stiling and Cornelissen, 2005 and Chaplin-Kramer et al., 2011. However, there are few meta-analysis studies on endophytic fungi (Mayerhofer et al., 2013), and a comprehensive, quantitative meta-analysis review of plant protection against insects with endophytic Bb has not been conducted to date. Hence, our research could be a stepping stone for future researchers in exploring new strategies understanding endophytic Bb efficacy. In the current

study, we quantified evidence on the ability of endophytic Bb to cause insect mortality by pooling 185 studies conducted over several years. We found strong evidence that supports the effect of endophytic Bb to reduce populations of diverse pest insects associated with several plant species.

Our results are supported by those of Bing and Lewis (1991, 1992a, 1992b, 1993) who demonstrated in the 1990s that Bb could be an endophyte in corn to control of European corn borer (*Ostrinia nubilalis*) after artificial inoculation of Bb into the corn plant. Since then, many studies have been conducted to evaluate the endophytic nature of Bb following artificial inoculation, and subsequent evidence on the ability of Bb to suppress pest populations (Quesada-Moraga et al., 2009). Additionally, Bb is known to occur as natural endophyte in several plant species and control insect pests to protect these plants (Brownbridge et al., 2012). However, debates have been raised over endophytic Bb application procedures, and in our studies, we discovered much variation across studies.

The activity of endophytic Bb could be influenced by several multitrophic interactions during the process of insect infection (Faeth and Bultman, 2002). In our study, we validated several influential variables or moderators that interact with endophytic Bb and affect its ability to cause mortality of insect pests. We found native isolates of Bb perform better that commercial isolates for control of insect pests. Similarly, Lecuona et al. (2001) found that when native strains of Bb were used, they were more effective against suppression of triatomine bugs, *Triatoma infestans* (Klug). Likewise, Qazzaz et al. (2015) reported that local native entomopathogenic Bb caused high insect mortality of Mediterranean fruit fly (*Ceratitis*

capitata) (77%). Lockwood (1993) suggested that in consideration of environmental and ecosystem safety, native isolates should be preferred in a microbial control system.

In our study the most significant endophytic Bb treatment for killing insects was directly dip of insects in concentrated Bb. Our results support those of Gurulingappa et al. (2011), who demonstrated that by dip third instar aphids in Bb mycelial extract for 5 s as a treatment, and controls in Czapek Dox liquid medium without Bb, high rates (80%) of aphid mortality occurred 4 days of post treatment. Furthermore, we found that combination treatments gave higher pest mortality rates. Combining a foliar spray of Bb + feeding insects plant tissue colonized with endophytic Bb, caused high insect mortality, but was insignificant due low study number. However, our results support those of Resquin et al. (2016) who reported combination treatments of foliar sprays of Bb and insect feedings with endophytically Bb-colonized melon, tomato, and alfalfa leaves resulted in a synergistic increase in insect mortality (75-80%) of African cotton leafworm (*Spodoptera littoralis*) compared to Bb foliar spray alone. Also, Ojiambo and Scherm (2006) reported that combination treatments with multiple bioagents caused synergistic, additive, or inhibitory effects on target performance compared to single bioagent application or inoculation. Similarly, Stiling and Cornelissen (2005) also suggested that multi-species combination bioagent treatments were more effective and consistent than single bioagent treatments. In our study, the most effective method of pest population suppression by endophytic Bb included direct application on insects or on plants (direct application). Our results support findings of Resquin et al. (2016) who demonstrated that Bb sprayed directly on plant tissue established Bb temporarily as an endophyte and caused systemic and non-systemic colonization of the plant and gave the best results for insect mortality, thereby improving the

overall Bb treatment efficacy. Likewise, Qazzaz et al. (2015) with direct Bb application on adult Mediterranean fruit fly (*Ceratitis capitata*) caused significantly higher insect mortality.

Furthermore, we noted that insects fed with plant tissue that was 50-100% colonized by Bb, resulted in high insect mortality rates. These results support those of Ojiambo and Scherm (2006), who suggested that application method had a significant impact on colonization of plants by a bioagent (Ojiambo and Scherm, 2006). Similar results were reported by Resquin-Romero et al. (2016), who demonstrated that alfalfa, tomato, and melon leaves, colonized by endophytic Bb, and fed to African cotton leafworm (*Spodoptera littoralis*) larvae caused significant mortality. The capacity of endophytic microbes to colonize plants and negatively affect insect pests has been a valuable tool in agriculture. The dual function of Bb to protect plants against insect pests and pathogens could be attributed to production of several toxic compounds produced by Bb; however, little is known about their impact on plants (Azevedo et al., 2000).

In our analysis, endophytic Bb treatments had the greatest impact on insect mortality of the fourth instar larval stage, while most research was conducted with the adult stage. From our results we conclude that the larval stage is most effective for evaluating Bb treatments due to their sensitivity to Bb, ease of handling, easy identification compared to adults, feeding behavior, and more simplified management steps needed to control insects at the larval stage (Lee, 2010).

In relation to plant factors, insect mortality was greatest with rose plants treated with endophytic Bb, with plants categorized as eudicots, and members of the Cucurbitaceae family. However, several other plant species and plant families were associated with high rates of

insect mortality with endophytic Bb treatments. Endophytic colonization of Bb has been documented by many researchers; however, the pattern of colonization may differ based on host plant tissues they colonize (Sridhar, 2012).

In our studies, endophytic Bb was effective in causing significant mortality of above and below ground insects. Insect death could be attributed to Bb pathogenesis and increased susceptibility of the insect to the fungus (Olleka et al., 2009; Beron and Diaz (2005). Timing of Bb application and targeting a susceptible stage of the insect pest are important for best outcomes (Erler and Ates, 2015). Beron and Diaz (2005) found that Bb isolates were more virulent to soil-dwelling insect pests. Bb pathogenesis and virulence may differ based on the isolate type used and among insect host species (Uma Devi, 2008). Similarly, Marcandier and Khachatourians (1987) demonstrated that relative humidity (RH) influenced the susceptibility of migratory grasshopper (*Melanoplus sanguinipes* (Fab.)) to Bb. The highest susceptibility was 76% RH compared to 33% RH. However, natural insect mortality was recorded at 100% RH.

In the current study, we found that in most cases of high insect mortality, researchers had reported evidence of mycosis caused by endophytic Bb. However, insect mortality was also reported without mycosis, which is in accordance with Marcandier and Khachatourians (1987). On studies with migratory grasshopper infected with Bb infection, they reported that at 100% RH, insects were killed, but no mycosis was observed on the insect cadaver.

Experimental conditions can impact biocontrol with endophytic Bb. In our analysis, controlled conditions, like growth chambers resulted in greater pest mortality than field applications of Bb. Most studies have reported that bioagents perform better under controlled conditions and fail to perform or have less significant results in pest suppression in field studies

(Wei et al., 2011). The poor field performance of bioagents is depended on several factors, including temperature, relative humidity, season of application, moisture, and several other co-related factors (Wei et al., 2011). In addition, there could be a change in the colonization capacity of the bioagent depending on delivery or application method used in the field, and competition with other microbes in the rhizosphere (Lugtenberg et al., 2001). Similarly, Hare and Andreadis (1983) reported studies with Bb on Colorado potato beetle on potato, climbing nightshade, carolina horsenettle, and tomato under both greenhouse and field conditions, and found that insects were more susceptible in the greenhouse than in the field. More effective results in growth chambers or greenhouses are mainly attributed to feasibility or manipulation of the environment to favor bioagents (Paulitz and Belanger, 2001). Controlled environments such as greenhouses, increased the lethal time for killing insects and caused higher mortality rates.

We found wide variation between and across studies related to application method, concentration of Bb and amount of Bb used in experiments, incubation period following inoculation, and plant age at inoculation. There is no standardized protocol or application method for Bb application, which gives researchers freedom to choose the application methodology they prefer, which can lead to different interpretations of results, even if they have studied same insect or crop species using the bioagent. By synthesizing data, we found that the highest rates of insect mortality were associated with concentrations of Bb applied at 1 x 10^5 conidia/ml, and amounts of Bb applied at 0.25% w/v dried mycelial extract in water. We also found that the best incubation period (time following application of Bb) for high insect mortality was 10 days, and that this incubation period was used most in the studies included in

this analysis. The most effective plant age for Bb application was the tight bud or true leaf stage, while the most studied plant age for treatments was 4 months.

The most effective (high insect mortality) studies with endophytic Bb were published in 2008-2011, but there were larger numbers of studies published in 2016-2019. The reason for this difference could depend on the editorial board, the journal to which the researcher applied or had the manuscript accepted, and the quality, significance, or novelty of data.

From the literature, it is clear that deleterious effects of Bb infection on insect pests could be due to several factors, including fungal production of toxic metabolites (Vurro and Gressel, 2007) volatile organic compounds (Patocka, 2016), or organic acids (Cheong, 2015; Bidochka and Khachatourians, 1991), which are toxic to insects. In addition, infection with Bb causes physiological stress of insects, including starvation, insect body nutrient depletion, or failure of insect defense mechanisms (Cheung and Grula, 1982; Broome et al., 1976). This occurs simultaneously with suppression of the insect immune defense and death by mycosis (Resquin-Romero et al., 2016; Lohse et al., 2014), followed by spread of the fungus to another host for survival.

Beauveria bassiana, could be a valuable tool in biocontrol studies due to its unique characteristics including high rate of asexual reproduction, ubiquitous nature, and ability to survive in different environments, such as insect or plant hosts and soil. Additionally, endophytic Bb protection is ecofriendly and may provide long-term as well as short-term insect control (Sandhu et al., 2012). The longevity and high persistence level of Bb in hosts and soil contribute to its long-term effect and suppression of insect pests (Sandhu et al., 2012). Bb can parasitize a wide variety of insect pests, and has been suggested as a nonselective bio-

insecticide (Sandhu et al., 2012). However, Bb does have constraints. Effectiveness is slow compared to chemical pesticides because chemicals act immediately and the effect of Bb may take 1-3 weeks post application to suppress the pest population. In addition, persistence, and efficacy of Bb in the host population could vary among different insect species, thus host-specific applications should be optimized to obtain long-term results. Furthermore, genotypic variability among Bb isolates (Devi, 2006) and variability in pathogenesis based on their geographical region (Sandhu et al., 2012) may result in inconsistent efficacy.

In our analysis we discovered that there is inconsistency between and across the studies conducted, which was supported by high and significant heterogeneity. This finding is supported by previous meta-analysis studies conducted on the efficacy of bioagents to suppress plant diseases (Ojiambo and Scherm, 2006). Cock et al. (2016) illustrated that possible inconsistencies in biocontrol-based protection efficacy could be due to the wide usage of single strains of bioagent, as well as indiscriminate use of bioagents. Ojiambo and Scherm (2006) suggested that wide application of bioagents without proper screening for target pathogens or survival in host ecosystems could cause problems. Chandrasekaran et al. (2016) suggested that inconsistent bioagent efficacy could be due to differential colonization abilities of bioagents or competition with other microbes. Additionally, Bb is influenced by several biotic and abiotic factors, including host species (insect or plant), host abundance, Bb application, delivery and timing, rate of application, and target region. Environmental conditions that impact efficacy include moisture, temperate, season, rainfall, and soil factors (Kikankie et al., 2010), all variables that could result in inconsistent in Bb efficacy in insect pest control (Bugeme et al. 2008; Kessler et al., 2003; Inyang et al., 2000). In addition, special care needs to be taken while

analyzing studies with meta-analysis because there may have been constraints, including retrieval of incomplete data, reporting studies that have publication bias in the data set, conflicting results of independent studies, and multiple small studies (Haidich 2010) that influences the analysis results.

In conclusion, this investigation confirmed that application of endophytic Bb can have a significant impact on a wide range of insect pests. Several influential variables were identified that impact the efficacy of endophytic Bb on insect mortality. These results provide a generalized overview of the research on the efficacy of endophytic Bb, and provide guidance for future research directions to better understand and develop effective techniques for pest control with this fungus.

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Appendix 2

All tables and figures for Chapter 2 are in Appendix 2.

Table 2. Moderators that influenced insect mortality caused by endophytic Beauveria bassiana.

Category	Moderator	Factor
Endophytic fungus: *Beauveria bassiana* (Bb)	1. Specific Bb Isolate	00010 (5652) c (2), 0002 (5644) a (2), 0003 (5645) b (1), 0007 (5649) a (2), 0008 (5650) c (2), 0009 (5651) (2), AF2 (1), APPRC-27 (2), APPRC-44BC (2), ATCC 74040 Naturalis (9), ATP01 (1), ATP02 (2), ATP03 (1), ATP04 (1), ATP05 (1), B.b.T-A (2), B1 (2), B16 (2), B4 (2), B7 (1), Bb03032 (1), Bb04a (1), Bb1022 (1), Bb1025 (1), Bb135 (1), Bb64 (1), BF4 (2), BG11 (7), DS-35-2 (1), DS-51-21 (2), EABb 01/33-Su (10), EABb 04/01-Tip (1), EABb 07/06-Rf (2), EABb 12/03-Pa (2), EABb 12/04-Rf (2), EABb 13/04-Rf (2), EABb04/01–Tip (1), FRH1 (7), G41 (10), GF4 (1), GHA (12), IBB010 (2), IGE3 (1), KF3 (1), Local strain (6), LPSC 1215 (8), LPSc 1225 (1), LPSc 1226 (1), LPSc 1227 (1), M1 (1), NK (45), S#05 (1), S#10H (2), S#34 (1), S#41 (1), S#44BC (1), S#45 (1), S#53 (1), SM3 (2), Overall (185)
	2. General Bb isolate type	Commercial (21), Native (128), NK-Not Known (36), Overall (185)
	3. Country of origin of Bb	Ethiopia (31), Argentina (3), Australia (4), Austria (1), Benin (9), Canada (2), China (2), Colombia (1), Egypt (6), France (2), Germany (2), Greece (2), Israel (2), Italy (2), New Zealand (14), Nigeria (2), NK–Not known (62), South Africa (2), Spain (5), Uganda (10), USA (21), Overall (185)
	4. General source of endophytic Bb	Soil (60), Plant (8), Coffee berries (1), Insect (77), NK-Not known (39), Overall (185)
	5. Specific source of Bb	Traditional olive orchard (10), Soil (30), Soil- banana plantations (10), Soil- tobacco plants (8), Soil-vineyard (2), Daisy (7), Coffee berries (1), African sugarcane stalk borer (3), Black pine bark beetle (7), Boll weevil (21), Click beetle (1), Codling moth (larva) (1), Earwig (6), Elephant weevil (4), Gall wasp (1), Insect (unidentified) (1), Maize stalk borer (3), Palm borer (1), Pine shoot moth (1), Pine Tree (1), Pink stalk borer (2), Poppy stem gall wasp larva (1), Red palm weevil (15), Sorghum chafer beetle (3), South American locust (3), Spruce bark beetle adult (1), Weevils (2), NK-Not known (39), Overall (185)
	6. Method of application for Bb on plants	Seed (7), Seedling dip (14), Banana plantlets dip (4), Soil drenching-double (2), Solid substrate (9), Injection-plant (14), Foliar-plant (8), Foliar-leaf (14), Topical-leaf (27), Foliar-flower buds (9), Root dip (15), Natural (1), NK-Not known (61), Overall (185)
	7. Method of application for Bb on insects	Whole plant (67), Topical + Inoculated leaf disc (3), Inoculated-pine needles (1), Inoculated leaf disc (44), Inoculated-flower buds (9), Topical on insect body (10), Direct insect dip (41), Foliar-insect (10), Overall (185)
	8. Concentration of Bb applied	0.5 x 10^7/g (2), 10^5 conidia/ml (3), 10^6 conidia/ml (9), 10^7 conidia/ml (12), 1 x 10^8/ml (93), 1 x 10^9/g (8), 1 x 10^9 conidia/ml (8), 1 x 10^{11}/g (5), 1 x 10^{11}conidia /ml (1), 1.06 x 10^{12}conidia /ml (3), 1.5 x 10^7conidia /ml (10), 1.5 x 10^9/ml (8), 1.5 × 10^{10} conidia/ml (4), 2.3 x 10^7cfu/ml (8), 1 lb (453.6 g)/ac (6), Desirable amount (5), Overall (185)
	9. Amount of Bb applied	1 ml (7), 2 ml (8), 4 ml (12), 10 ml (21), 20 ml (6), 100 ml (6), 200 ml (2), 300 ml (10), 200 mg endophytic date-palm tissue/kg wheat kernels (4), 0.25% (4), 1.5% (8), 2% (5), 3% (8), NK (84), Overall (185)
	10. Plant colonization range (%) of Bb	0-50% (24), 50-100% (50), NK (111), Overall (185)
	11. Bb mechanism against insects	Mycosis (98), No mycosis (8), NK – Not known (79)
Insect factors	12. Insect pest species	Adult spider mite (2), African pink stalk borer (11), Aphids (2), Banana weevil (10), Cotton aphid (4), Cotton bollworm (13), Cotton leaf worm (12), Diamondback moth (3), Fall army worm (4), Grapevine mealybug (2), Grasshopper (3), Green peach aphid (12), Greenhouse whitefly (2), Lygus bug (6), pseudostem weevil (27), Red palm weevil (16), Silverleaf whitefly (2), South American bollworm moth (8), Spotted stem borer (26), Stalk borer (1), Vine mealybugs (8), Western flower thrips (11), Overall (185)

"Table 2. Continued"

Category	Moderator	Factor
Insect factors	13. Insect pest Order	Coleoptera (53), Hemiptera (38), Lepidoptera (78), Orthoptera (3), Thysanoptera (11), Trombidiformes (2), Overall (185)
	14. Insect pest Family	Aleyrodidae (4), Aphididae (18), Crambidae (26), Curculionidae (53), Miridae (6), Noctuidae (49), Plutellidae (2), Pseudococcidae (10), Tetranychidae (2), Thripidae (11), Tristiridae (3), Yponomeutidae (1), Overall (185)
	15. Insect pest food preference	Monophagous (37), Oligophagous (29), Polyphagous (119), Overall (185)
	16. Insect pest mouthparts	Chewing (134), Piercing-sucking (51), Overall (185)
	17. Insect pest feeding behavior	Foliage (175), Rhizome and pseudostem (10), Overall (185)
	18. Insect pest damage	Leaf and sooty mold (20), Leaf skeletonization (1), Foliage feeder (8), Foliage and buds (6), Bore holes (21), Fruit & foliage (12), Defoliation (10), Growing tips and flower buds (11), Plant stems and leaves (2), Leaf and stem (3), Stem (1), Tunneling (90), Overall (185)
	19. Insect stage	Egg (1), Larval stage (11), First instar larvae (21), Second instar larvae (9), Third instar larvae (35), Fourth instar larvae (31), Fifth instar larvae (1), Nymph (12), Pupa (1), Adult (63), Overall (185)
	20. Insect pest destructive stage	Larval (88), Adult and larva (54), Nymphs and adults (40), Hoppers, nymphs, and adults (1), Larvae, nymphs, and adults (2), Overall (185)
	21. Insect habitat	Above and below ground (mainly rhizome) (10), Above ground (175), Overall (185)
Plant factors	22. Plant species	Alfalfa (4), Arabidopsis (14), Banana (37), Bean (2), Date palm (4), Faba bean (12), Grapevine (10), Maize (11), Melon (3), NK (58), Oilseed rape (1), Pine (1), Rose (9), Strawberries (6), Sweet sorghum (1), Tobacco (8), Tomato (4), Overall (185)
	23. Plant taxa - group	Angiosperms - Eudicots (73), Angiosperms - Monocot (53), Monterey pine (1), NK–Not known (58), Overall (185)
	24. Plant Family	Arecaceae (4), Brassicaceae (15), Cucurbitaceae (3), Fabaceae (18), Musaceae (37), NK–Not known (58), Pinaceae (1), Poaceae (12), Rosaceae (15), Solanaceae (1), Vitaceae (10), Overall (185)
	25. Plant age	True leaf stage (9), Leaf stage (12), 2 leaf stage (2), 5 leaf stage (8), 7 leaf stage (1), Tight bud stage (9), 14 days (10), 17 days (5), 35 days (14), 1.5 month and 7 days (2), 56 days (83), 2 months (4), 3 months (3), 112 days (1), 4 months (27), 483 days (8), 2 years (1), 52-year-old grape vine (2), NK-Not known (64), Overall (185)
	26. Plant materials fed to the insects	Leaf (34), Pine needles (1), Direct application on insect and leaf (3), Direct application on insect (61), Date palm pulp (4), Flower buds (9), Pseudostem (33), Stem (1), Whole plant (39), Overall (185)
Experimental factors	27. Country where research was conducted	Argentina (11), Australia (4), Benin (11), China (2), Egypt (6), Ethiopia (26), Germany (21), Greece (1), India (27), Iran (9), Mexico (11), New Zealand (15), South Africa (2), Spain (17), Uganda (10), USA (12), Overall (185)
	28. Experimental conditions	Field (11), Growth chamber (30), Climatic chamber (8), Screen house (10), Greenhouse (79), Laboratory (47), Overall (185)
	29. Incubation period after inoculation with Bb	1 day (1), 2 days (3), 3 days (12), 4 days (9), 5 days (5), 6 days (2), 7 days (18), 8 days (2), 9 days (2), 10 days (35), 5-10 days (3), 11 days (7), 12 days (6), 14 days (18), 15 days (5), 11-15 days (3), 18 days (3), 16-20 days (3), 21 days (2), 24 days (12), 21-25 days (3), 28 days (2), 30 days (7), 26-30 days, (3), 31-35 days (3), 36-41 days (3), 41-45 days (3), 45 days (8), NK-Not known (2), Overall (185)

"Table 2. Continued"

Category	Moderator	Factor
	30. Bb growth observed in culture after plating	5 days (8), 7 days (34), 10 days (3), 7-10 days (12), 14 days (12), 15 days (5), 30 days (1), 45 days (5), Daily observation (26), NK-Not known (79), Overall (185)
	31. Publication years	2004-2007 (11), 2008-2011 (14), 2012-2015 (43), 2016-2019 (117), Overall (185)
	32. Journal	*Biocontrol Science and Technology* (3), *AMB Express* (1), *Archives of Phytopathology and Plant Protection* (2), *Journal of Berry Research* (12*), Biological Control* (8), *Crop Protection* (11), *Current Science* (12), Doctoral dissertation (Addis Ababa University) (26), *Egyptian Journal of Biological Pest Control* (8), *Entomologia Experimentalis et Applicata* (4), *Frontiers in Microbiology* (15), *International Journal of Plant Protection* (27), *Journal of Invertebrate Pathology* (24), *International Journal of Pest Management* (11), *Microbial Pathogenesis* (2), *New Zealand Plant Protection* (1), *Journal of Plant Protection Research* (8), *Scientific Journal of King Faisal University* (4), *Southwestern Entomologist* (4), *Toxins* (2), Overall (185)

*Figures in parenthesis are number of studies (n).

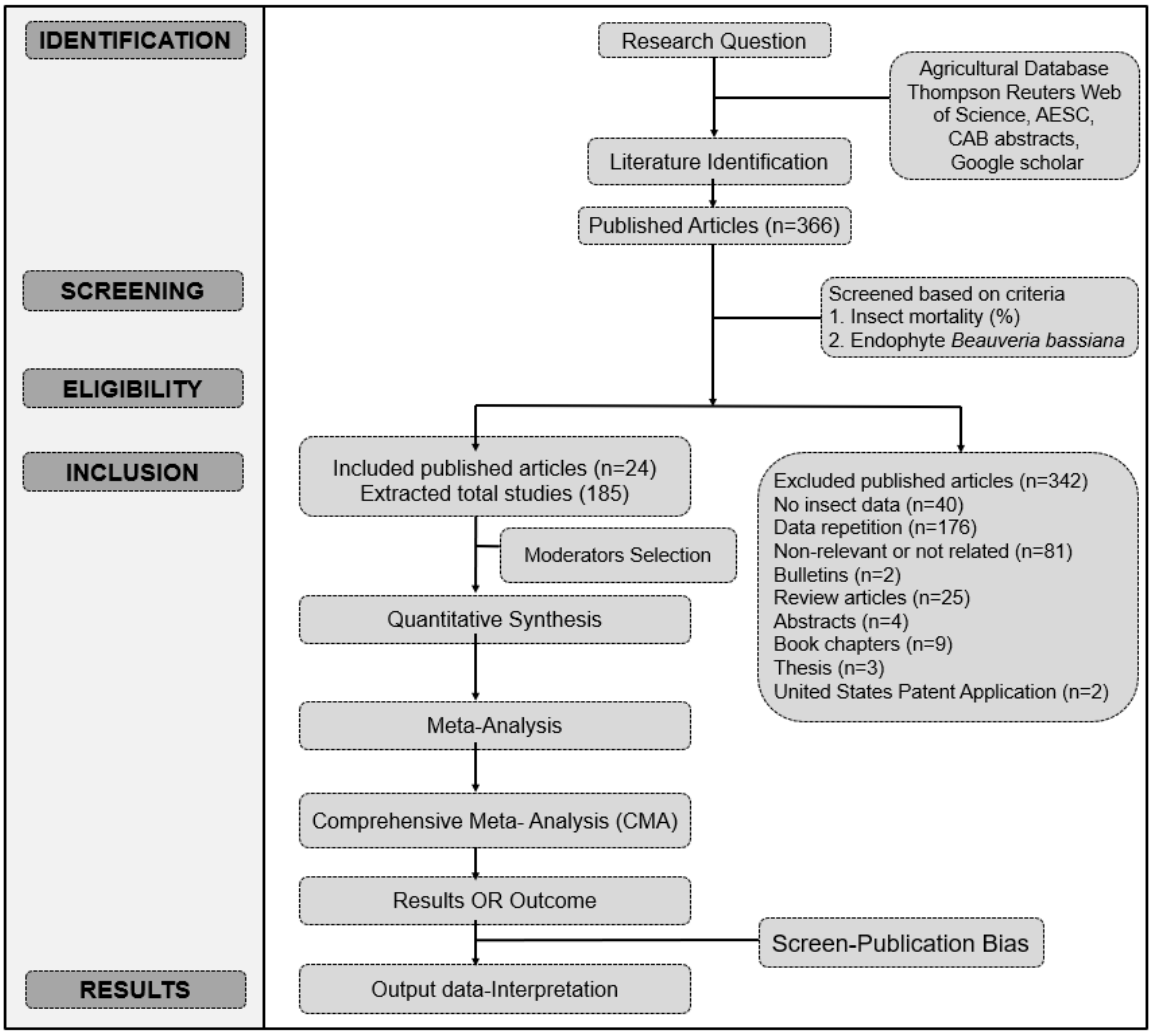
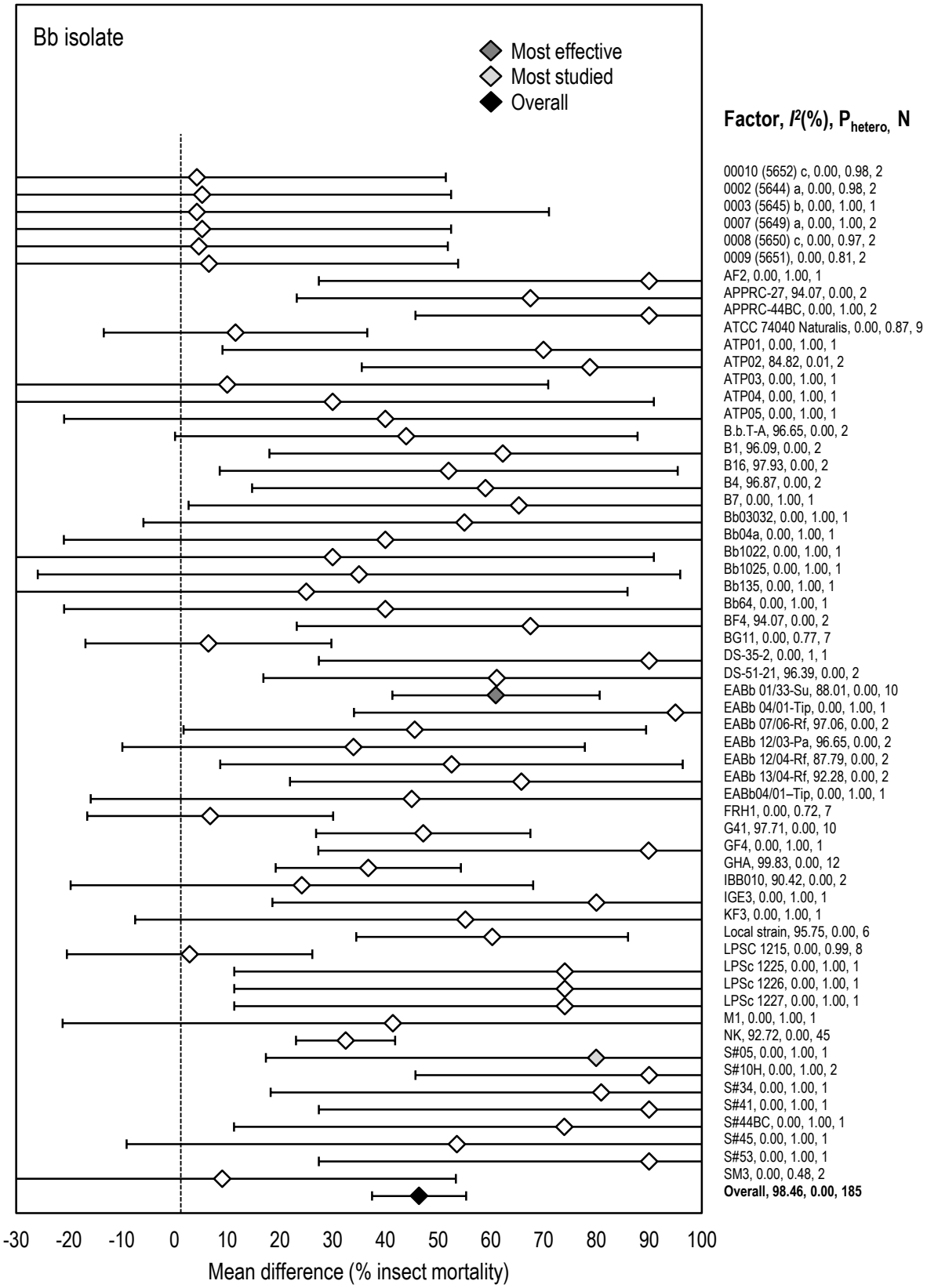


Figure 2.1. Schematic representation of meta-analysis procedure followed in the study[1].

[1] AESC = Agriculture & Environmental Science Database; CAB=International Comprehensive Database for Agriculture and Veterinary Medicine.

Figure 2.2. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific Bb isolate.

Moderator: Comparison among studies on specific Bb isolates used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (Bb isolate) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.


Bb isolate
Most effective
Most studied
Overall
Factor, I^2(%), $P_{hetero,}$ N
00010 (5652) c, 0.00, 0.98, 2
0002 (5644) a, 0.00, 0.98, 2
0003 (5645) b, 0.00, 1.00, 1
0007 (5649) a, 0.00, 1.00, 2
0008 (5650) c, 0.00, 0.97, 2
0009 (5651), 0.00, 0.81, 2
AF2, 0.00, 1.00, 1
APPRC-27, 94.07, 0.00, 2
APPRC-44BC, 0.00, 1.00, 2
ATCC 74040 Naturalis, 0.00, 0.87, 9
ATP01, 0.00, 1.00, 1
ATP02, 84.82, 0.01, 2
ATP03, 0.00, 1.00, 1
ATP04, 0.00, 1.00, 1
ATP05, 0.00, 1.00, 1
B.b.T-A, 96.65, 0.00, 2
B1, 96.09, 0.00, 2
B16, 97.93, 0.00, 2
B4, 96.87, 0.00, 2
B7, 0.00, 1.00, 1
Bb03032, 0.00, 1.00, 1
Bb04a, 0.00, 1.00, 1
Bb1022, 0.00, 1.00, 1
Bb1025, 0.00, 1.00, 1
Bb135, 0.00, 1.00, 1
Bb64, 0.00, 1.00, 1
BF4, 94.07, 0.00, 2
BG11, 0.00, 0.77, 7
DS-35-2, 0.00, 1, 1
DS-51-21, 96.39, 0.00, 2
EABb 01/33-Su, 88.01, 0.00, 10
EABb 04/01-Tip, 0.00, 1.00, 1
EABb 07/06-Rf, 97.06, 0.00, 2
EABb 12/03-Pa, 96.65, 0.00, 2
EABb 12/04-Rf, 87.79, 0.00, 2
EABb 13/04-Rf, 92.28, 0.00, 2
EABb04/01–Tip, 0.00, 1.00, 1
FRH1, 0.00, 0.72, 7
G41, 97.71, 0.00, 10
GF4, 0.00, 1.00, 1
GHA, 99.83, 0.00, 12
IBB010, 90.42, 0.00, 2
IGE3, 0.00, 1.00, 1
KF3, 0.00, 1.00, 1
Local strain, 95.75, 0.00, 6
LPSC 1215, 0.00, 0.99, 8
LPSc 1225, 0.00, 1.00, 1
LPSc 1226, 0.00, 1.00, 1
LPSc 1227, 0.00, 1.00, 1
M1, 0.00, 1.00, 1
NK, 92.72, 0.00, 45
S#05, 0.00, 1.00, 1
S#10H, 0.00, 1.00, 2
S#34, 0.00, 1.00, 1
S#41, 0.00, 1.00, 1
S#44BC, 0.00, 1.00, 1
S#45, 0.00, 1.00, 1
S#53, 0.00, 1.00, 1
SM3, 0.00, 0.48, 2
Overall, 98.46, 0.00, 185
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% insect mortality)

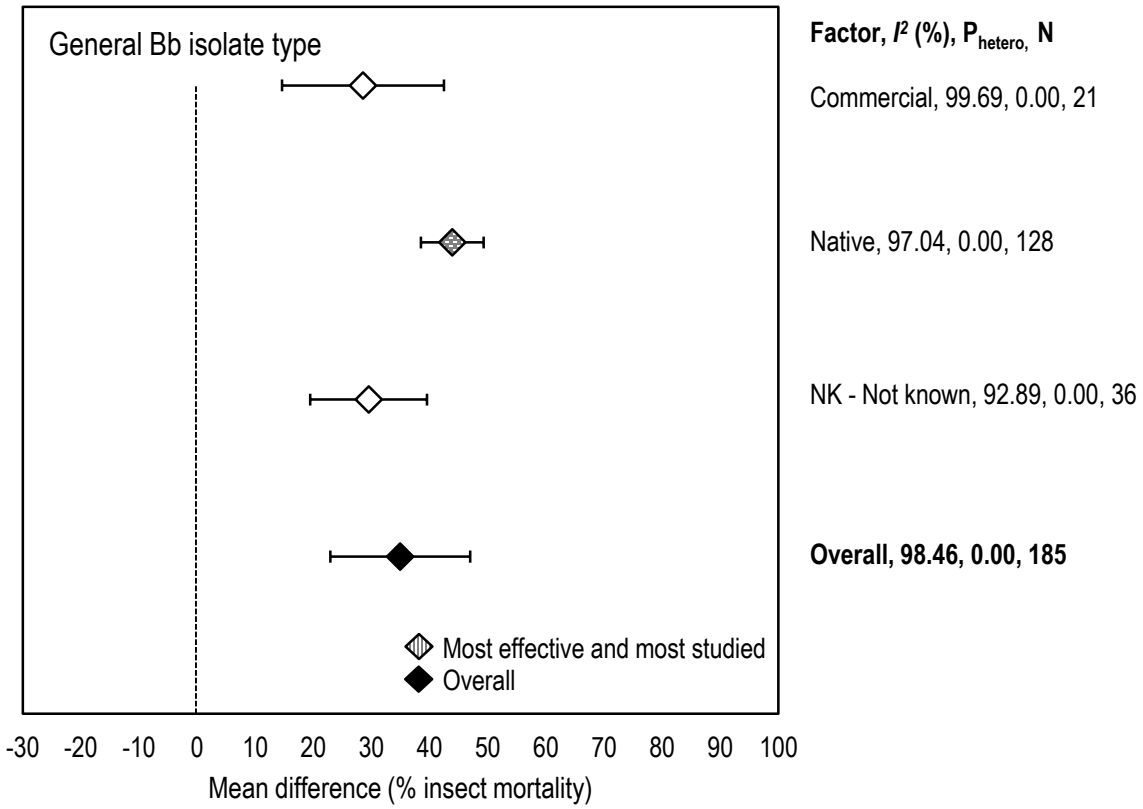


Figure 2.3. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on general type of Bb isolate.

Moderator: Comparison among studies on general type of Bb isolate used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (general type of Bb isolate) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

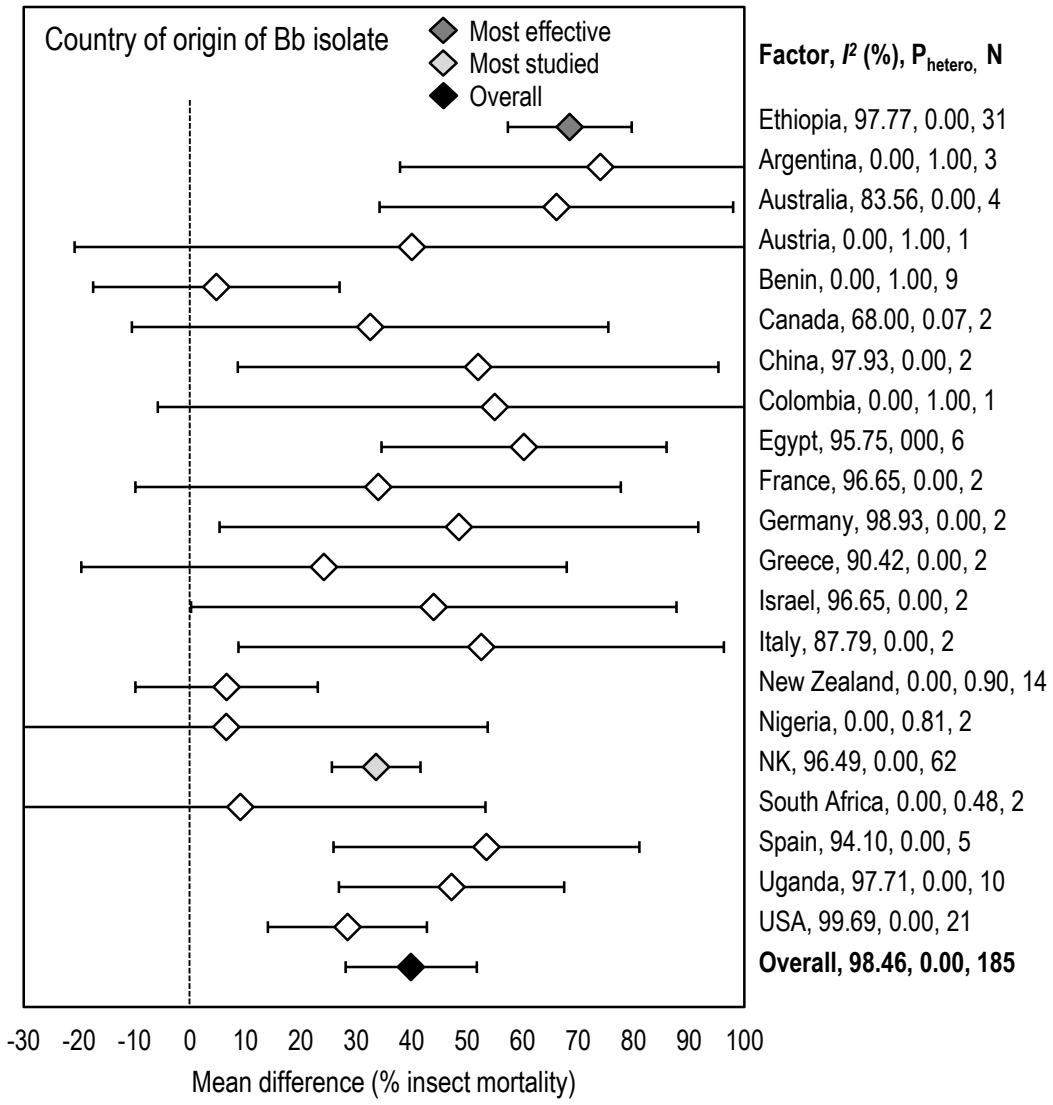


Figure 2.4. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on the country of origin of Bb isolates.

Moderator: Comparison among studies on country of origin of Bb isolates used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (country of origin of Bb isolate) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

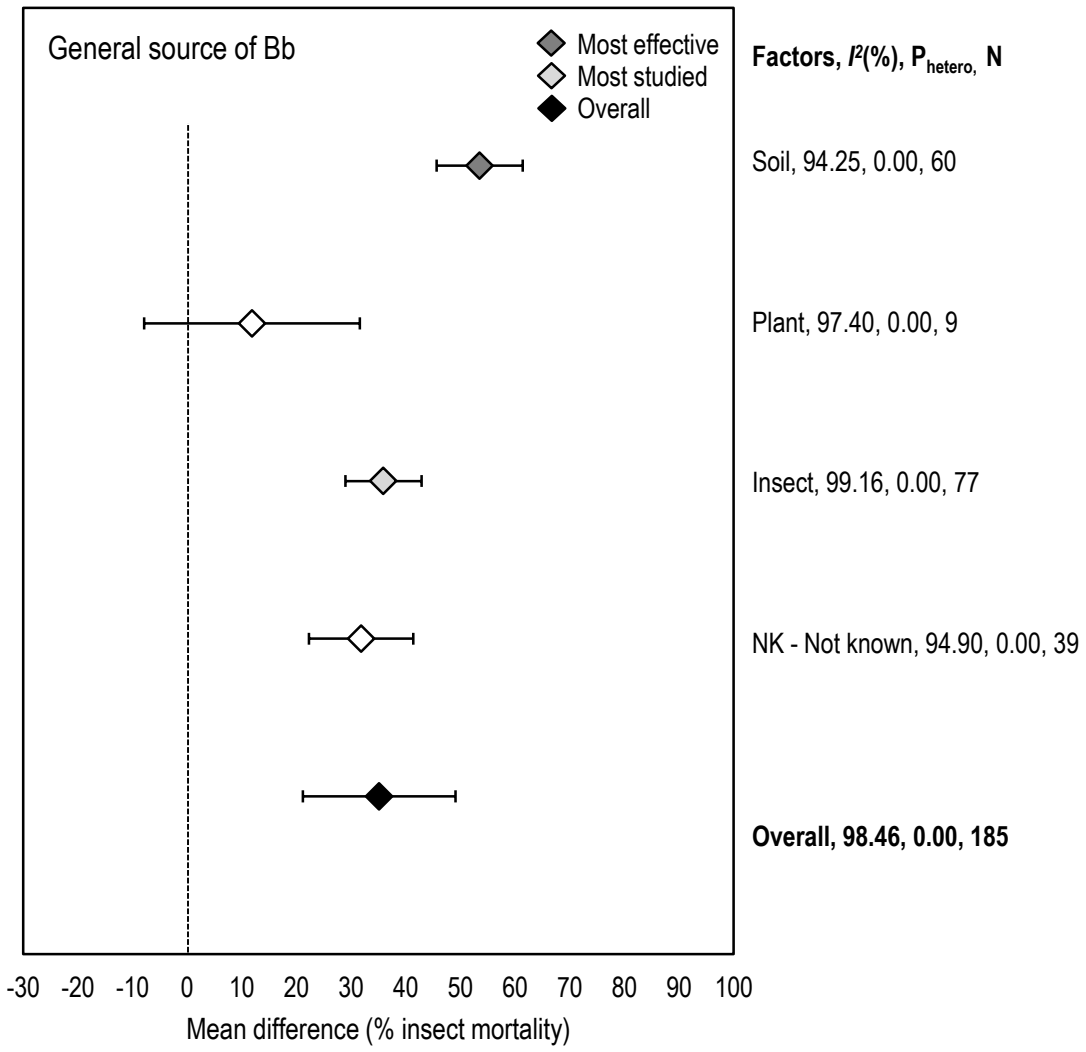
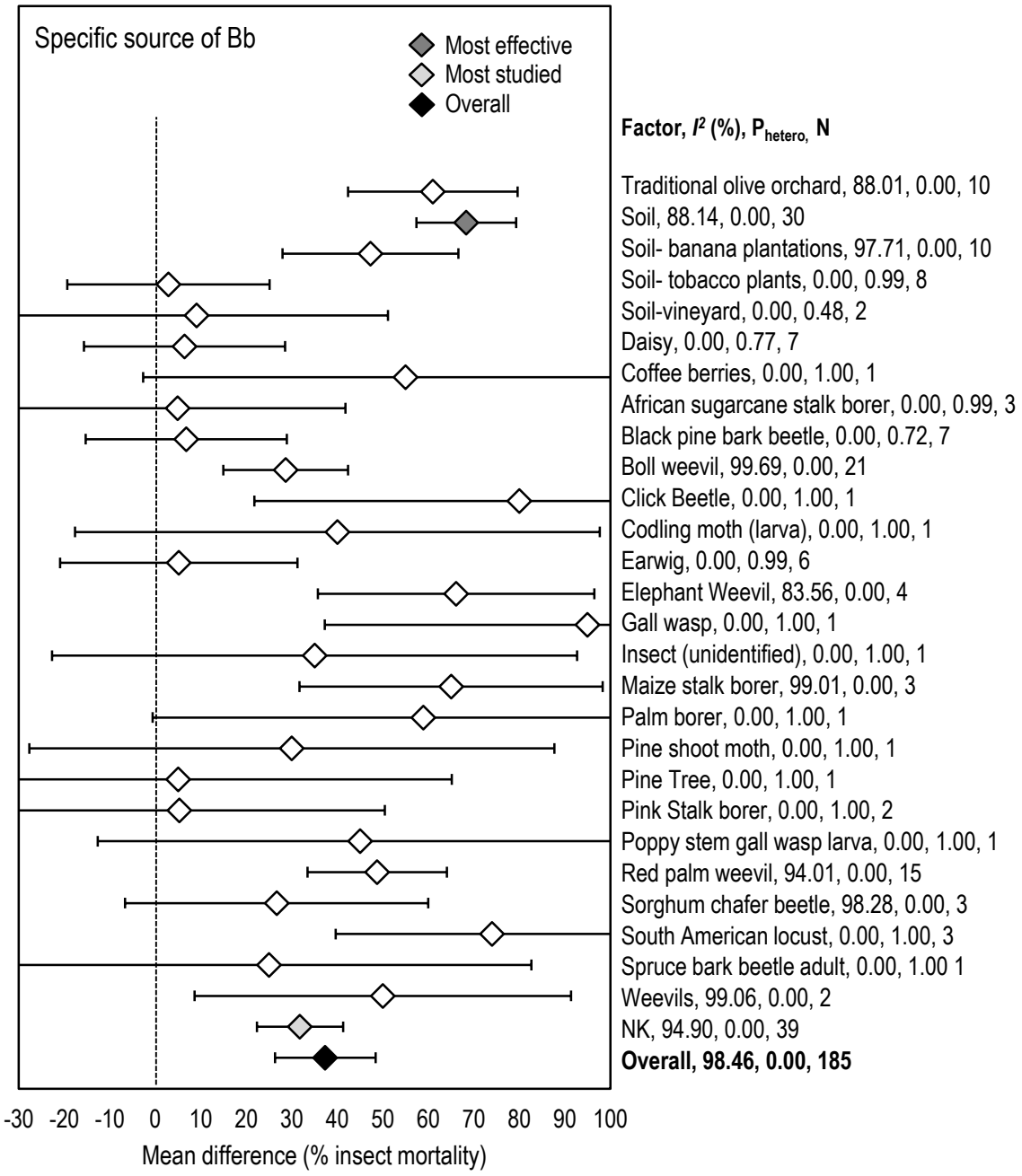


Figure 2.5. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on the general source of Bb isolates.

Moderator: Comparison among studies on general source of Bb isolates used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (general source of Bb isolate) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

Figure 2.6. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific source of Bb isolate.

Moderator: Comparison among studies on specific source of Bb isolates used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (specific source of Bb isolates) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.


Specific source of Bb
Most effective
Most studied
Overall
Factor, I^2 (%), P_{hetero}, N
Traditional olive orchard, 88.01, 0.00, 10
Soil, 88.14, 0.00, 30
Soil- banana plantations, 97.71, 0.00, 10
Soil- tobacco plants, 0.00, 0.99, 8
Soil-vineyard, 0.00, 0.48, 2
Daisy, 0.00, 0.77, 7
Coffee berries, 0.00, 1.00, 1
African sugarcane stalk borer, 0.00, 0.99, 3
Black pine bark beetle, 0.00, 0.72, 7
Boll weevil, 99.69, 0.00, 21
Click Beetle, 0.00, 1.00, 1
Codling moth (larva), 0.00, 1.00, 1
Earwig, 0.00, 0.99, 6
Elephant Weevil, 83.56, 0.00, 4
Gall wasp, 0.00, 1.00, 1
Insect (unidentified), 0.00, 1.00, 1
Maize stalk borer, 99.01, 0.00, 3
Palm borer, 0.00, 1.00, 1
Pine shoot moth, 0.00, 1.00, 1
Pine Tree, 0.00, 1.00, 1
Pink Stalk borer, 0.00, 1.00, 2
Poppy stem gall wasp larva, 0.00, 1.00, 1
Red palm weevil, 94.01, 0.00, 15
Sorghum chafer beetle, 98.28, 0.00, 3
South American locust, 0.00, 1.00, 3
Spruce bark beetle adult, 0.00, 1.00 1
Weevils, 99.06, 0.00, 2
NK, 94.90, 0.00, 39
Overall, 98.46, 0.00, 185
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% insect mortality)

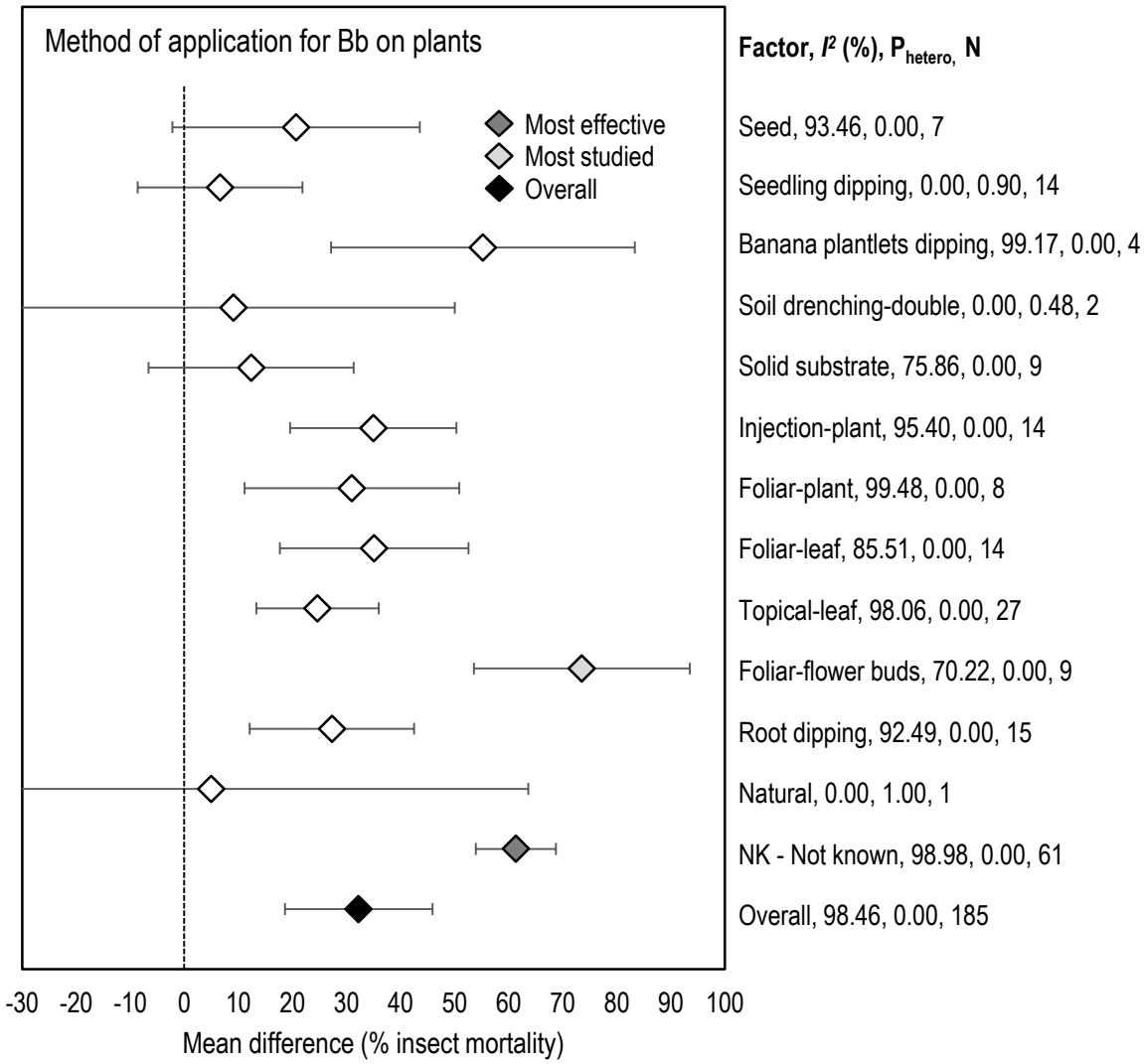


Figure 2.7. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on method of application for Bb on plants.

Moderator: Comparison among studies on method of application for Bb on plants used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (method of application for Bb on plants) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

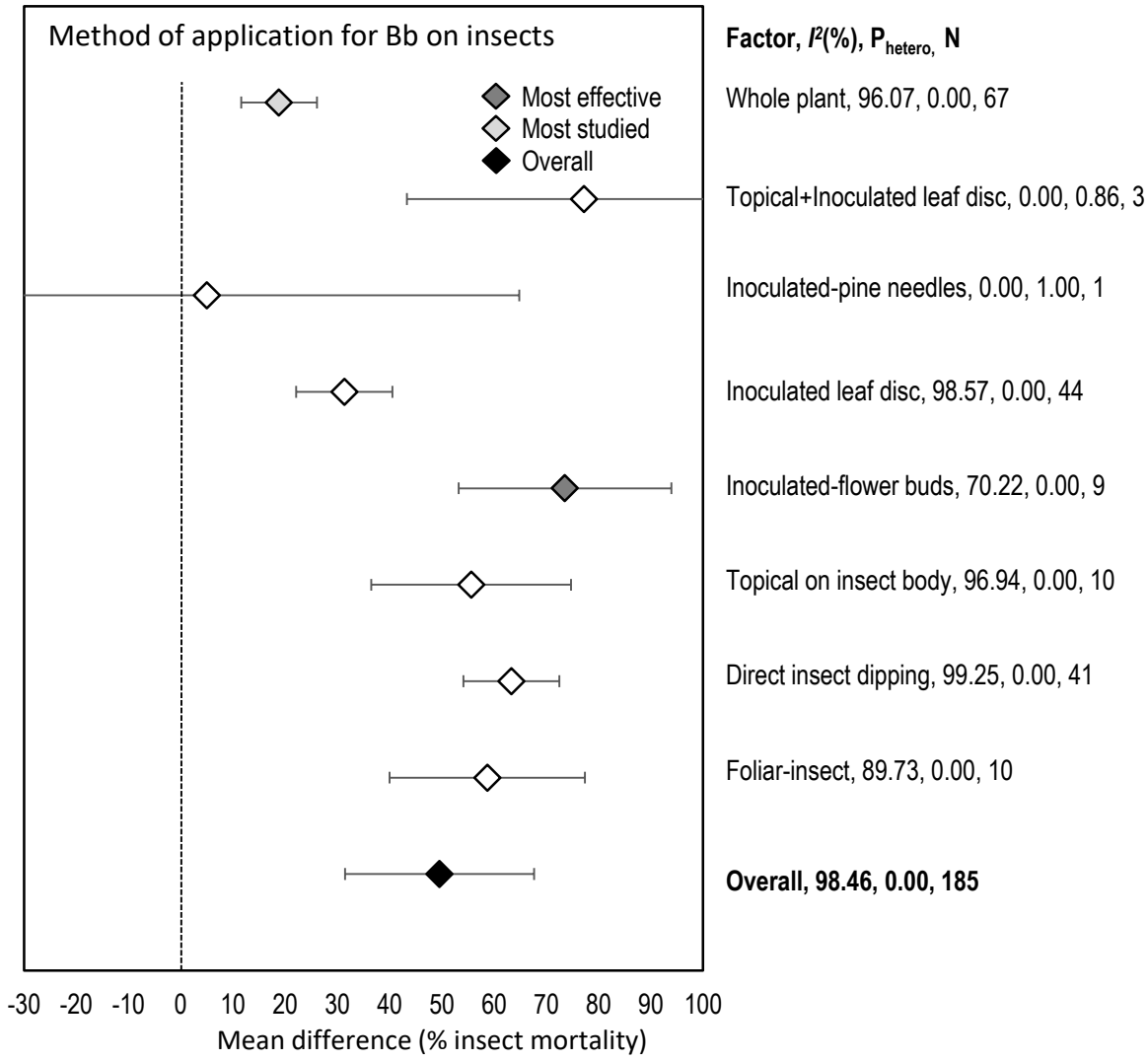


Figure 2.8. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on method of application for Bb on insects.

Moderator: Comparison among studies on method of application for Bb on insects used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (method of application for Bb on insects) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

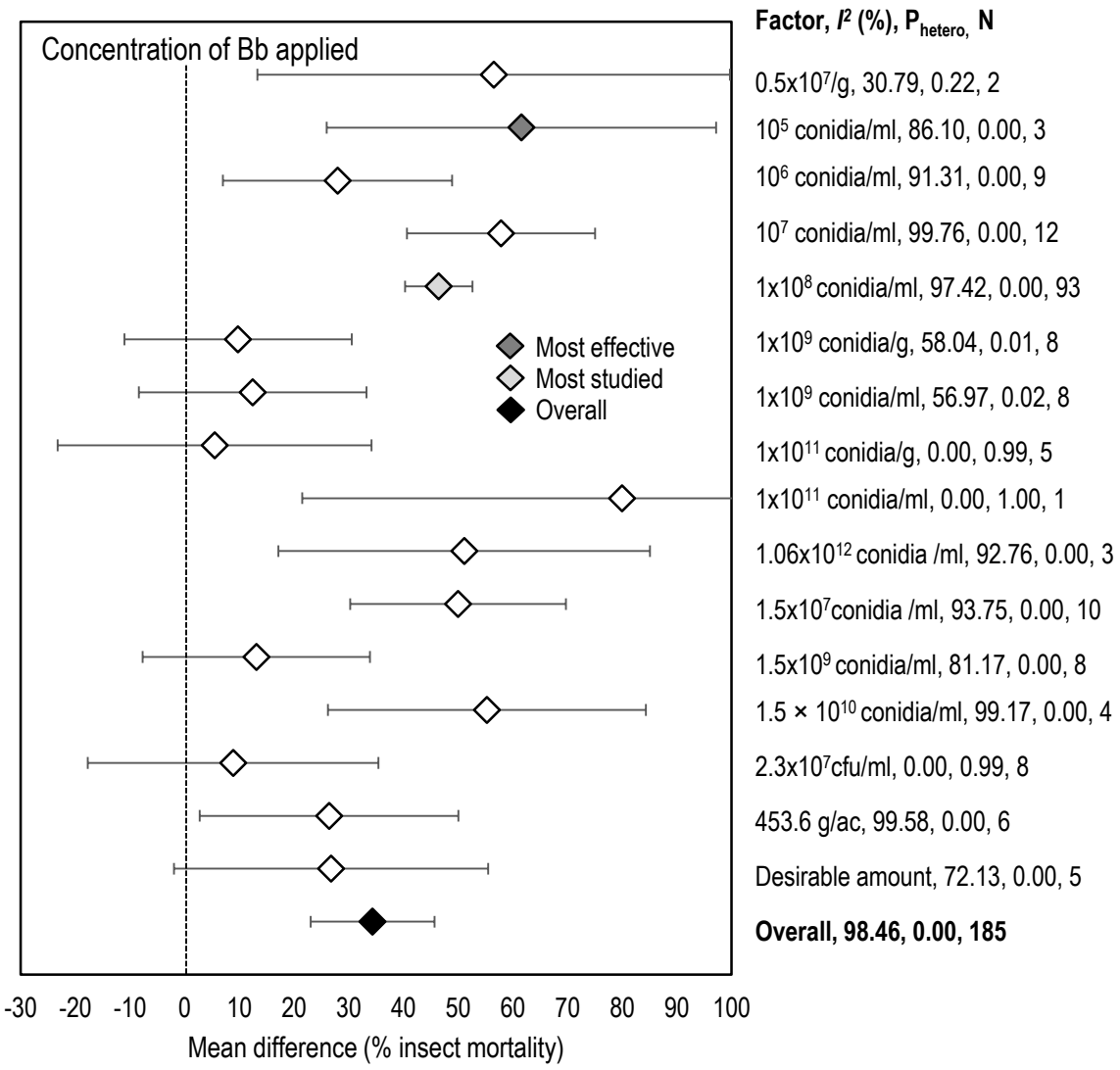


Figure 2.9. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on concentration of Bb applied.

Moderator: Comparison among studies on concentration of Bb applied in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (concentration of Bb applied) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

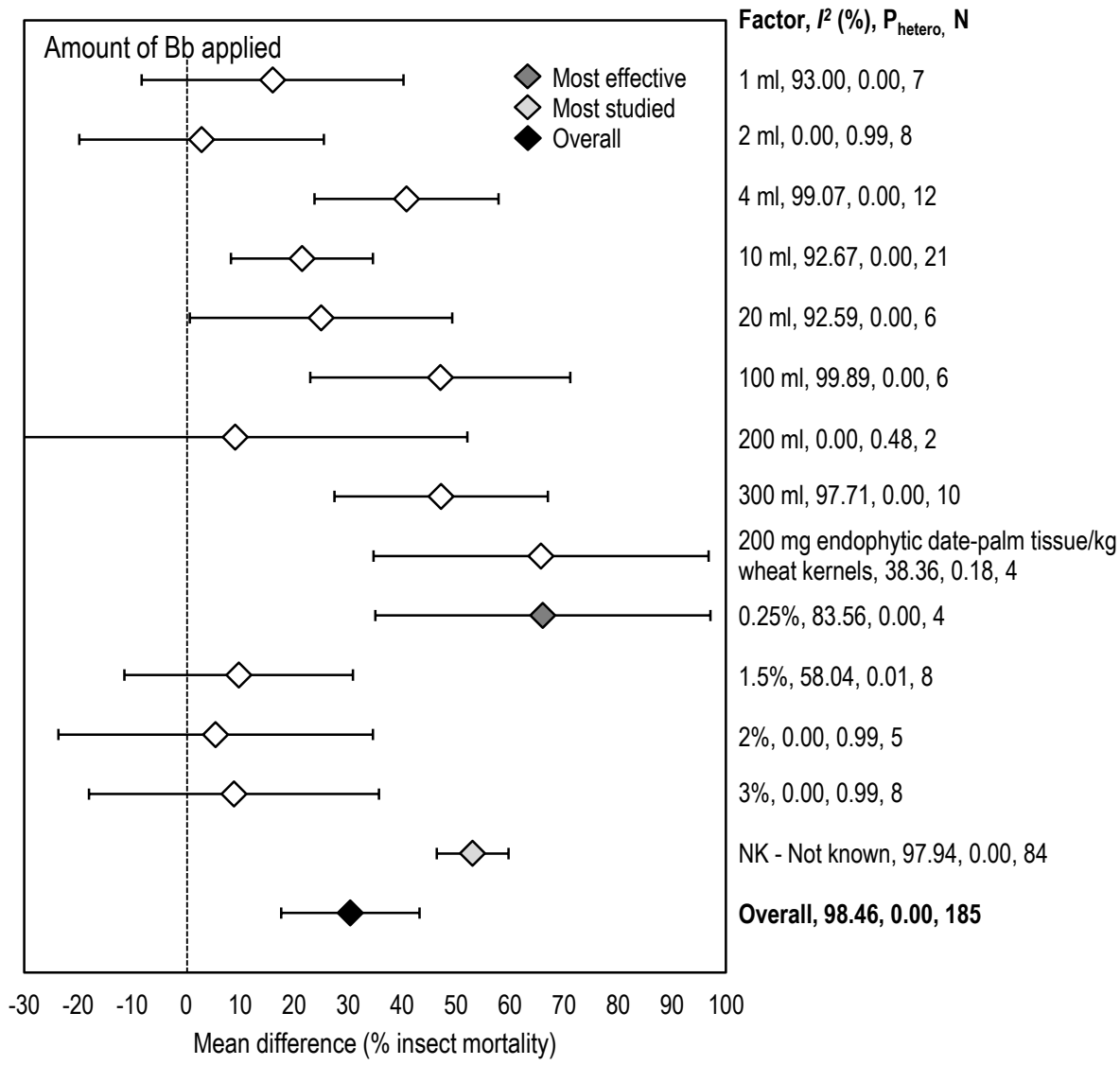


Figure 2.10. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on amount of Bb applied.

Moderator: Comparison among studies on amount of Bb applied in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (amount of Bb applied) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when $P>0.05$ there is no significance; if $P<0.05$ there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

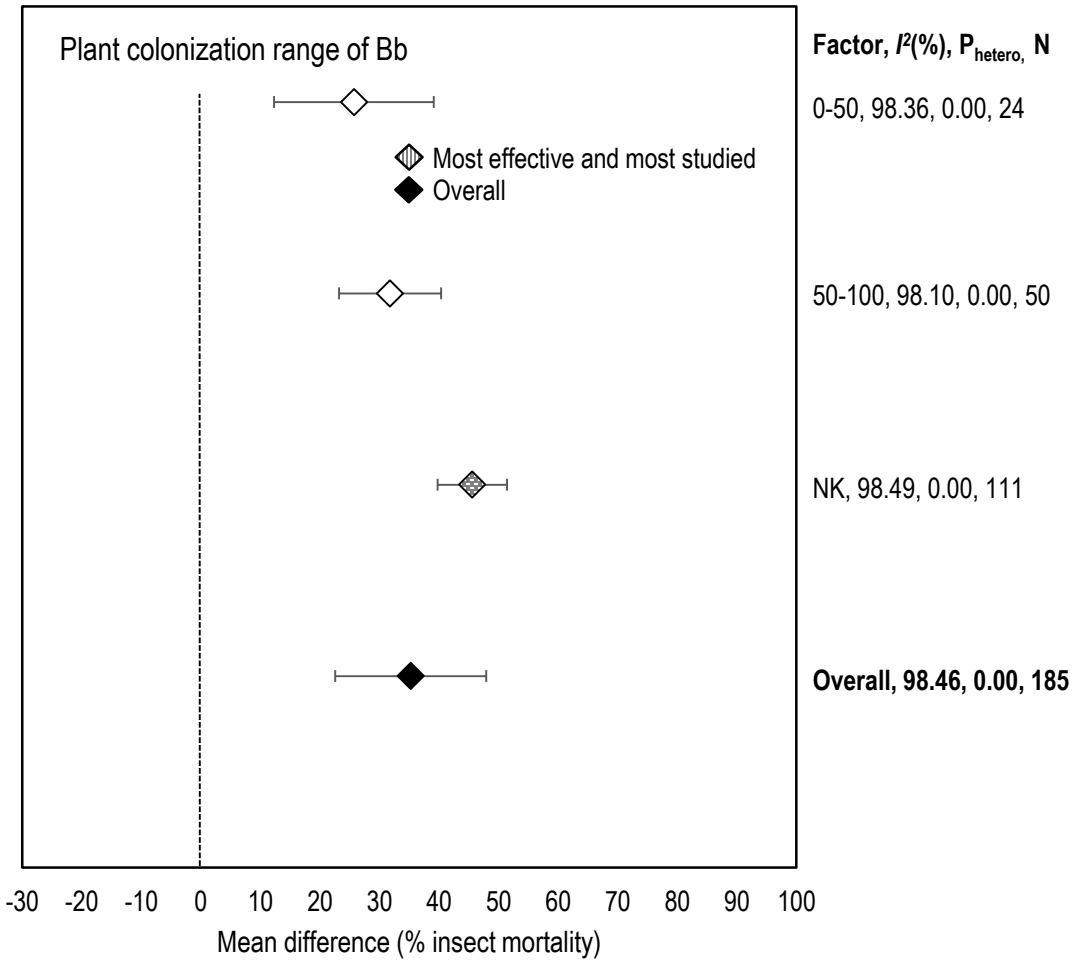


Figure 2.11. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant colonization range (%).

Moderator: Comparison among studies on plant colonization range used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (plant colonization range (%) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

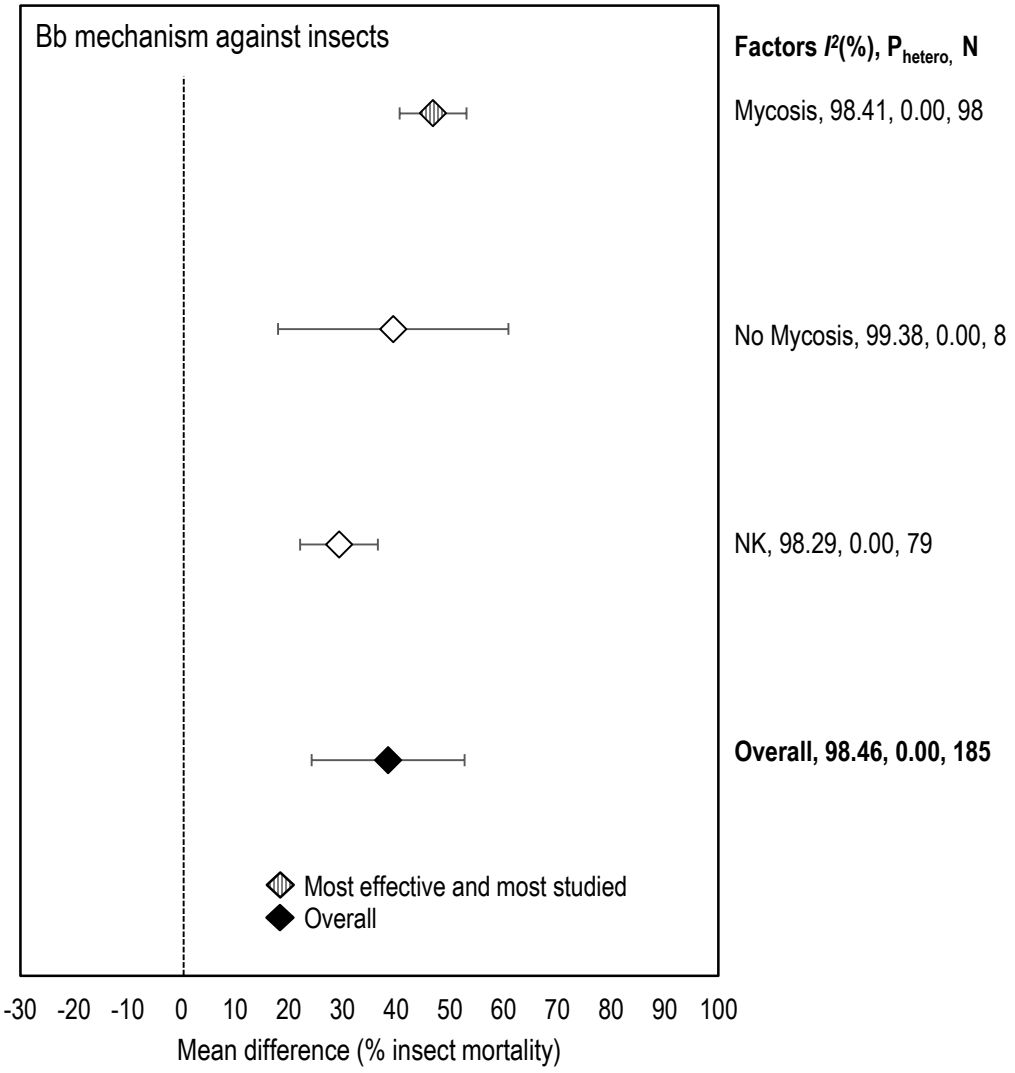
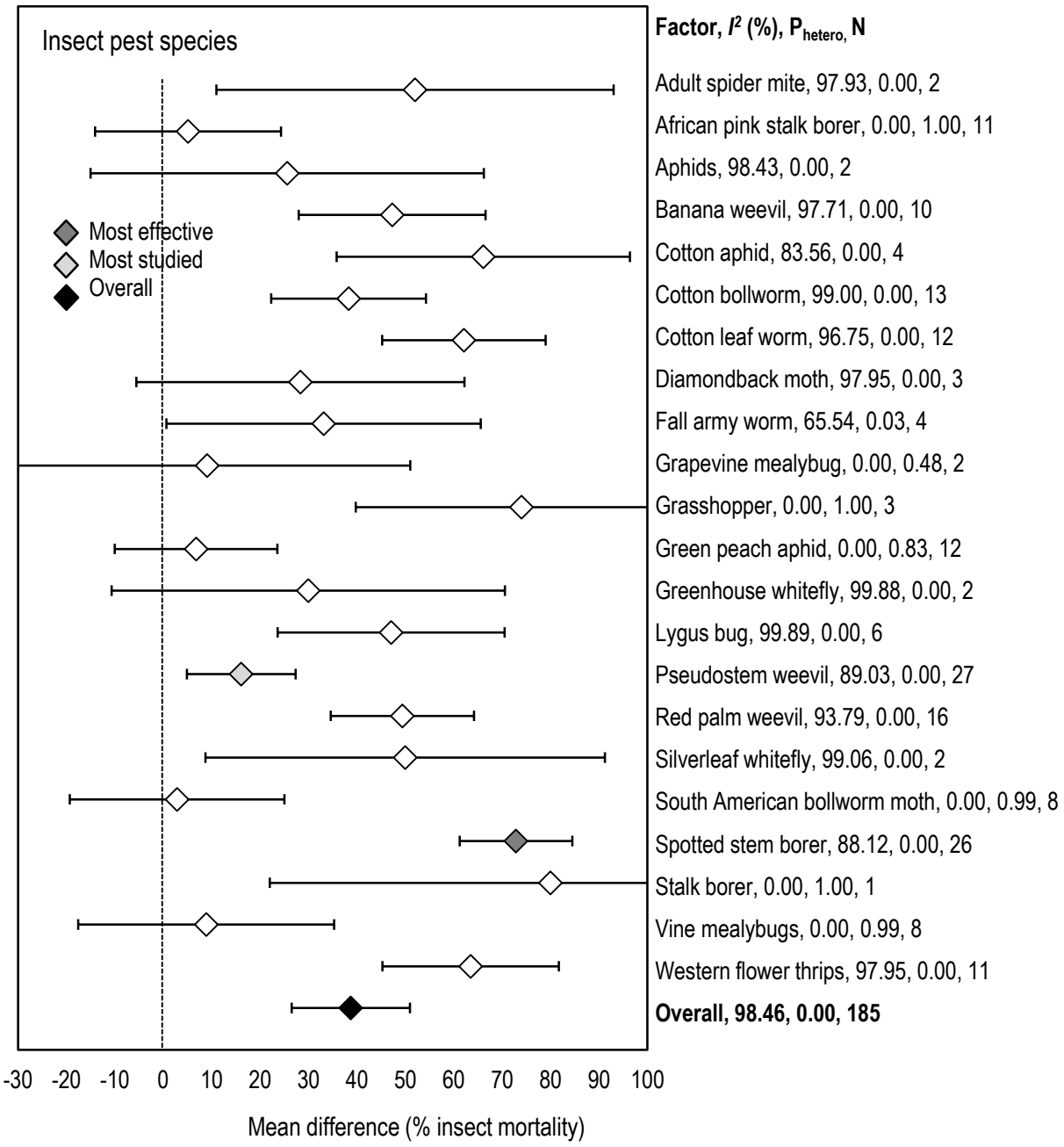


Figure 2.12. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on mechanism of Bb against insects.

Moderator: Comparison among studies on mechanism of Bb against insects in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (mechanism of Bb against insects) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

Figure 2.13. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on insect pest species.

Moderator: Comparison among studies on insect pest species used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (insect pest species) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.


Insect pest species
Most effective
Most studied
Overall
Factor, I^2 (%), P_{hetero}, N
Adult spider mite, 97.93, 0.00, 2
African pink stalk borer, 0.00, 1.00, 11
Aphids, 98.43, 0.00, 2
Banana weevil, 97.71, 0.00, 10
Cotton aphid, 83.56, 0.00, 4
Cotton bollworm, 99.00, 0.00, 13
Cotton leaf worm, 96.75, 0.00, 12
Diamondback moth, 97.95, 0.00, 3
Fall army worm, 65.54, 0.03, 4
Grapevine mealybug, 0.00, 0.48, 2
Grasshopper, 0.00, 1.00, 3
Green peach aphid, 0.00, 0.83, 12
Greenhouse whitefly, 99.88, 0.00, 2
Lygus bug, 99.89, 0.00, 6
Pseudostem weevil, 89.03, 0.00, 27
Red palm weevil, 93.79, 0.00, 16
Silverleaf whitefly, 99.06, 0.00, 2
South American bollworm moth, 0.00, 0.99, 8
Spotted stem borer, 88.12, 0.00, 26
Stalk borer, 0.00, 1.00, 1
Vine mealybugs, 0.00, 0.99, 8
Western flower thrips, 97.95, 0.00, 11
Overall, 98.46, 0.00, 185
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% insect mortality)

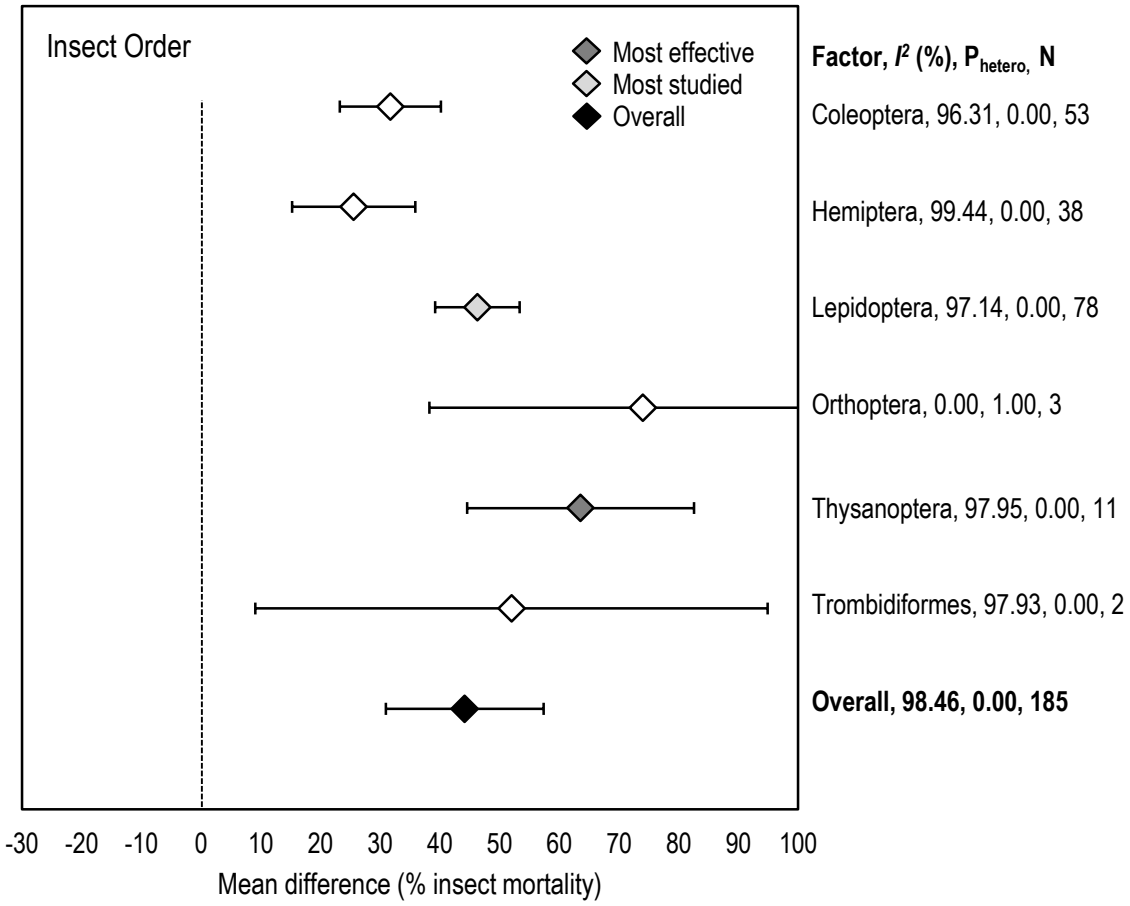


Figure 2.14. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on insect Order.

Moderator: Comparison among studies on insect Order used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (insect Order) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

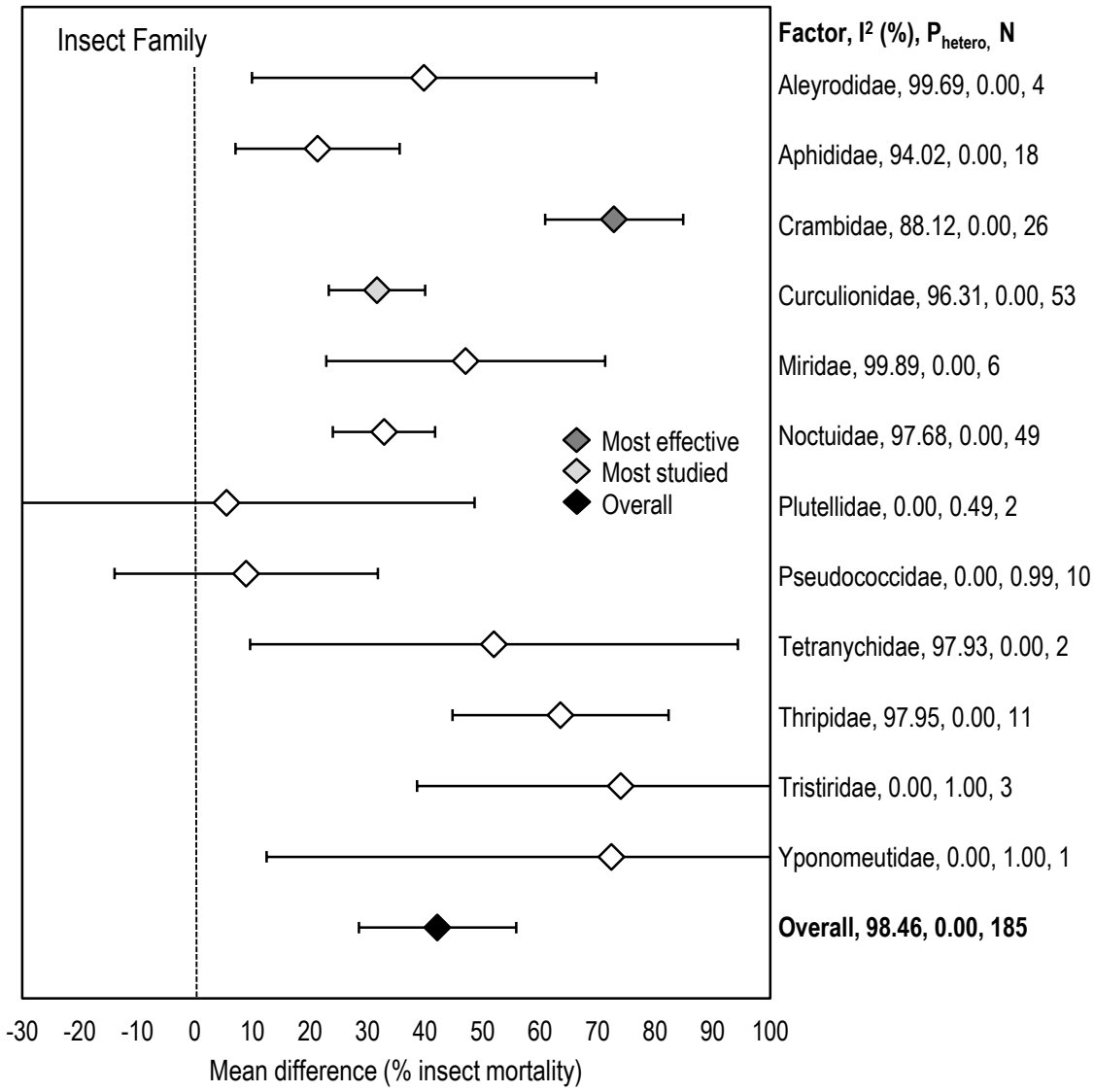


Figure 2.15. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on insect pest Family.

Moderator: Comparison among studies on insect pest Family used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (insect pest Family) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when $P>0.05$ there is no significance; if $P<0.05$ there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

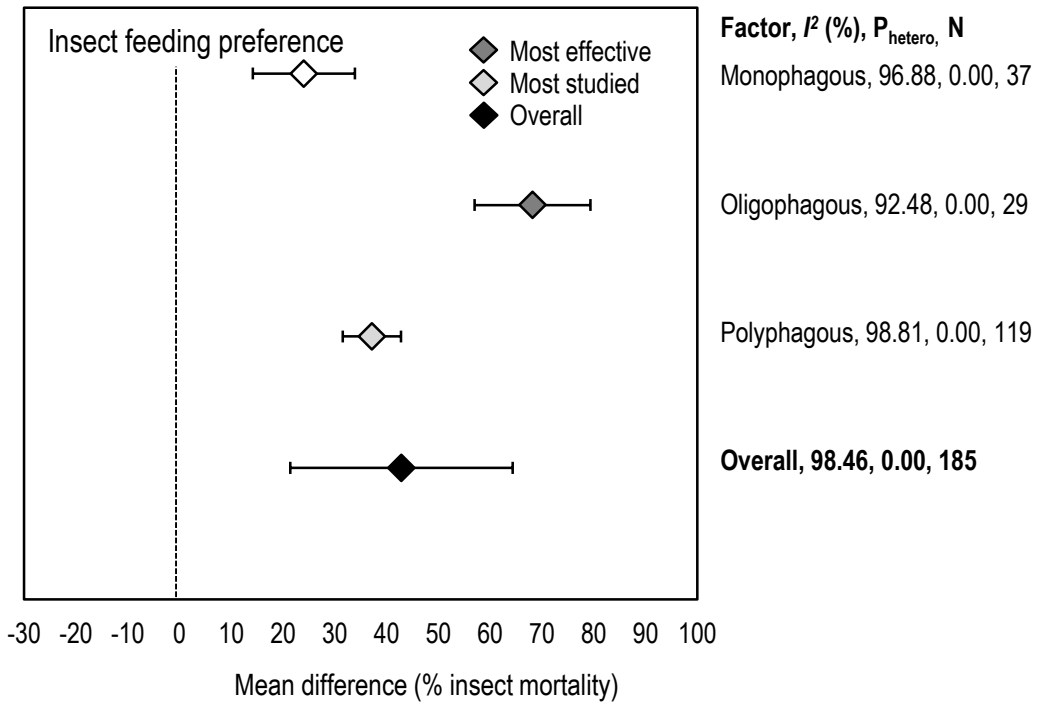


Figure 2.16. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on insect feeding preference.

Moderator: Comparison among studies on insect feeding preference in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (insect feeding preference) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

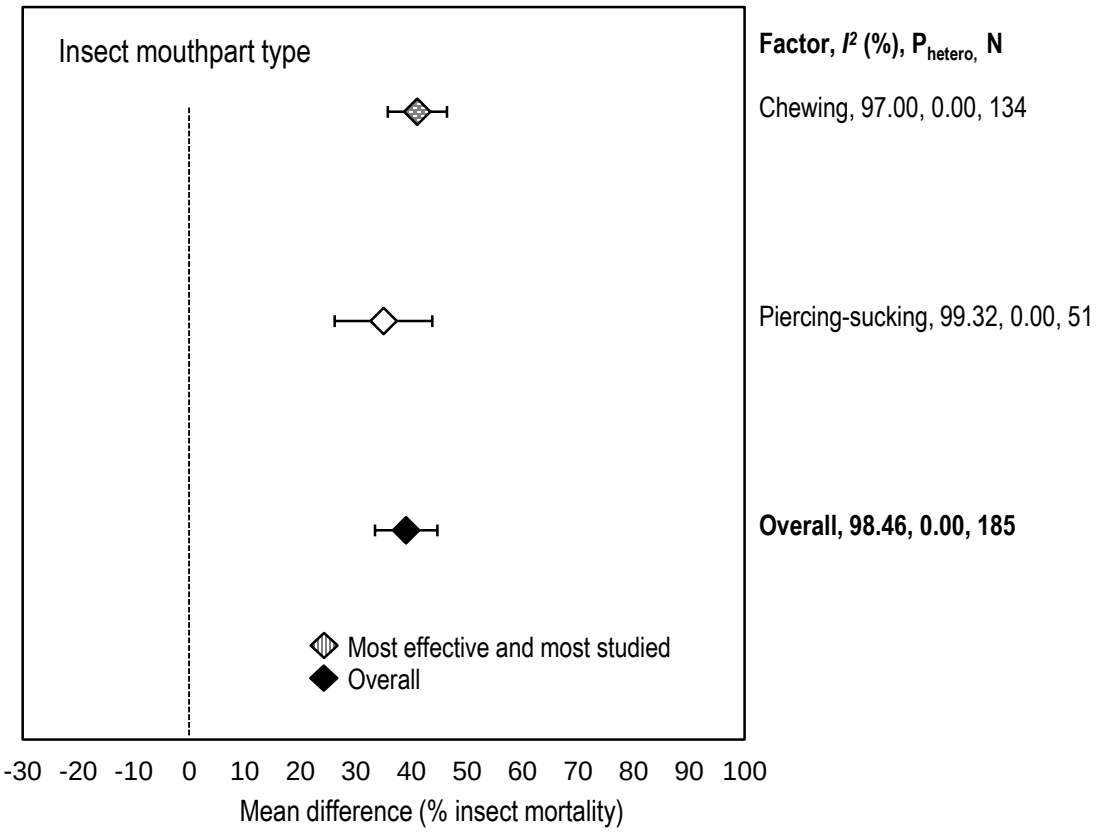


Figure 2.17. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on type of insect pest mouthpart.

Moderator: Comparison among studies on type of insect pest mouthparts in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (type of insect pest mouthparts) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

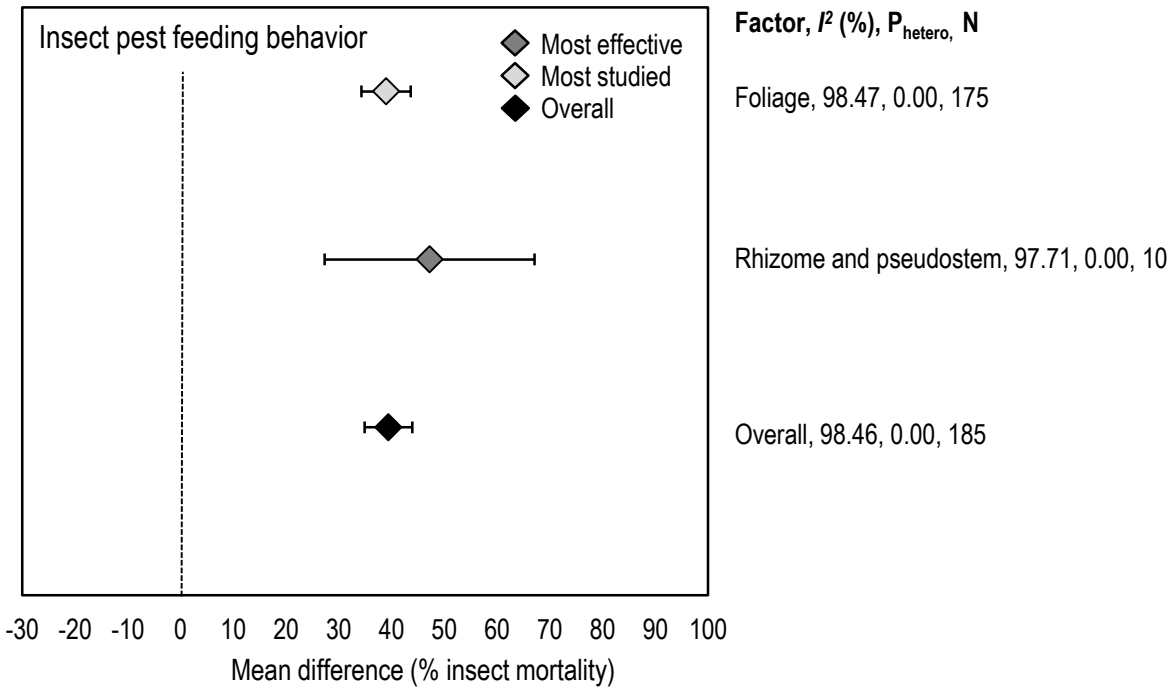


Figure 2.18. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on insect pest feeding behavior.

Moderator: Comparison among studies on insect pest feeding behavior in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (insect pest feeding behavior) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

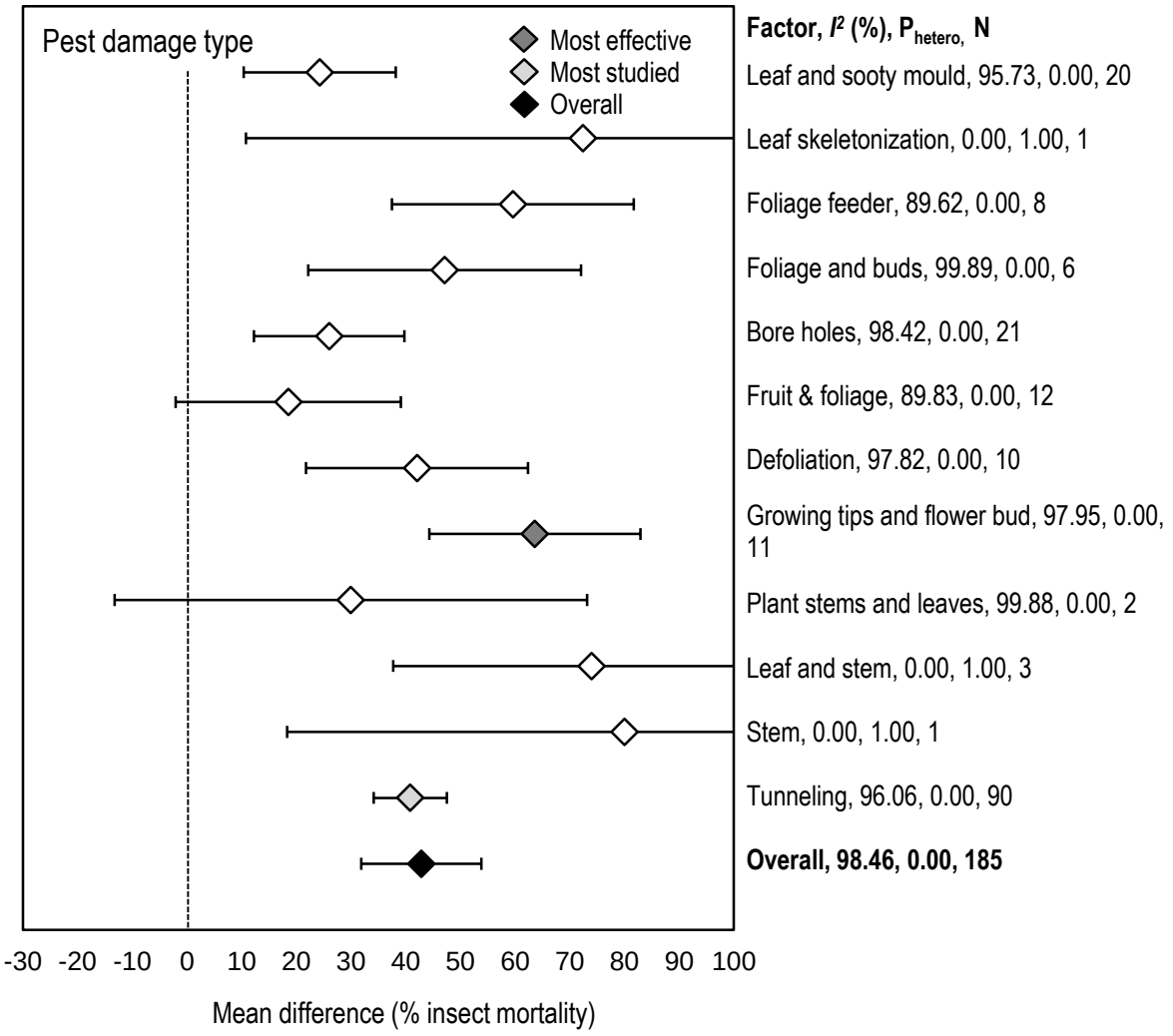


Figure 2.19. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on type of insect pest damage.

Moderator: Comparison among studies on type of insect pest damage in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (type of insect pest damage) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

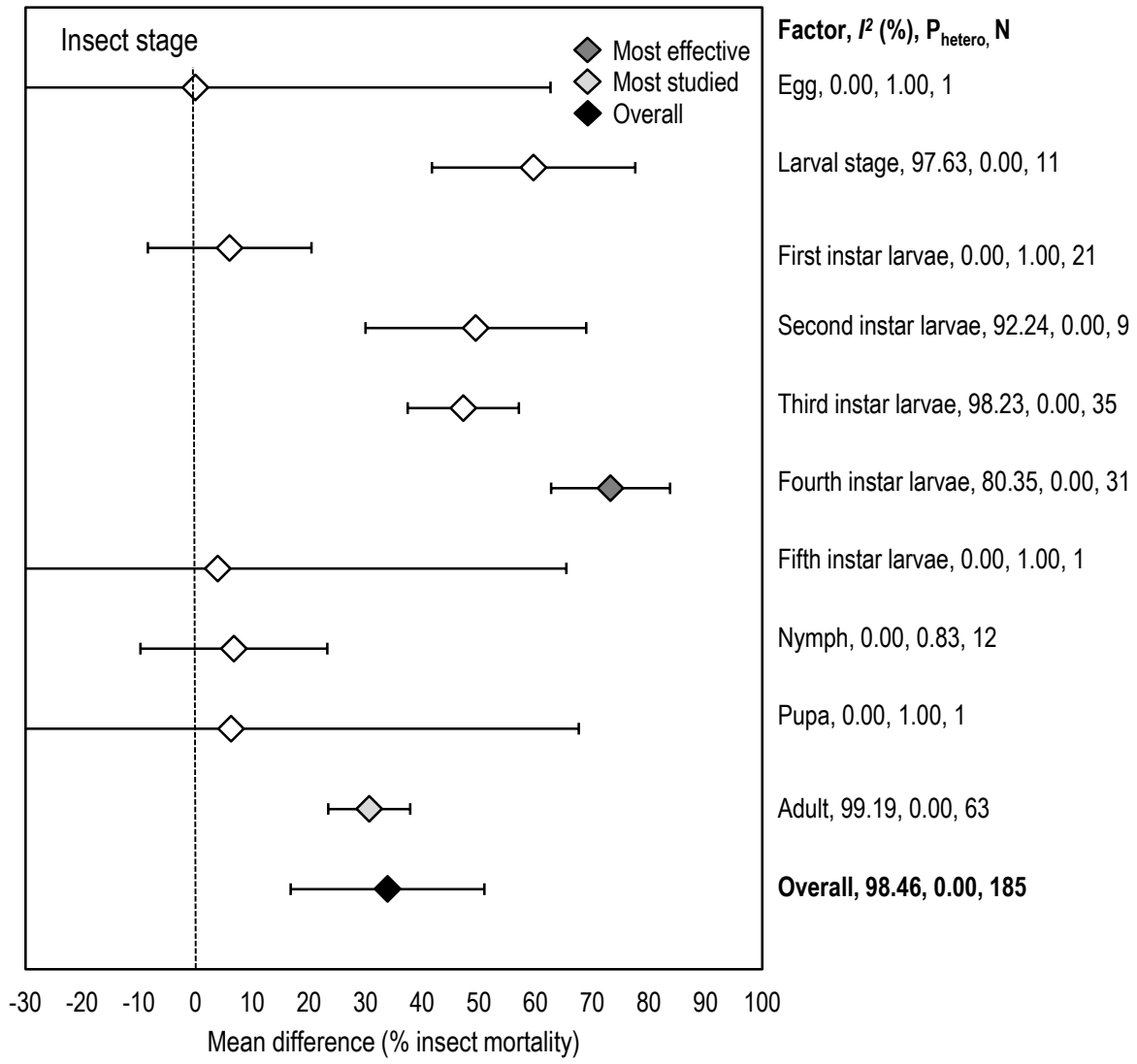


Figure 2.20. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on insect stage.

Moderator: Comparison among studies on insect stage used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (insect stage) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

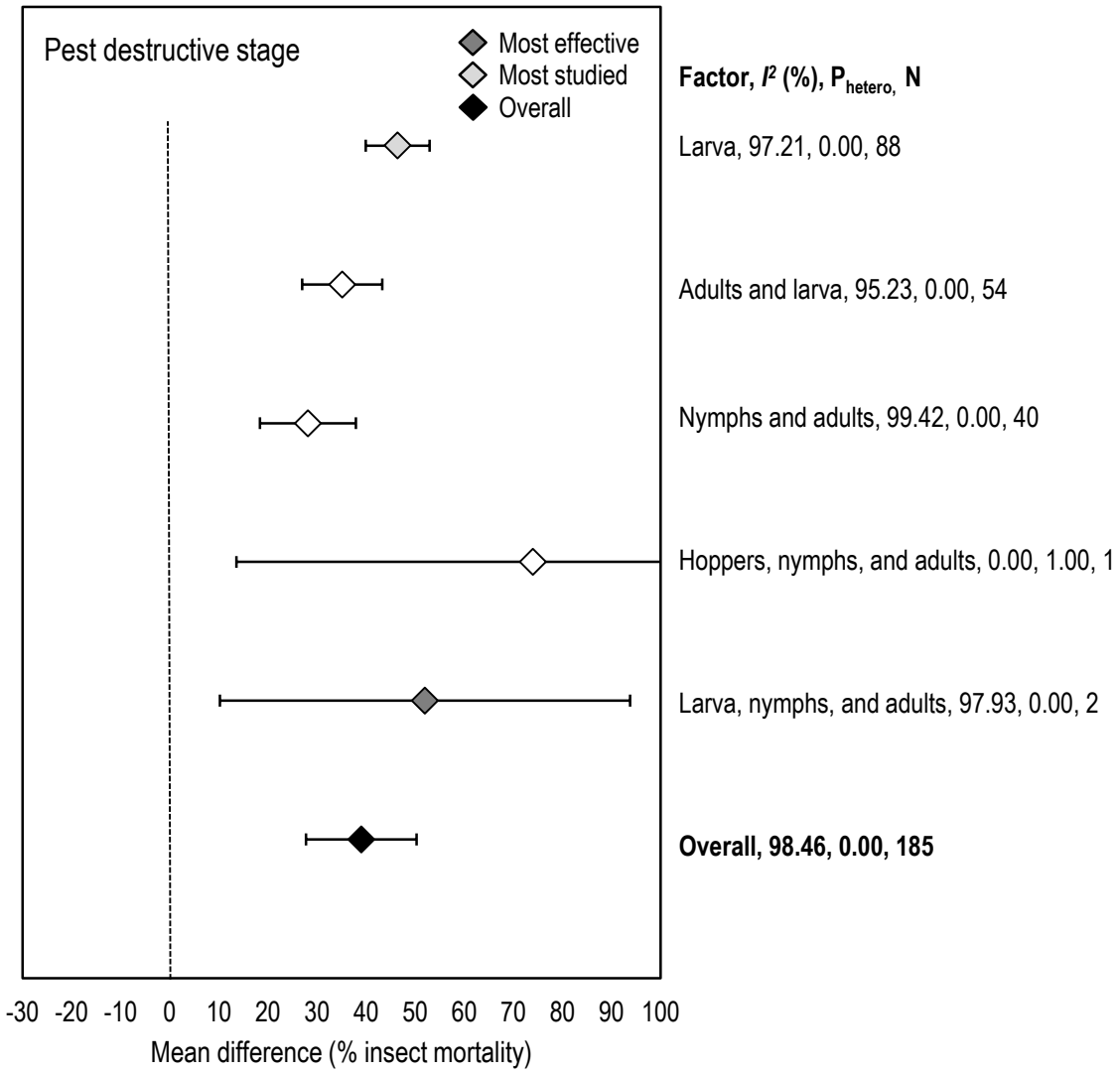


Figure 2.21. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on insect pest destructive stage.

Moderator: Comparison among studies on insect pest destructive stage used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (insect pest destructive stage) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

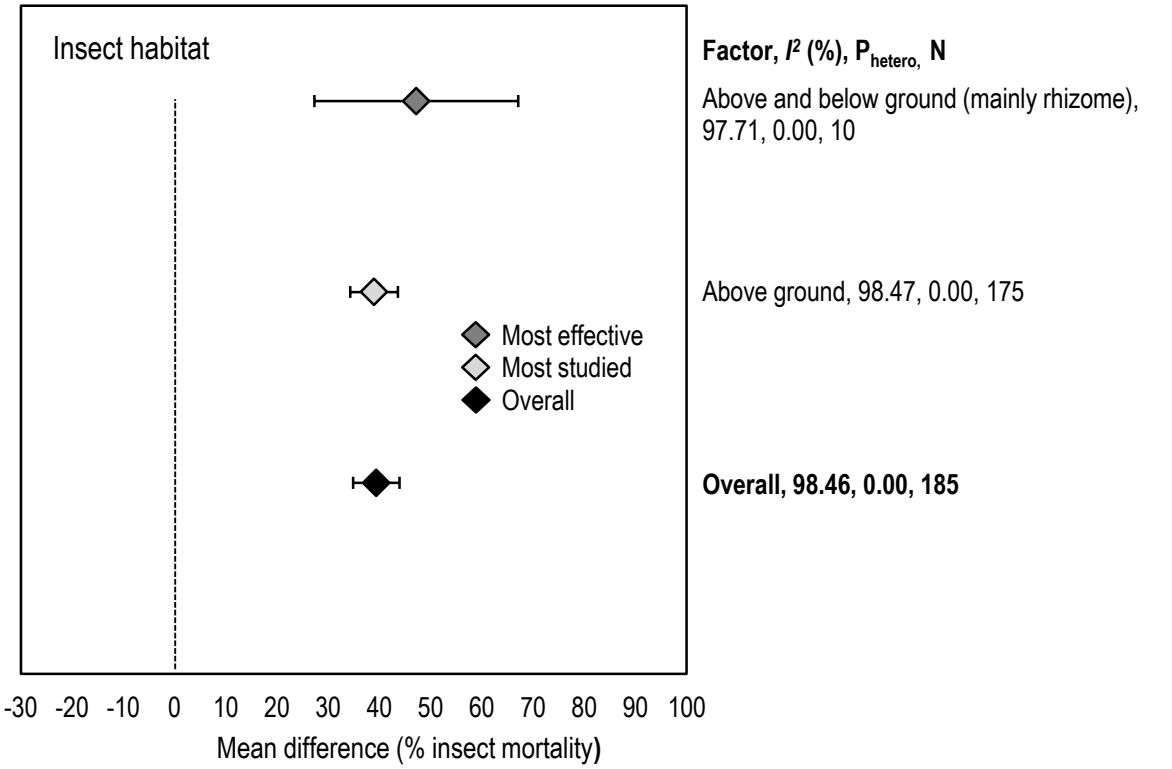


Figure 2.22. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on insect pest habitat.

Moderator: Comparison among studies on insect pest habitat used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (insect pest habitat) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when $P>0.05$ there is no significance; if $P<0.05$ there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

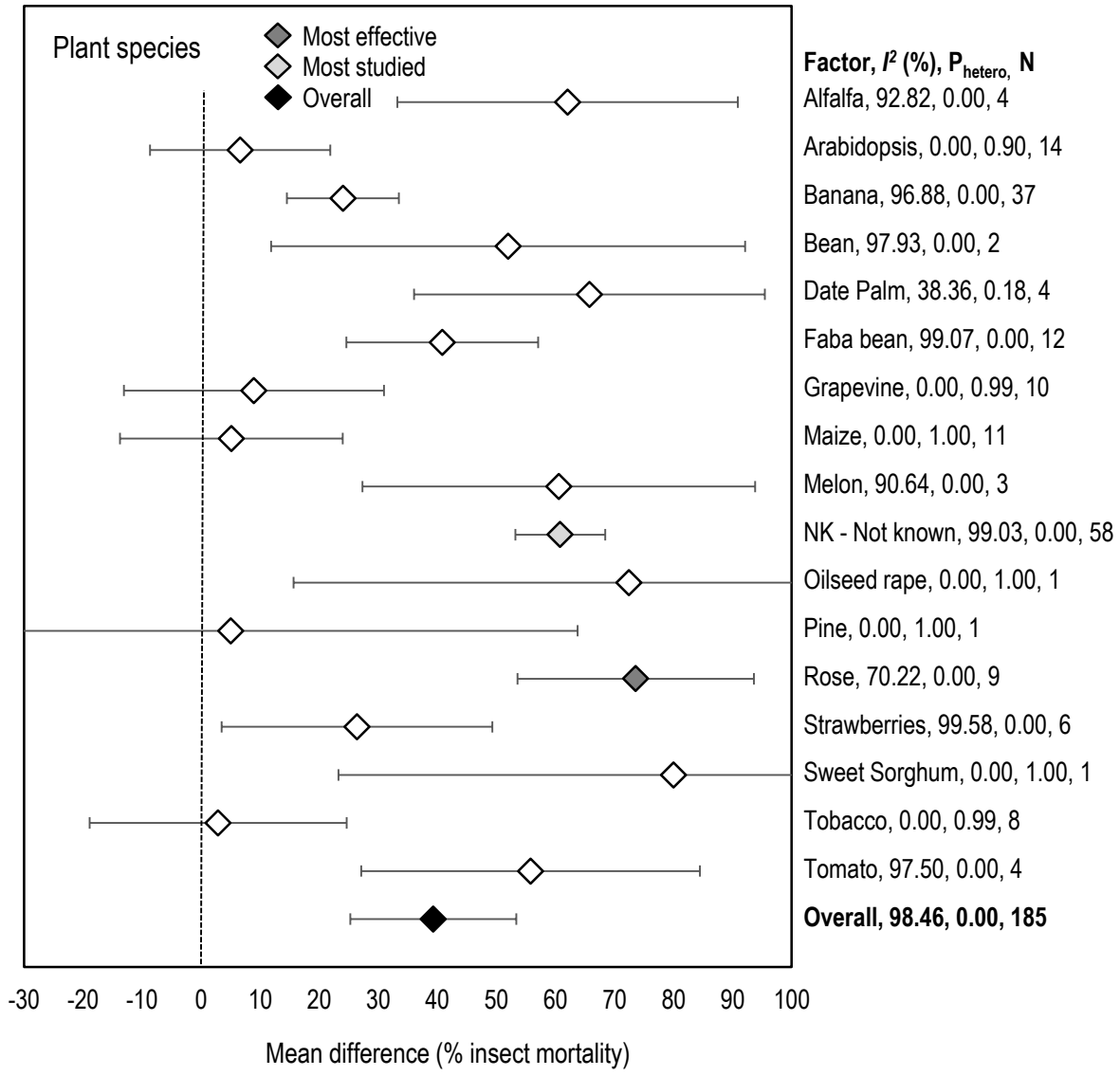


Figure 2.23. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant species.

Moderator: Comparison among studies on plant species used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (plant species) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

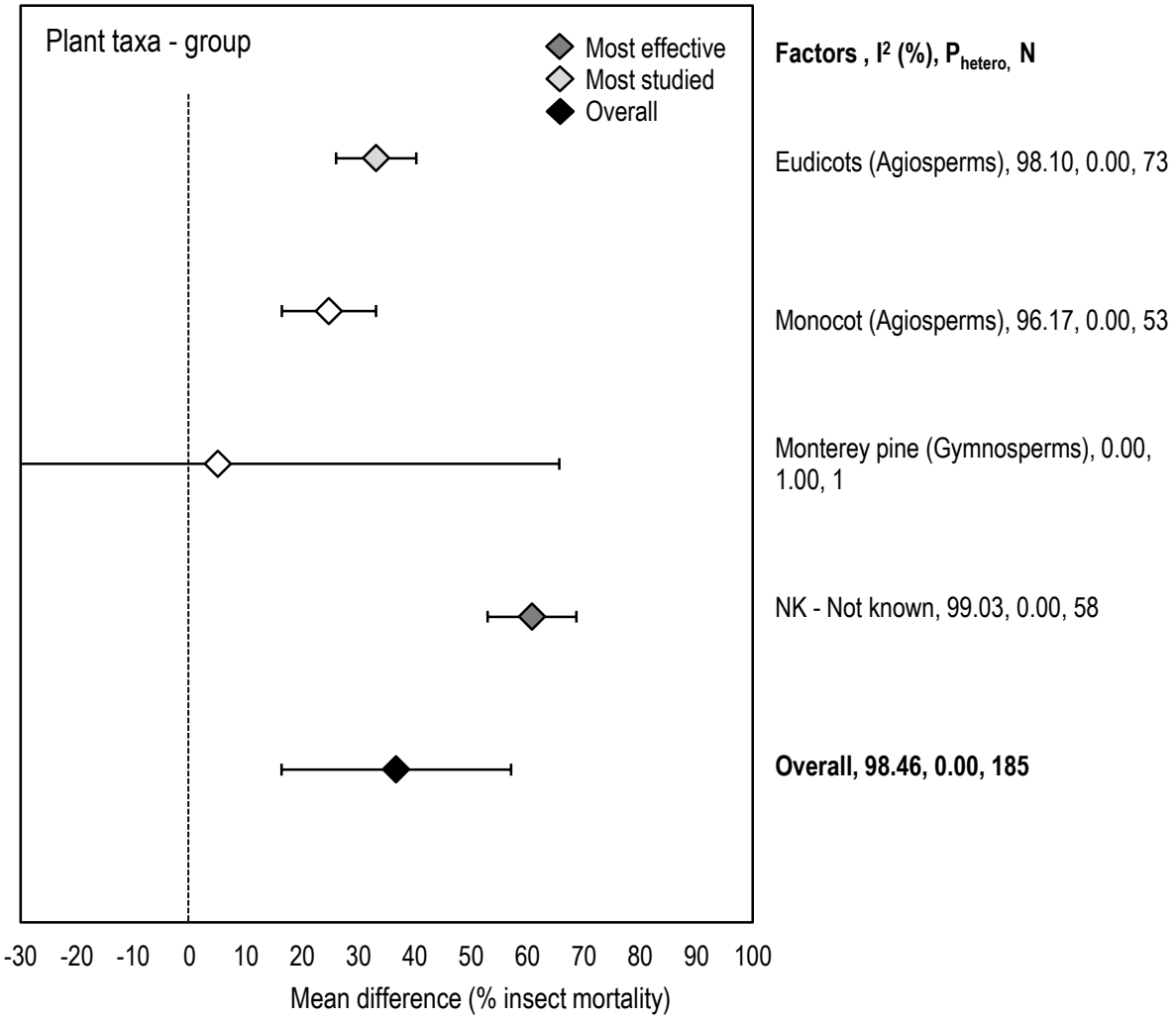


Figure 2.24. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant taxa - group.

Moderator: Comparison among studies on plant taxa - group used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (plant taxa - group) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

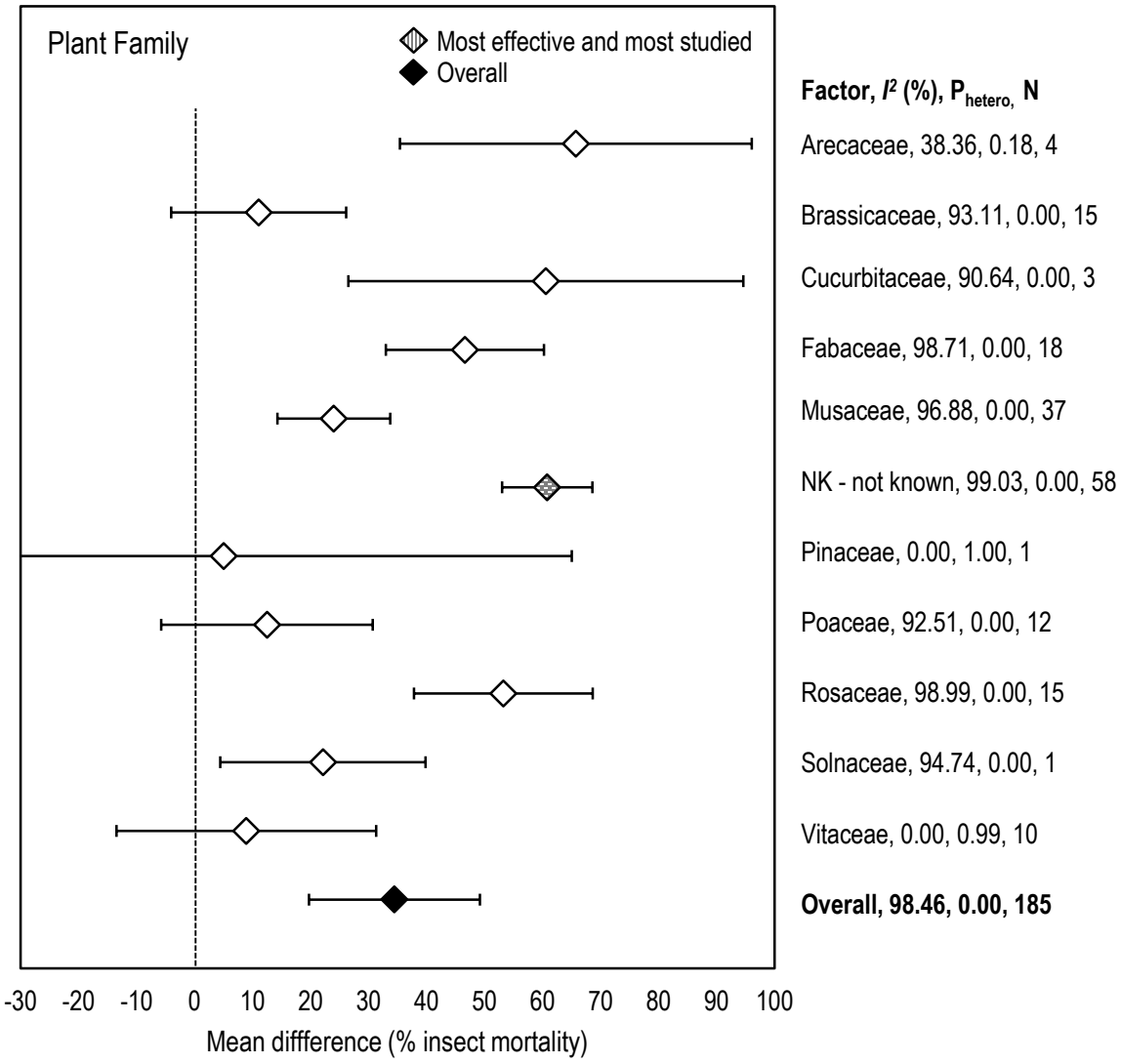


Figure 2.25. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant Family.

Moderator: Comparison among studies on plant Family used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (plant Family) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

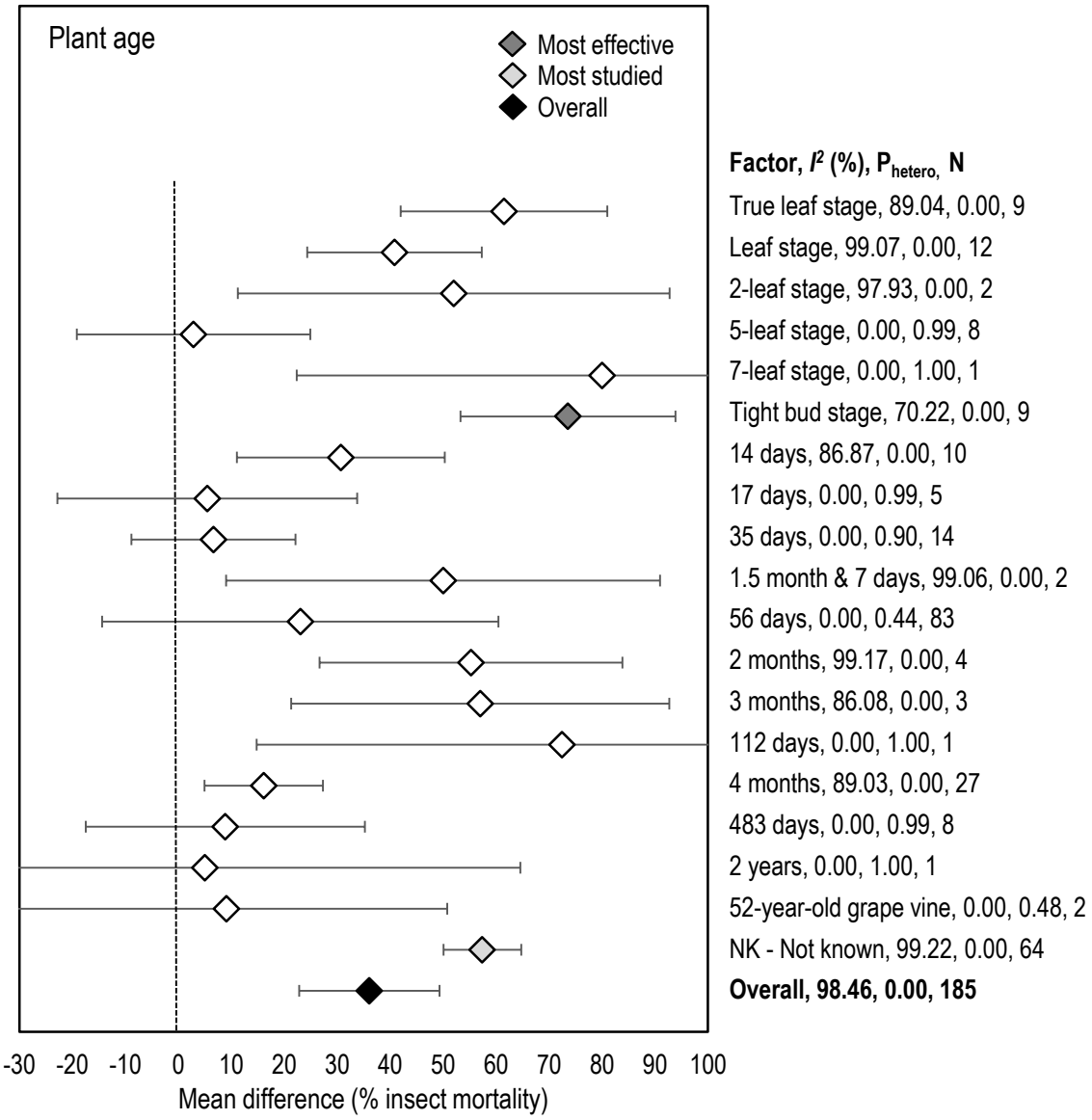


Figure 2.26. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant age.

Moderator: Comparison among studies on plant age used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (plant age) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

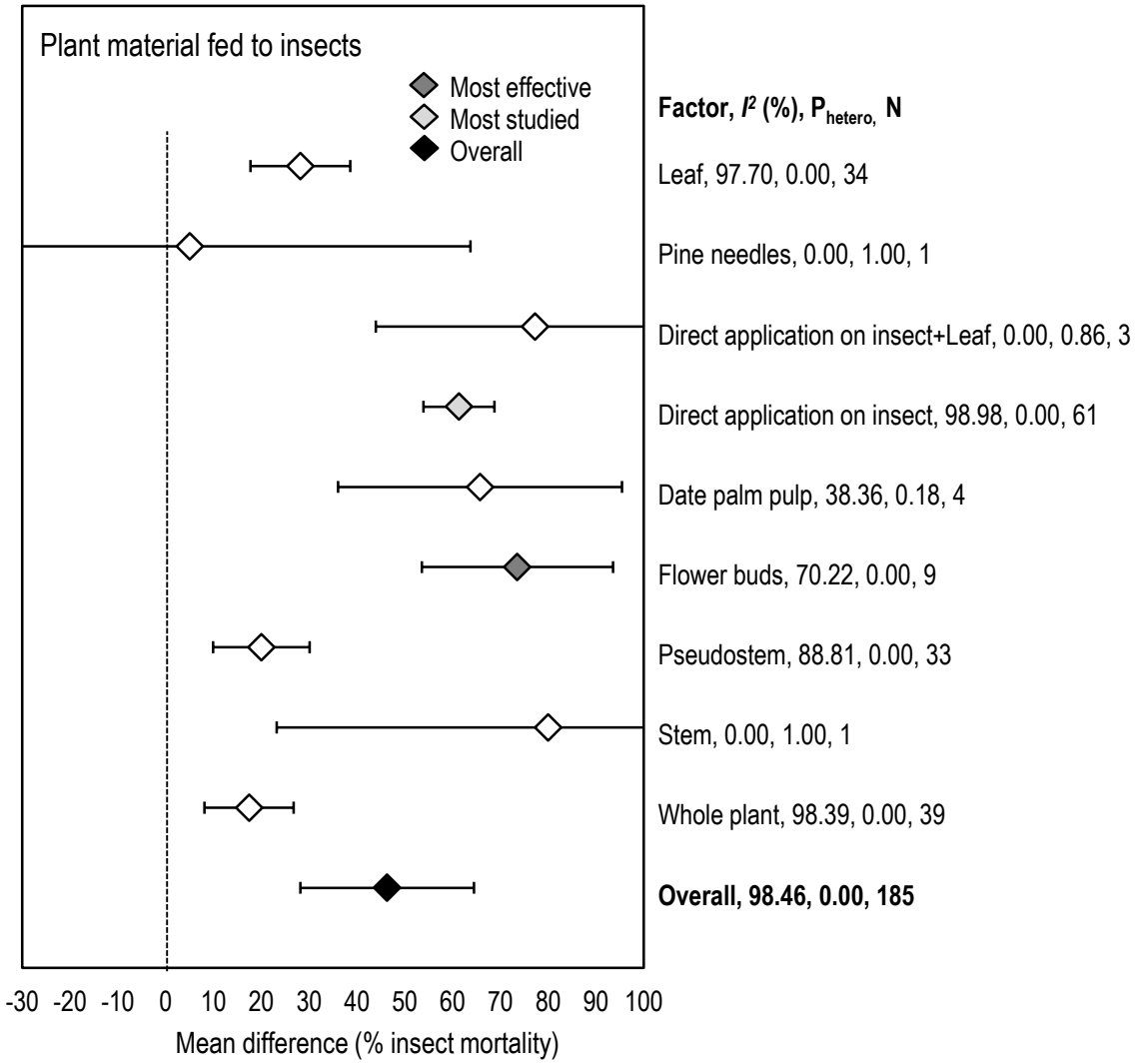


Figure 2.27. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant materials fed to insects.

Moderator: Comparison among studies on plant materials fed to insects in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (plant materials fed to insects) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

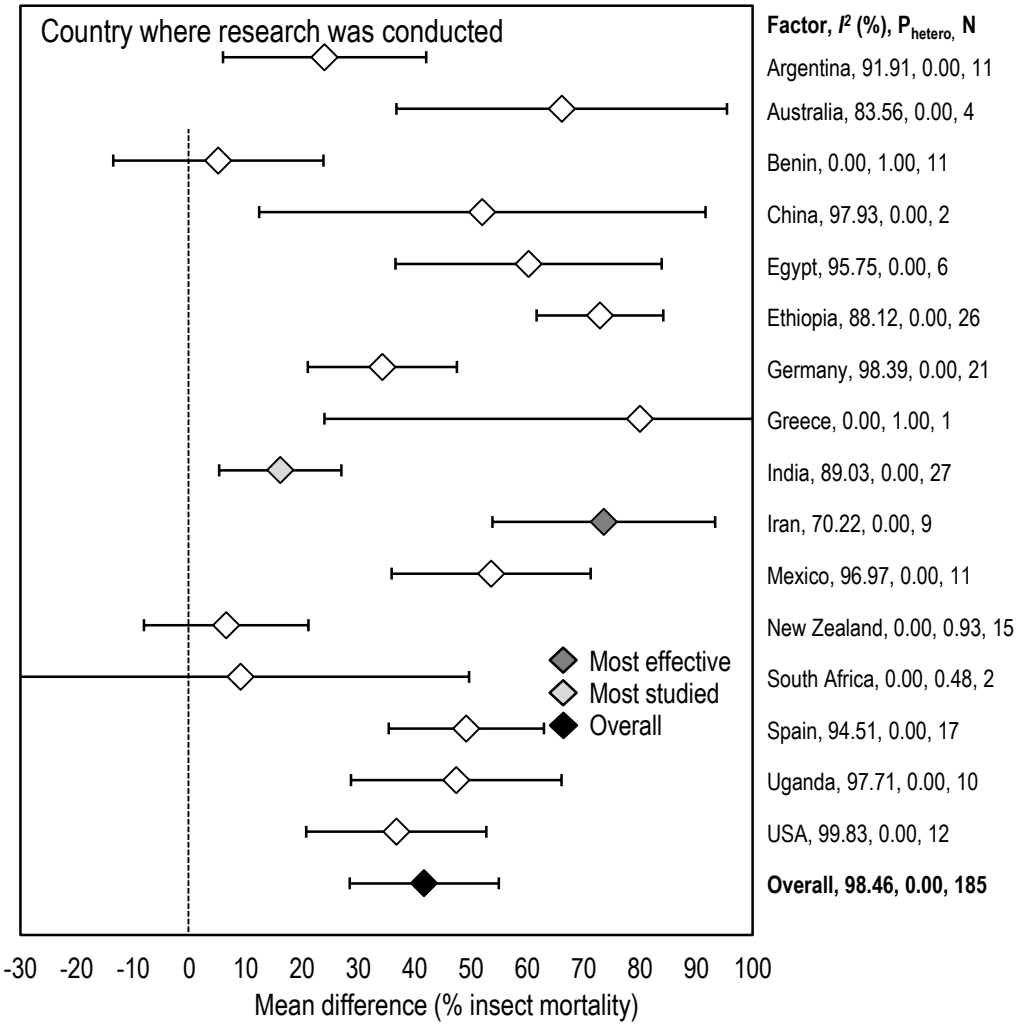


Figure 2.28. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on country where research was done.

Moderator: Comparison among studies on country where research was done in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (country where research was conducted) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

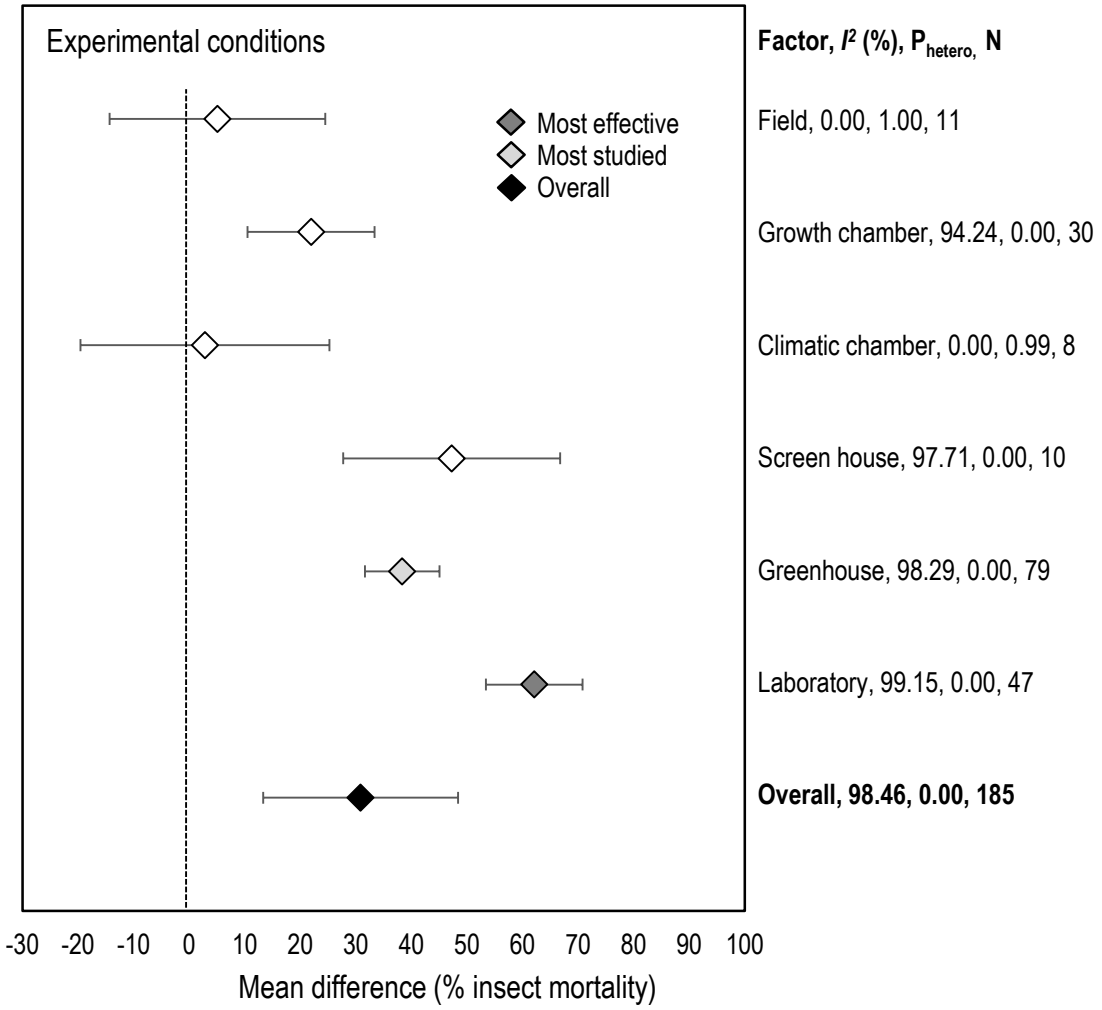
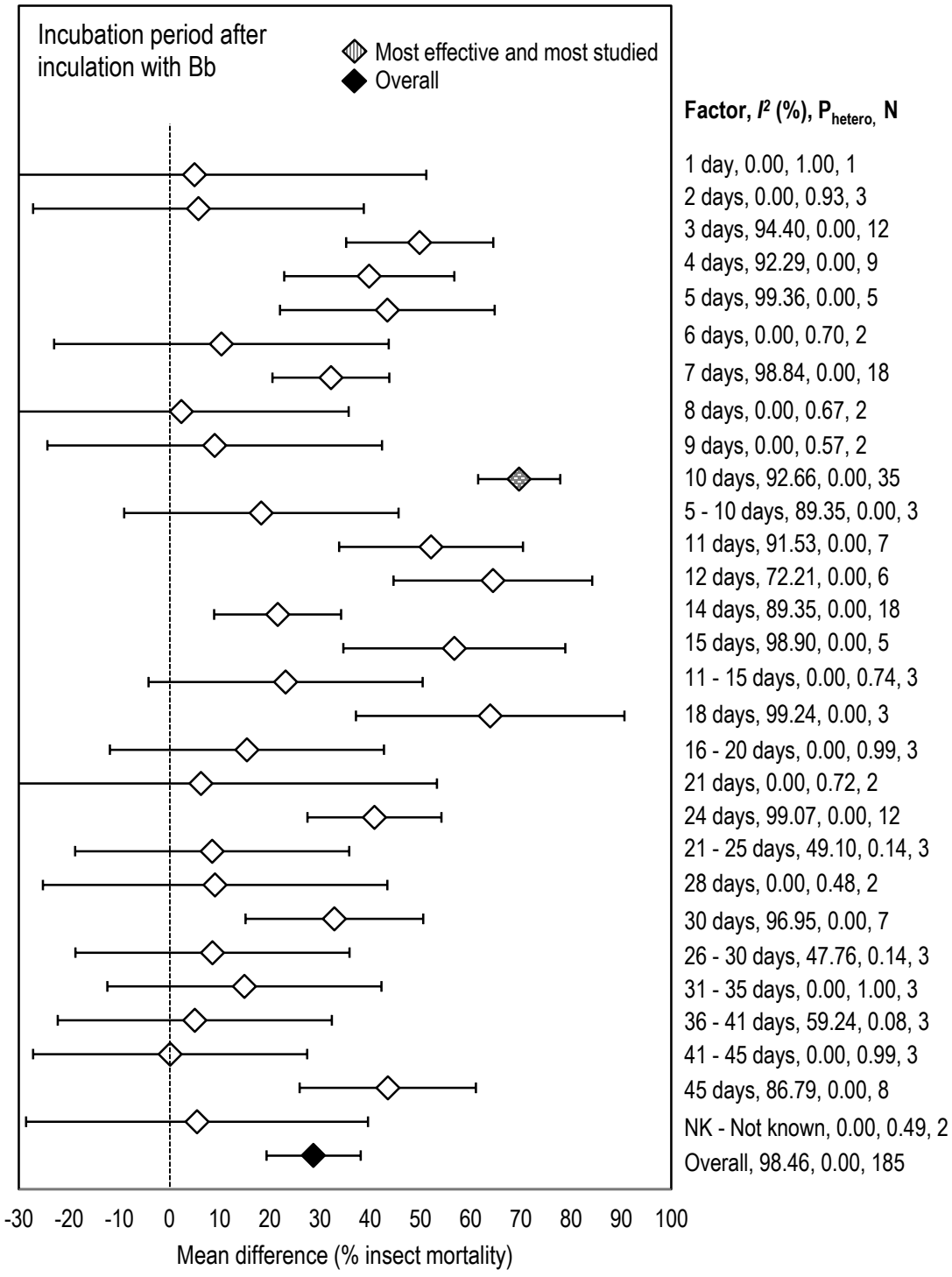


Figure 2.29. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on experimental conditions.

Moderator: Comparison among studies on specific experimental conditions used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (experimental conditions) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

Figure 2.30. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on incubation period after inoculation with Bb.

Moderator: Comparison among studies on incubation period after inoculation with Bb used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (incubation period after inoculation with Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.


Incubation period after inculation with Bb
Most effective and most studied
Overall
Factor, I^2 (%), $P_{hetero,}$ N
1 day, 0.00, 1.00, 1
2 days, 0.00, 0.93, 3
3 days, 94.40, 0.00, 12
4 days, 92.29, 0.00, 9
5 days, 99.36, 0.00, 5
6 days, 0.00, 0.70, 2
7 days, 98.84, 0.00, 18
8 days, 0.00, 0.67, 2
9 days, 0.00, 0.57, 2
10 days, 92.66, 0.00, 35
5 - 10 days, 89.35, 0.00, 3
11 days, 91.53, 0.00, 7
12 days, 72.21, 0.00, 6
14 days, 89.35, 0.00, 18
15 days, 98.90, 0.00, 5
11 - 15 days, 0.00, 0.74, 3
18 days, 99.24, 0.00, 3
16 - 20 days, 0.00, 0.99, 3
21 days, 0.00, 0.72, 2
24 days, 99.07, 0.00, 12
21 - 25 days, 49.10, 0.14, 3
28 days, 0.00, 0.48, 2
30 days, 96.95, 0.00, 7
26 - 30 days, 47.76, 0.14, 3
31 - 35 days, 0.00, 1.00, 3
36 - 41 days, 59.24, 0.08, 3
41 - 45 days, 0.00, 0.99, 3
45 days, 86.79, 0.00, 8
NK - Not known, 0.00, 0.49, 2
Overall, 98.46, 0.00, 185
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% insect mortality)

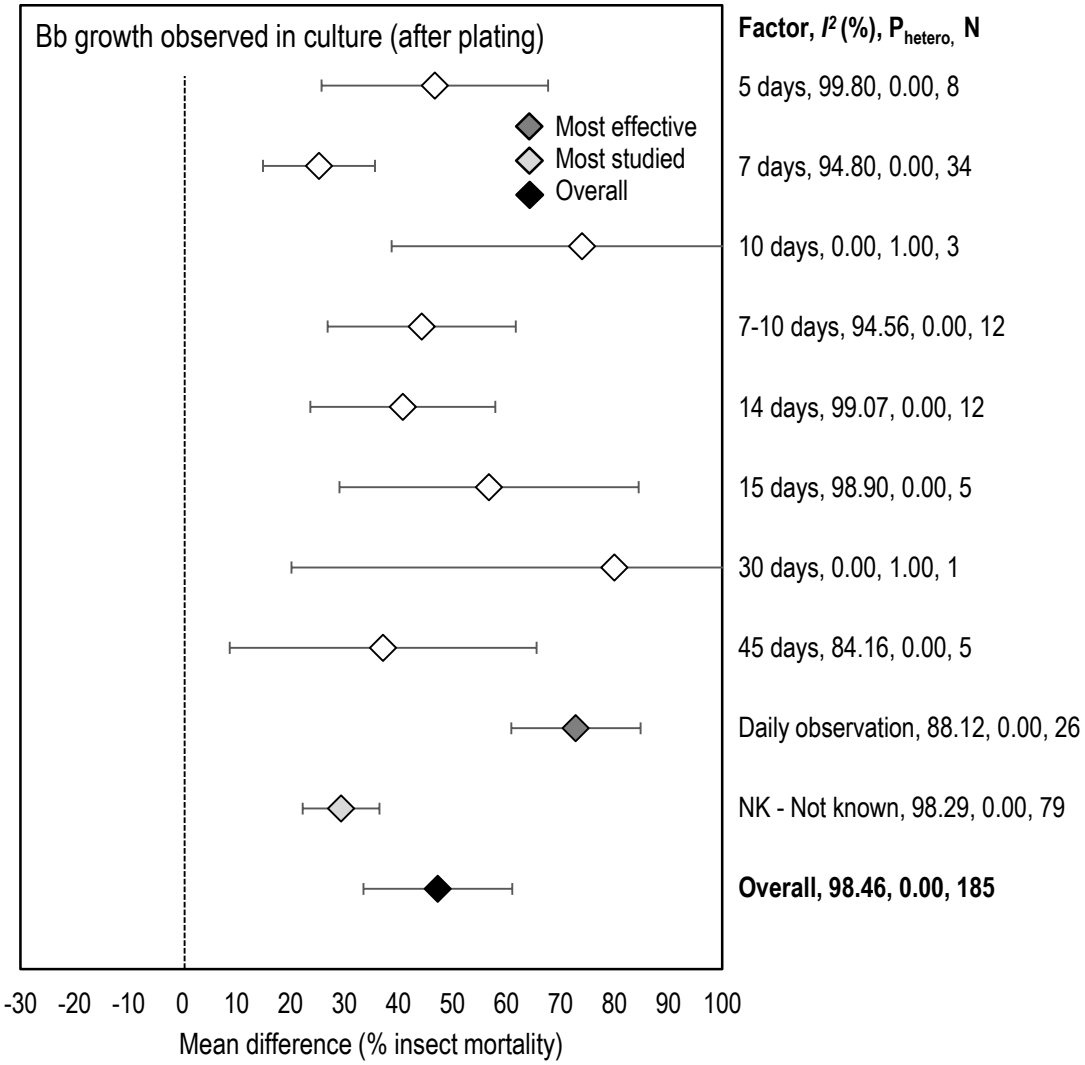


Figure 2.31. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on Bb growth observed in culture after plating specimen.

Moderator: Comparison among studies on Bb growth observed in culture after plating specimen used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (Bb growth observed in culture after plating specimen) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

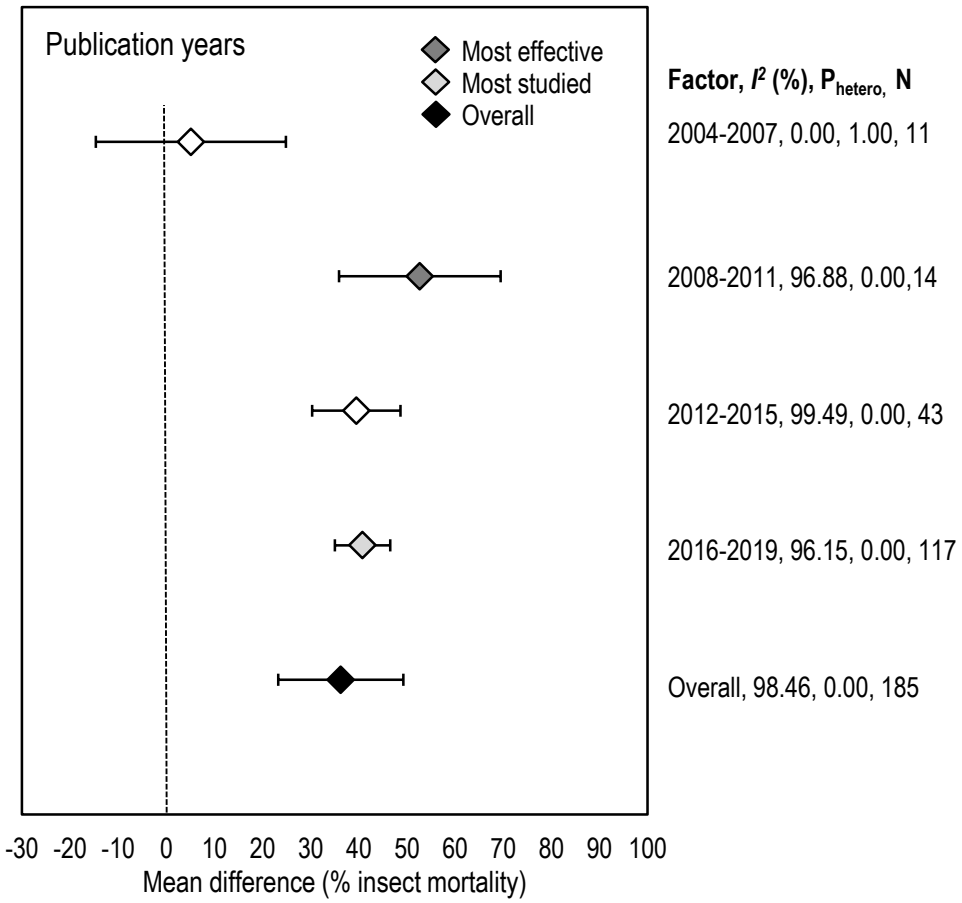


Figure 2.32. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on publication years.

Moderator: Comparison among studies on publication years used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (publication years) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

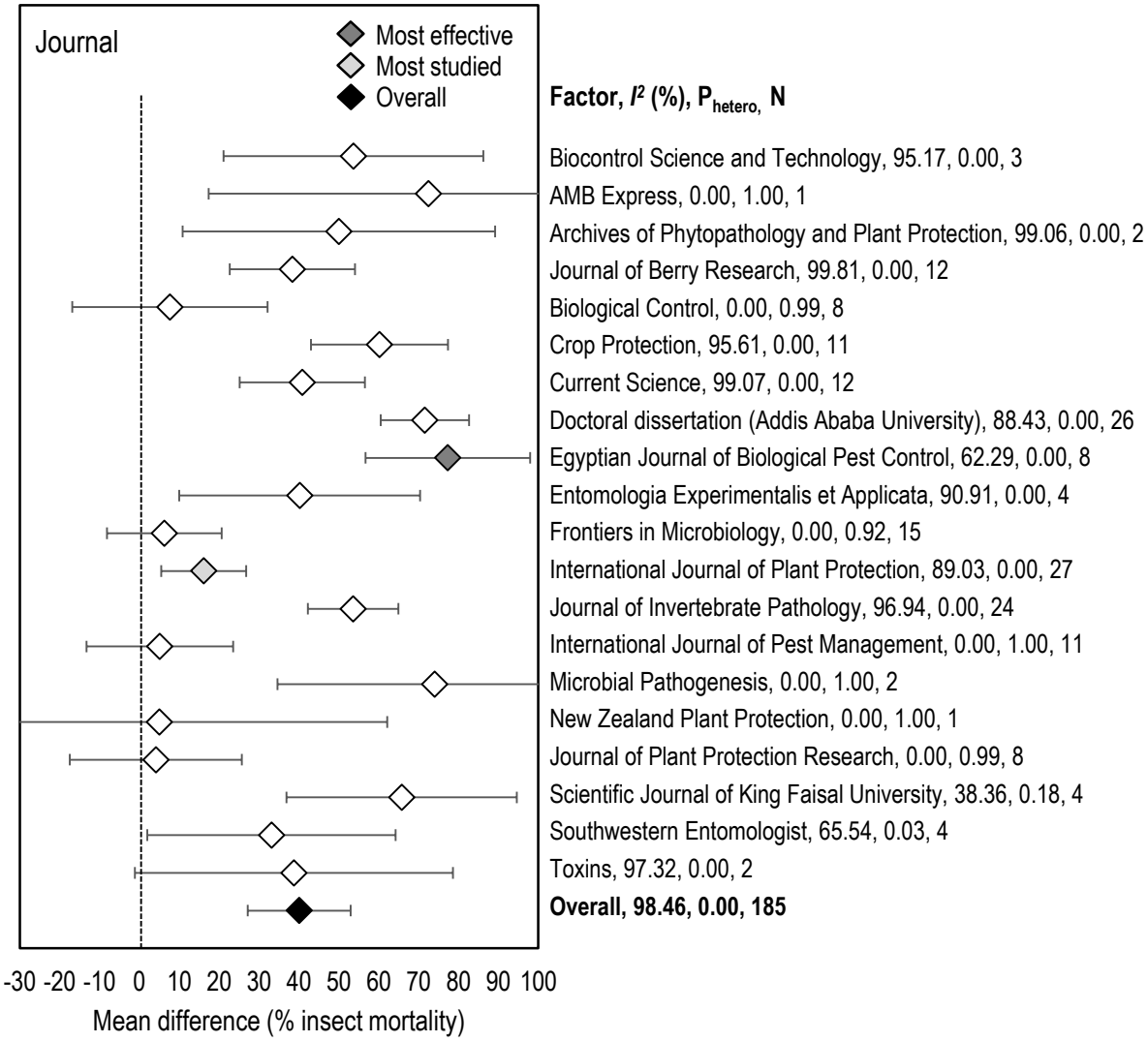


Figure 2.33. Mean difference and 95% confidence intervals of percent insect mortality caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on journal in which articles were published.

Moderator: Comparison among studies on journal in which articles were published used in insect mortality studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent insect mortality (endophytic Bb treatment vs. control). For each factor (journal in which articles were published) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on insect mortality.

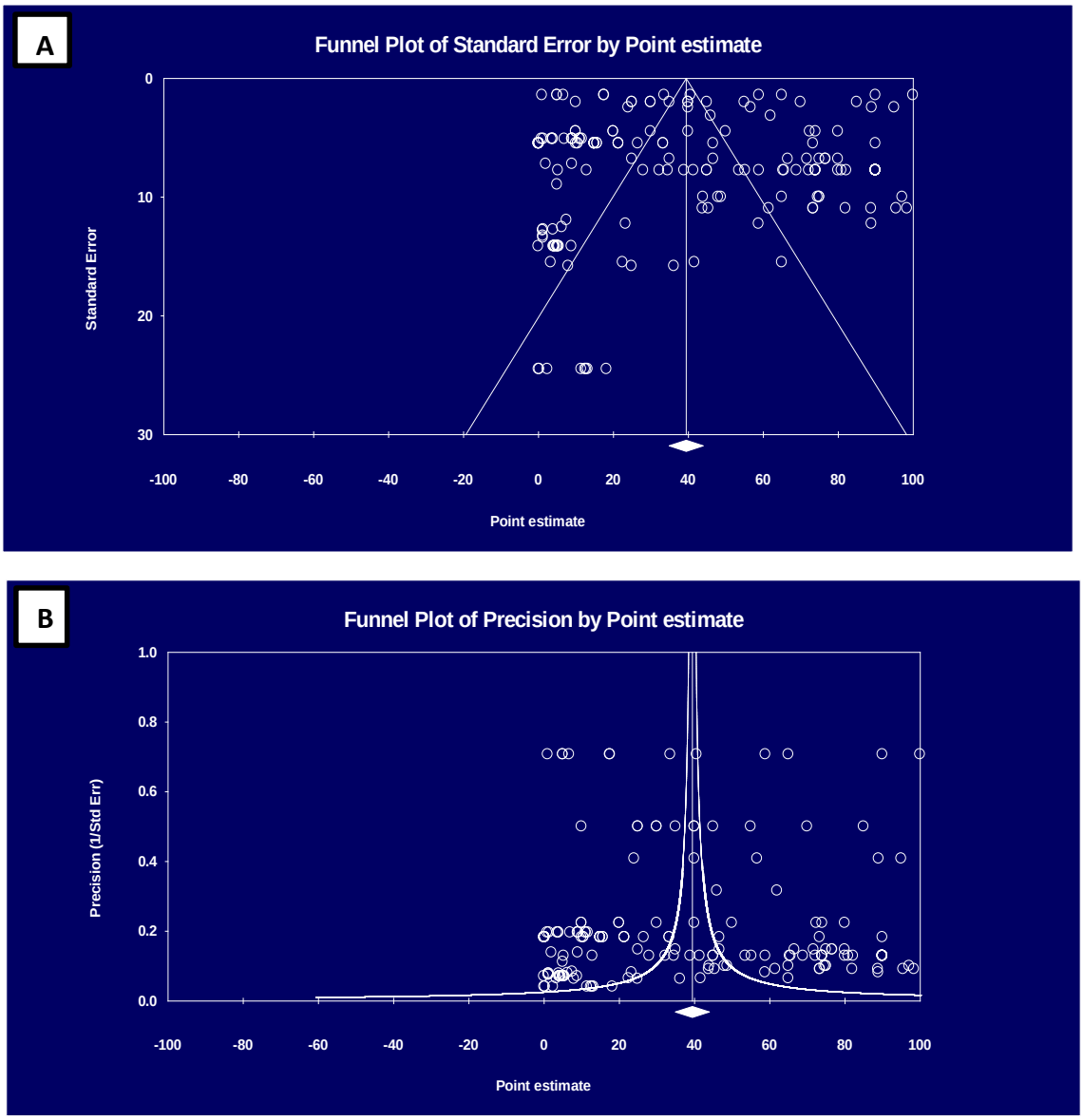


Figure 2.34. Publication bias (PB) represented in the form of funnel plots for A) Standard error by point estimate, and B) Precision by point estimate.

The horizontal axis shows point estimate or mean differences (%) at 95% confidence intervals for endophytic *B. bassiana* (Bb) effect on percent insect mortality analyzed with meta-analysis extracted from different research studies. The vertical axis represents (A) Standard error or (B) Precision (1/Standard error). The circles represent each individual study (total = 185).

CHAPTER 3

META-ANALYSIS ON THE EFFICACY OF ENDOPHYTIC INSECT PATHOGENIC FUNGUS *BEAUVERIA BASSIANA* AGAINST DIVERSE PLANT PATHOGEN SPECIES

Abstract

The endophytic, entomopathogenic fungus, *Beauveria bassiana* (Bb), can protect plants against insects. However, less is known about the ability of endophytic Bb to protect against plant pathogens. To fill this knowledge gap, a meta-analysis of published research was conducted using a random-effects model. A total of 1,287 published articles were extracted from Web of Science database and research studies were screened for reports on endophytic Bb and percent pathogen suppression, as measured by percent reduced pathogen growth, disease severity, or disease incidence. Moderator analysis was performed and 34 factors were identified that influenced the effect of endophytic Bb on plant pathogens. Moderators were categorized into four groups, based on whether the primary effect was on endophytic Bb, plant pathogen, plant host, or other experimental factors. Overall, 351 effect sizes were extracted. Across all studies, endophytic Bb suppressed plant pathogens by 31%. The percentage of total variation across studies due to heterogeneity, rather than chance, was 98.26%. Among moderators, the most effective isolate was Bb India with 75% pathogen suppression, and commercial isolates were more effective than native isolates, although more studies included native isolates. Analysis of sources of isolates indicated that those from Ethiopia were most effective (61% suppression), isolates from soil (67%) and plants (63%) were more effective than those from insects (30%), and foliar sprays (57%) on leaves (61%) were more effective than other types of application and treatment sites. A concentration of 1 x 10^8 spores/ml gave the highest rate of pathogen suppression (60%). A plant colonization range of 0-50% with Bb was more effective in suppressing pathogens than a range of 50-100%, (53% vs. 29%, respectively). For plant age at Bb treatment, 14 days resulted in the highest pathogen suppression (60%). The meta-analysis

indicated that endophytic Bb can significantly suppress *Sclerotinia sclerotiorum* (77%), *Zucchini yellow mosaic virus* (ZYMV) (64%), and various species of *Fusarium* (24% to 62%). Among taxonomic classifications of plant pathogens, endophytic Bb was most effective in suppressing viruses (64%) followed by oomycetes (Chromista) (32%), fungi (31%) and bacteria (15%). Among fungal and fungal-like pathogens, suppression was greater with Bb against ascomycetes (33%), followed by oomycetes (32%) and basidiomycetes (22%). Among plant species, Bb suppressed pathogens of squash most (64%), but was more effective on monocots (42%) than eudicots (27%), and annuals (59%) than perennials (19%). Regarding location of the research, studies in Jordan reported the most pathogen suppression (55%), and protected environments (greenhouse, 43%) resulted in more pathogen suppression with Bb than field studies (8.6%). These findings, across the last 15 years of publications, provide a quantitative review of the suppressive effect of endophytic Bb on different plant pathogen species, and enhance our current understanding of the potential of endophytic Bb to control plant diseases. This meta-analysis provides a valuable tool in further development of endophytic Bb as a sustainable plant disease management strategy.

Introduction

Endophytic protection against plant pathogens is an emerging topic, and much information has accumulated in the past two decades. However, there are gaps in our understanding of plant-endophyte protection that need to be addressed. To address these knowledge gaps, a meta-analysis was conducted to examine the effect of endophytic *Beauveria bassiana* (Bb) on plant pathogens.

Meta-analysis is a quantitative, statistical procedure that combines, synthesizes, and summarizes results of multiple independent studies, to understand the results of any investigation in the context of all other related studies (Gurevitch and Hedges, 1999; Rosenberg et al., 2004). A comprehensive conclusion from many experimental studies can then be drawn (Haidich, 2010; Nelson et al., 2015; Poeplau and Don, 2015). In a meta-analysis, a single measure is selected as the effect size, and it is estimated across all studies, which allows one to identify, combine, and compare the results of multiple studies directly (Rosenberg et al., 2004). The primary purpose of a meta-analysis is to determine whether the treatment of interest (effect size) is consistent or inconsistent across the body of data and to quantify the variance from study to study (Borenstein et al., 2009). Most recently, meta-analysis has gained significant attention in agricultural studies (Poeplau and Don, 2015), especially in plant pathology for the evaluation, synthesis, and validation of data for plant health management (Scherm et al., 2014).

Microbial plant pathogens affect growth and cause diseases in plants. Plant pathogens interfere with biological processes, including photosynthesis, plant development (fruiting, flower), and absorption and translocation of water and nutrients (Al-Sadi, 2017). Plant pathogens include fungi, bacteria, phytoplasmas, viruses, viroids, and nematodes. Based on symptoms that pathogens cause in plants, diseases can be categorized as leaf blights, leaf spots, root rots, fruit rots, fruit spots, dieback, and wilts. (Al-Sadi, 2017). Plant diseases cause enormous economic and food production losses in agriculture (Tewari and Sharma, 2019).

In the past decade, global losses caused by insect pests, diseases, and weeds have increased, despite development of modern technologies for control (Singh et al., 2014). Disease

is controlled primarily using conventional chemicals, but these have been associated with environmental pollution and health hazards (Wilson and Tisdell, 2001; Asogwa and Dongo, 2009; Aktar et al., 2009). Development of eco-friendly endophytic-based protection systems to control plant diseases could be an effective alternative to minimize chemical usage.

that the fact that the ubiquitous insect-pathogenic fungus *Beauveria bassiana* (Hypocreales; Ascomycota) can become endophyte in certain plants (Vega et al., 2008); and provide protection against insect pests (Arab and El-Deeb, 2012) is well established. In addition, there are also studies on plant pathogen control with this fungus (Ownley et al. 2010), including studies on *Rhizoctonia solani* (Bishop, 1999; Seth, 2001); *Pythium myriotylum* (Ownley et al. 2008); *Fusarium oxysporum* f. sp. *lycopersici* (Rathinam et al., 2014); *Plasmopara viticola* (Jaber et al. 2013) *Zucchini yellow mosaic virus* (ZYMV) (Jaber and Salem, 2014); and *Xanthomonas axonopodis* pv. *malvacearum* (Ownley et al., 2008). The potential of *B. bassiana* as a dual biocontrol, against insect pests and plant pathogens, offers exciting possibilities for development of sustainable plant protection strategies. Additionally, endophytic Bb-based protection is effective, safe, eco-friendly (Shanthakumar, 2013), and economical for plant health management.

Many studies on *B. bassiana* efficacy against plant pathogens have accumulated, but overall comparisons cannot be drawn from single studies. When we compare several independent studies, variations may exist among and between studies where the same organism was evaluated by different methods and treatments. The variability between independent studies testing endophytic Bb against plant pathogens could lead to different interpretations of results, leading to different conclusions, and recommendations for disease

management. In addition, there are various ways to measure pathogen suppression, such as pathogen growth, disease severity, or disease incidence. For meta-analysis, a single effect measure is chosen and estimated for all studies, which allows comparisons of studies and an overall estimation of the effect measure to draw conclusions. Combining the outcomes of multiple studies leads to increased statistical power and allows detection of even minor effects in a study (Rosenberg et al., 2004).

Despite several studies on the effect of endophytic Bb against plant pathogens in recent years, information is lacking on the biology of endophytic Bb isolates, its natural association with plants, its interactions with phytopathogens and mechanisms to suppress them, the diversity of endophytic Bb isolates, and details about Bb ecological niches. Furthermore, the durability of endophytic Bb-based plant protection against different plant pathogens and long-term effects are unknown. A quantitative review of endophytic Bb and its effect on different phytopathogen taxa is essential to understand the durability and sustainability of endophytic Bb in plant disease management. More answers are needed to increase effectiveness of Bb isolates, including improved delivery and application methods. Additionally, no meta-analysis studies have been reported on suppression of different plant pathogen taxa by endophytic Bb to date. Thus, there is a timely need to summarize the overall efficacy of endophytic Bb on plant pathogens and the factors that influence pathogen suppression from many independent studies to draw stronger conclusions for future success.

In the current study, the outcome of many research studies, conducted during the past two decades, on the effect of endophytic Bb on phytopathogens was analyzed. The specific objectives of this research included the following:

1. To conduct a quantitative meta-analysis and determine the estimate for the overall summary effect sizes of percent suppression of plant pathogens by endophytic Bb;
2. To synthesis and identify factors (external and internal) that influence effect sizes by moderator analysis;
3. To assess publication bias;
4. To determine whether the effects of endophytic Bb suppression of phytopathogens are more pronounced or consistent under experimental conditions over time; and
5. To determine the relation of endophytic colonization to plant pathogen suppression by Bb.

Combining the results of different, independent studies is an advanced approach that can contribute to recommendations for future studies. In this research, we focused on understanding how the endophytic colonization rate of *B. bassiana* is related to suppression of pathogen activity by the endophyte. These findings may assist researchers in exploring new sustainable biocontrol technologies or approaches by providing updated information on data published on endophytic Bb, and identifying the factors that influence the interaction of endophytic Bb and plant pathogens. This meta-analysis may be useful in the expansion and development of effective plant disease management strategies.

Materials and Methods

Literature search and data extraction (Fig. 3.1)

Data from the literature was extracted during 2018 (March) and 2019 (April and May) using several online databases. An extensive search was performed and the final search terms were filtered to "*Beauveria bassiana*" AND "endophyte" AND "plant pathogens" in the agriculture

database Thompson Reuters Web of Science search engine, which produced 171 results. From the Web of Science Core Collection, other search terms were used, including "*Beauveria bassiana*" AND "endophyte" AND "insects" and 84 results were collected; "*Beauveria bassiana*" AND "endophyte" AND "plant pathogens" returned 182 results; "*Beauveria bassiana*" AND "endophyte" AND "plant diseases" collected 14 results. In 2019, a search with Google Scholar and the search terms "*Beauveria bassiana*" AND "endophyte" AND "plant diseases" collected 670 results. CABI's International Comprehensive Database for Agriculture and Veterinary Medicine, with search terms "*Beauveria bassiana*" AND "endophyte" AND "plant pathogens" found 6 results. The AESC-Agriculture & Environmental Science Database with search terms "*Beauveria bassiana*" AND "endophyte" AND "insect pests" collected 80 results, and "*Beauveria bassiana*" AND "endophyte" AND "plant pathogens" also returned 80 results. In total, 1,287 published articles were extracted, analyzed, and evaluated for data.

The collected articles were screened, and a set of criteria were established for including the data in the meta-analysis. First, the studies needed to have percent disease severity or incidence, or percent plant pathogen growth, and second, the studies must have used endophytic isolates of Bb to manage or evaluate plant disease or plant pathogen growth. Thirty-two publications met the criteria established for the meta-analysis. Publications that did not meet the criteria, i.e. 1,255, were excluded. Articles were excluded as follows: did not include data repetition (n=373), data was insufficient - no plant disease or plant pathogen data (n=4), data was not relevant (n=450), review articles (n=110), book (n=6), book chapters (n=135), thesis or doctoral dissertation (n=81), abstracts (n=25), bulletins (3), and U.S. Patent or

application (n=68) (Fig. 3.1). From the final screened published articles, 351 effect sizes were extracted (351 studies).

Data extraction

From each final published article, the treatment means (Bb + phytopathogen), control means (only phytopathogen), and sample sizes of treatment and controls were obtained with respect to plant disease severity or disease incidence or pathogen growth. The sample size was taken as n=2, if LSD or standard errors were mentioned in the publication. If it was not mentioned in the article, the sample size was assumed to be n=1. If the data was presented in the form of graphs, the Web Plot Digitizer (Rohatgi, 2011) was used to extract mean values. A schematic representation of the meta-analysis procedure followed in this study is presented (Fig. 3.1).

Moderator variables

Moderator variables are used to determine if any significant heterogeneity exists in the meta-analysis (Auge et al. 2016). Pathogen suppression by endophytic Bb can be influenced by several external and internal factors. To determine their role on the effect of endophytic Bb on pathogen suppression, these factors were selected as moderators in this study (Table 3). Moderators were grouped into four categories, based on whether they were more likely to affect endophytic Bb, the plant pathogen, the plant host, or were other experimental factors that would influence results. Each moderator was divided further into several factors. Each moderator and factor were analyzed separately to understand the variation in effect sizes. The first group of moderators, which mostly affected endophytic Bb, included the following: Bb isolate used, type of Bb isolate (native versus commercial), general source of Bb isolate (soil, insect, plant), specific host source of Bb isolate, country of origin, application method used to

apply Bb, concentration and amount of Bb applied, site of Bb application on the plant, form of Bb application, endophytic colonization range (%) of host plant by Bb, and age of plant when Bb was applied. The second group of moderators mainly influenced the plant pathogen and included specific plant pathogen and plant disease; taxonomy: kingdom, phylum, class, order, and family; survival mechanism, and saprophytic habitat of plant pathogen; and pathogen survival spores and structures. The third group of moderators influenced the plant host, and included plant species, plant family, taxonomic group, life span, horticultural classification, and crop use. The fourth factor included other experimental factors and experimental conditions, incubation period after inoculation with plant pathogen, publication years, and journal in which articles were published. The moderator mean effect sizes or factors were significant if the P value was <0.05 and CI (95%) did not overlap with 0 or 100, then we assumed a common among study variance (Borenstein et al., 2009; personal communication, February 20, 2018).

Effect size and meta-analysis

The extracted data was analyzed and computed using the statistical package Comprehensive Meta-Analysis, Version 3 (CMA) software (Version 3, Biostat, Englewood, NJ, USA; 2014), and the methods of Borenstein et al. (2009). A random-effects model (REM) was selected instead of a fixed-model (FM), because meta-analysis studies in agriculture, the true effect is assumed to vary across the studies and heterogeneity exists in the true effect size.

Effect size calculations

Data extracted from each article was analyzed and the effect sizes were calculated as the raw mean difference (MD) by formula (Borenstein et al. 2009)

$$\boldsymbol{MD = Xt - Xc},$$

Where, Xc is the control mean (mean percent pathogen activity of control) and Xt is the treatment mean (mean percent pathogen activity of treated), and mean difference = percent pathogen suppression. Pathogen activity is defined as pathogen growth, disease severity, or disease incidence. Variance (V) of the data was calculated by extracting the samples sizes of the treatment and controls of each article. Non- parametric variance was used to weight variance within studies of each individual study (Auge et al. 2014)

$$\boldsymbol{V} = (\boldsymbol{nt} + \boldsymbol{nc}) / (\boldsymbol{nt} \times \boldsymbol{nc}),$$

Where V= Variance, nt = treatment sample size (number of plants or samples used in the treatment), nc= control sample size (number of plants or samples used in the control)

Statistical analysis and heterogeneity analysis

The data was considered non-parametric because several articles did not report standard errors or standard deviations. Q statistic (measure of weighted squared deviations) was used to determine the presence or absence of heterogeneity and I^2 to quantify heterogeneity. The I^2 value was calculated by ratio of true variation (heterogeneity) to total variance (heterogeneity) across the studies (Higgins and Thompson, 2002). The total heterogeneity (Qt) comprise expected variation or within-study heterogeneity (Qw, i.e., sample error mean) and excess variation or between study variation (Qb, i.e., true heterogeneity in effect sizes among studies.

$$\boldsymbol{I^2} = \left[\frac{\boldsymbol{Qt} - \boldsymbol{df}}{\boldsymbol{Qt}}\right] \times \mathbf{100}\%,$$

Where (df) is degrees of freedom and denotes the expected variation Qw and (Qt - df) the excess variation, true heterogeneity, while Qt denotes total heterogeneity. If df exceeds Qt, I^2 value is set to 0 (%).

The percentage of total variation across studies was measured by I^2 measures. Negative (-) I^2 values indicate that the true heterogeneity lies between 0 and 100%, while 0 (%) indicates no true heterogeneity, and positive (+) values indicate true heterogeneity, with larger variation across the studies and larger values reflecting a larger proportion of the observed variation due to true heterogeneity. An I^2>75% is considered substantial heterogeneity (Haidich, 2010).

When Q-test (P values) for heterogeneity were < 0.1, homogeneity assumptions were considered invalid (Iacovelli et al., 2014). Variation among-studies or moderators was assumed. The total summary effect, between studies variance (T_2), and actual variance (T) were also determined. The summary effect sizes were used to illustrate true heterogeneity.

To determine the amount of variation between the studies and estimate the overall result of the information from individual studies, forest plots were used (Lewis and Clarke, 2001). If P<0.05, studies were significant for heterogeneity, the null hypothesis was rejected, and there was no common effect shared between studies. This is a positive result, often with larger studies, which are more likely to have been published (Haidich 2010). If P>0.05 the studies were non-significant, and the null hypothesis was accepted, which states that all studies share a common effect. Non-significance is often associated with smaller studies and are considered negative results (Haidich 2010).

Publication bias

Publication Bias (PB) arises when studies with positive results are more likely to be published than those with negative results (Rothstein et al. 2006), and this leads to a conclusion biased towards significance or positivity (Duval and Tweedie, 2000). A potential major disadvantage of meta-analysis due to PB is due to bias between publication of significant and non-significant

studies. Publication is more likely for larger studies that are significant, novel, have stronger data, with low variability, and low standard errors. In contrast, smaller studies that are non-significant and have large standard errors and variability are less likely to be published (Madden and Paul, 2011; Borenstein et al., 2009; Higgins et al., 2009; Rothstein et al., 2005).

Publication bias always exists in a meta-analysis, and cannot be eliminated from the dataset, but the problem can be minimized by an analysis that determines the missing number of studies based on symmetric assumptions by visualization with a funnel plot. The missing values can be estimated by the "trim and fill" approach, which provides an overall effect on the inferences in the meta-analysis due to publication bias (Duval and Tweedie, 2000). In this study CMA analysis was used and several parameters were analyzed and check for PB in the dataset. Parameters included values and data from funnel plots, which illustrate whether studies dispersion is symmetrically. Classic Fail-safe N, and Orwin Fail-safe N determine possible missed studies, and their overall effect. Inclusion of missed studies would shift the effect size toward the null. Other tests included Kendall's tau b value (significance or non-significance). The Egger regression intercept used to assess bias by using precision. Duval and Tweedie's Trim and Fill method was used to determine the missing studies, where they fall, and to fill them to compute the combined effect. This demonstrates how the summary effect size would shift if apparent bias were removed (Shrestha et al., 2016).

Results

In this study, 351 effect sizes (studies) with 32 moderators were extracted and meta-analyzed with respect to percent suppression of plant pathogens with endophytic Bb compared to an untreated control. Overall of the summary effect sizes, the suppression of pathogens with

endophytic Bb averaged 31% with 95% confidence that the true value across studies is expected to fall between 27.35 (Lower Confidence Interval) to 33.67 (Upper Confidence Interval).

Heterogeneity measures

To assess heterogeneity between studies, several parameters, Q value, P value, T_2, T, and I^2 were determined. In the current study, the overall P value was ≤ 0.05, indicating significant difference between studies. This could be due to larger studies with higher power. Additionally, Q statistics were greater than expected variance (degrees of freedom), evidence that all the studies do not share a common effect and there is heterogeneity. With this evidence, the null hypothesis, which states that all studies share common effect sizes, was rejected and we concluded that all 351 studies do not share a common true effect size. Between studies variance (T_2), the actual true effect, was 868.38 indicating real differences among studies. Actual variance (T) was 29.46 and the summary effect was 30.51, therefore we expect that 95% of the true effects will fall in the range of 30.51 ± 29.46. I^2, which plays a key role in measuring heterogeneity, was 98.26% among our studies, which depicts that 98.26% of the observed variance is real in the studies, indicating a high percent of inconsistency among studies included in this meta-analysis.

Endophytic Bb factors

In this study, 37 moderators were quantitatively analyzed that impacted suppression of plant pathogens by endophytic Bb (Table 3). Moderators were categorized as fungus factors (11), plant pathogen factors (15), plant factors (7), and other experimental factors (5). Each group of

factors was divided into sublevels. Factors that impacted endophytic Bb the most were considered the most crucial for suppressing plant pathogens.

Bb isolate (Fig. 3.2)

Pooling all data for Bb isolates used in the studies, Bb India gave the highest, statistically significant pathogen suppression (75%). Other isolates with more than 50% pathogen suppression, significant heterogeneity (P<0.05), and generally smaller confidence intervals (CI) were Bb 6 (69%), Bb 2R-3-3-1 (63%), ATP05 (62%), Bb SDTu (62%), Bb 5-1 (61%), EABb04/01-Tip (60%), ATP01 (60%), J18 (59%), Bb-MTCC (58%), BG11 (54%), Bb 2R-4-5 (52%) and ATCC 74040 (52%).The most studied isolates were Bb 11-98 (113, 14% pathogen suppression), ATCC 74040 (56 studies), and GHA (28 studies, 5% suppression). Overall, endophytic Bb caused 37% suppression of plant pathogens (351 studies, I^2 = 98.26 %, P<0.05).

General Bb isolate type (Fig. 3.3)

When commercial and native isolates were compared (isolate type), commercial isolates were slightly more effective (37%) than native isolates (29%). However, there were more studies with native (267) than commercial isolates (84). Both factors had high heterogeneity among and between studies (P<0.05), with larger studies and narrow CI. Overall, endophytic Bb caused suppressed pathogens by 32% (351 studies, I^2 = 98.26%, P<0.05).

Country of origin of Bb (Fig. 3.4)

Endophytic Bb isolates from Ethiopia were highly effective in pathogen suppression with (61%). Similarly, isolates from Spain (60%), New Zealand (59%), Italy (50%), India (50%), Colombia (48%), South Korea (42%) reported high rates of suppression. All recorded high heterogeneity among and between (P<0.05), and most were larger studies with narrower CI. The most studied

included USA (170, 14% suppression), Italy (48), Ethiopia (24), and South Korea (27). Non-significant (P>0.05) include Egypt, Indonesia and Russia with no or low heterogeneity (I^2) and are consistent with smaller study number with large CI compared to others. Overall, for this comparison, endophytic Bb caused 38% pathogen suppression (351 studies, I^2 = 98.26%, P<0.05).

General source of Bb (Fig. 3.5)

General sources of endophytic Bb included soil, plants, and insects, as well as an unknown (NK) source factor. Isolates from soil were highly effective (67% pathogen suppression, 10 studies), while the most studied were isolated from insects (240), but these were less effective (30%). Isolates from plants had 63% suppression, but study number was small (7) and confidence intervals were larger. All factors high heterogeneity among and between studies (P<0.05). Overall, endophytic Bb caused 42% pathogen suppression (351 studies, I^2 = 98.26%, P<0.05).

Specific source of Bb (Fig. 3.6)

Factors for specific sources of endophytic Bb were divided into distinct organisms for the insect and plant categories. The soil factor was not subdivided and isolates had significant pathogen suppression (67%) as reported for general source of Bb. Although 80% suppression was found for maize isolates, this was non-significant and had a low study number. Isolates from maize cob had significant 59% suppression and those from lawn daisy had 54%. Specific insect sources with more than 40% pathogen suppression were sorghum chafer (62%), red spider mite (62%), poppy stem gall wasp larva (60%), maize stalk borer (60%), and black pine bark beetle (49%). Isolates with the most studies were from click beetle (113, 14% suppression), NK-Not known (94, 26% suppression), and boll weevil (84, 37% suppression). All of those mentioned (except

maize isolates) had high heterogeneity among and between studies (P<0.05). Overall, for this factor, endophytic Bb caused 47% pathogen suppression (351 studies, I^2 = 98.26%, P<0.05).

Method of application for Bb (Fig. 3.7)

High and significant pathogen suppression (57%) was found with foliar applications of endophytic Bb. Other methods with significant heterogeneity (P>0.05) and consistent results (relatively low CI) included studies that did not involve plants [non-volatile culture filtrate (54%), dual plate technique (47%), agar disks (26%)] and those that did [root dip (37%), seed + root dip + soil (26%), root drench (25%), seed + soil (22%), seed coating (22%)]. The most studied included root drench (107), seed coating (62), and foliar spray (53). Overall, endophytic Bb suppressed pathogens 31% (351 studies, I^2 = 98.26%, P<0.05).

Site of Bb application on plants (Fig. 3.8)

Endophytic Bb applied on leaves had significant and high pathogen suppression with 61%. In addition, suppression was significant when the application site was not reported or plants were not involved (NK- not known, 42%), followed by roots (26%), seed (22%), and soil (18%). These application sites had high and significant heterogeneity and smaller CI than others. The most studied included applications on roots (116), seed (67), NK- not known (63), and leaves (49). Overall, endophytic Bb caused 24% pathogen suppression for this factor (351 studies, I^2 = 98.26%, P<0.05).

Concentration of Bb applied (Fig. 3.9)

There were many factors for this moderator, but only a few resulted in significant and consistent pathogen suppression by endophytic Bb. The most effective concentration was 1 x 10^8 spores/ml with 60%, this concentration was also the most studied (56). Other

concentrations with significant (P<0.05) and large (I^2 >50%) heterogeneity, larger study numbers and narrower CI, included 1 x 10^6 spores/ml (57%, 16 studies), 1 x 10^7 spores/ml (43%, 47 studies), 1 x 10^6 CFU/ml (33%, 14 studies), NK-unknown concentration (33%, 45 studies), and 1 x 10^9 spores/ml (19%, 14 studies). In addition to a concentration of 1 x 10^8 spores/ml, other concentrations with Overall, for the factor concentration of endophytic Bb, pathogen suppression was 26% (351 studies, I^2 = 98.26%, P<0.05).

Amount of Bb applied (Fig. 3.10)

Like concentration, only a few 'amounts of Bb applied' that suppressed pathogens had high (I^2>50) and significant (P<0.05) heterogeneity, and narrow CI. The most effective amount of Bb was 5 µl conidia with 66% suppression, followed by 3 ml conidia (61%), 15 ml conidia (46%), 300 ml CFU (29%), NK-Not known (29%), 1 ml conidia (25%), and 10 ml conidia (23%). The amounts with the most studies were 1 ml conidia (58) and NK-not known (58). However, all recorded high heterogeneity among and between with and showed significant difference with narrow CI, larger studies with large sample sizes. Overall, endophytic Bb suppressed pathogens by 28% (351 studies, I^2 = 98.26%, P<0.05).

Form of Bb application (Fig. 3.11)

Endophytic Bb was highly effective and statistically significant when Bb was applied as a conidial suspension, with 38% pathogen suppression. This form also had the most studies (178). Except for NK- form not known (35%, 48 studies), other forms had non-significant heterogeneity, were inconsistent (wide CI) or had low rates of pathogen suppression. Overall, endophytic Bb caused 26% pathogen suppression (351 studies, I^2 = 98.26%, P<0.05).

Colonization range of Bb in plants (Fig. 3.12)

The most effective treatments for pathogen suppression reported lower rates of plant colonization by endophytic Bb (0-50% colonization, 53% suppression; 50-100% colonization, 29% suppression). Studies without a plant colonization range reported (NK-Not known) had 26% suppression. The number of studies for the three groups were 166 (NK), 141 (50-100% plant colonization), and 44 (0-50% colonization). All three groups had high and significant (P<0.05) heterogeneity among and between studies, narrow CI, and had large study numbers. Overall, endophytic Bb caused 35% pathogen suppression (351 studies, I^2 = 98.26%, P<0.05).

Plant age at which Bb was applied (Fig. 3.13)

Endophytic Bb amount applied to 14-day-old plants was highly effective and statistically significant in pathogen suppression (60%), followed by 7 days (53%), 5-6 true leaves (46%), NK (42%), and seed (22%), and seedling stage (14%). The most studied included seed (72), NK (63), 13 days (54, non-significant), 5-6 true leaves (36), and 14 days (34). There was high heterogeneity among and between studies with significant differences (P<0.05) and narrow CI for larger studies with large sample sizes. Overall, treatment with endophytic Bb resulted in 28% pathogen suppression (351 studies, I^2 = 98.26%, P<0.05).

Plant Pathogen (PP) factors

Site of plant pathogen application (Fig. 3.14)

Application of the plant pathogen on cotyledons led to the most effective pathogen suppression when treated with endophytic Bb (64%). Less pathogen suppression occurred with Bb when the pathogen was applied to roots (43%), leaves (24%), and soil (18%). In studies where the pathogen application site was unknown or not reported (NK), pathogen suppression

was 36%. Study number was greater with leaves (90), followed by soil (85), NK (77), roots (44), and cotyledons (32). These five factors had more studies, with high and significant heterogeneity among and between studies, and with narrow CI. Across all pathogen application sites, pathogens were suppressed by 34% with endophytic Bb (351 studies, I^2 = 98.26%, P<0.05).

Specific plant disease (Fig. 3.15)

Significant and effective (>30%) pathogen suppression was found for studies on white mold (77%), *Zucchini yellow mosaic virus* (ZYMV) (64%), cob rot (62%), late blight of tomato (59%), crown and root rot (48%), and NK-Not known (43%). The pathogen in studies categorized as NK was *Bacillus cereus*, a human bacterial pathogen associated with plants. Factors with the most studies were bacterial blight (63, non-significant for pathogen suppression), root-rot disease (44), crown and root rot (44), and *Zucchini yellow mosaic virus* (ZYMV) (32). Except for bacterial blight, all had high and significant heterogeneity among and between studies with narrow CI for those with larger studies. Non-significant effects (P>0.05) were found for charcoal rot, crown rot, vascular streak dieback, vascular wilt of cotton, pythium damping-off, downy mildew, powdery mildew, black mold of cereals, purple blotch, blackleg or Phoma stem canker, branch canker pathogen, bacterial blight, maize leaf blight, and potato late blight, with no or low heterogeneity, smaller study numbers and large CI compared to others. Overall, endophytic Bb caused pathogen suppression of 34% (351 studies, I^2 = 98.26%, P<0.05).

Specific plant pathogen (Fig. 3.16)

Endophytic Bb was highly effective and statistically significant in pathogen suppression of potyviruses with 64%. Other pathogens that were significantly suppressed by endophytic Bb,

with narrow CI, were *Sclerotinia sclerotiorum* (77%), *Fusarium verticillioides* (62%), *F. culmorum* (52%), *F. oxysporum* (45%), *F. moniliforme* (46%), *Bacillus cereus* (human pathogen associated with plants, 43%), *Phytophthora infestans* (40%), *Botrytis cinerea* (29%), *Fusarium lycopersici* (24%), and *Rhizoctonia solani* (23%). The most studied were *Xanthomonas axonopodis* pv. *malvacearum* (63), *Rhizoctonia solani* (53), *and Potyvirus* (32). Endophytic Bb was non-significant (P>0.05) in suppression of *F. pallidoroseum*, *F. vasinfectum* Race 4, *Macrophoma* sp., *Macrophoma theicola, Macrophomina phaseolina, Pythium myriotylum, Cladosporium herbarum, Alternaria porri, Plasmopara viticola, Podosphaera aphanis, Setosphaeria turcica, Leptosphaeria maculans, Oncobasidium theobromae,* and *X. axonopodis* pv. *malvacearum* with no or low heterogeneity. Overall, pathogens were suppressed by 36% with endophytic Bb (351 studies, I^2 = 98.26%, P<0.05).

Pathogen kingdom (Fig. 3.17)

Four plant pathogen kingdoms were found among the studies. Endophytic Bb was highly effective and statistically significant in suppression of viruses and viroids (64%), followed by oomycetous pathogens (Chromista, 32%), Fungi (31%), and Bacteria (15%). Most studies were fungal pathogens (211), followed by bacteria (73), pathogens in Chromista (35), and viruses and viroids (32). High and significant heterogeneity among and between studies was found for all four factors. Overall pathogen kingdoms, endophytic Bb suppressed pathogens by 35% (351 studies, I^2 = 98.26%, P<0.05).

Pathogen phylum (Fig. 3.18)

Endophytic Bb was highly effective and statistically significant in suppressing pathogens in the phyla: Viruses (64%), followed by Firmicutes (*Bacillus*) (43%), Ascomycota (33%), Pseudofungi

(oomycetes) (32%), Basidiomycota (22%), and Proteobacteria (Gram-negative bacteria) (9.8%). The number of studies was greatest with pathogens in Ascomycota (157), followed by Proteobacteria (63), Basidiomycota (54), Pseudofungi (35), Viruses (32) and Firmicutes (10). All groups except Proteobacteria had high and significant heterogeneity and had a significant effect on suppression of pathogens with endophytic Bb. Overall, Bb caused 33% pathogen suppression (351 studies, I^2 = 98.26%, P<0.05).

Pathogen taxonomic class (Fig. 3.19)

Endophytic Bb was highly effective and statistically significant in suppressing pathogens that were classified as viruses (64%), Bacilli (human pathogen, 43%) Sordariomycete (37%), Leotiomycete (34%), Oomycete (32%), Dothideomycete (24%), and Agaricomycete (22%). Treatments with endophytic Bb were not effective in suppressing pathogens in the class Gammaproteobacteria (9.8%). Most studies involved pathogens in the class Sordariomycete (86), followed by Agaricomycete (54), Gammaproteobacteria (63), Dothideomycete (38), Oomycete (35), Leotiomycete (33), Firmicutes (10), and Viruses (32). Across taxonomic classifications of pathogens, endophytic Bb treatment resulted in 33% pathogen suppression (351 studies, I^2 = 98.26%, P<0.05).

Pathogen taxonomic order (Fig. 3.20)

At the taxonomic level of order, endophytic Bb was highly effective and significant (P<0.05) in suppressing plant pathogens that were Viruses (64%); or in the fungal orders: Peronosporales (54%), Pleosporales (54%), Helotiales (37%), Hypocreales (37%), Ceratobasidiales (22%), and Botryosphaeriales (17%); or in the bacterial order Bacillales (43%). Most studies involved pathogens in the order Hypocreales (86). Endophytic Bb was not effective (P>0.05) in

suppression of pathogens in four taxonomic orders Capnodiales, Erysiphales, Pythiales, Xanthomonadales, or very wide CI and smaller study numbers compared to others. Overall, 31% pathogen suppression by endophytic Bb was found was found for this moderator (351 studies, I^2 = 98.26%, P<0.05).

Pathogen taxonomic family (Fig. 3.21)

For classification of pathogens by taxonomic family, endophytic Bb was highly effective and significant (P<0.05) in suppressing pathogens in Potyviridae (64%), Peronosporaceae (54%), Bacillaceae (43%), Sclerotiniaceae (37%), Nectriaceae (37%), Ceratobasidiaceae (22%), and Botryosphaeriaceae (17%). Most studies were in Nectriaceae (86). Endophytic Bb did not suppress pathogens in Erysiphaceae, Leptosphaeriaceae, Mycosphaerellaceae, Pythiaceae, Pleosporaceae, and Xanthomonadaceae with no or low heterogeneity, or smaller study numbers and large CI compared to others. Overall, endophytic Bb caused 31% pathogen suppression (351 studies, I^2 = 98.26%, P<0.05).

Pathogen survival mechanism (Fig. 3.22)

Endophytic Bb was highly effective and statistically significant for pathogen suppression based on the survival mechanism of the plant pathogen, including aphid-borne and seedborne virus (64%), followed by human pathogen (43%), seedborne + soilborne pathogen (37%), soilborne pathogen (24%), seedborne pathogen (17%). Most studies were in the factors soilborne (111), seedborne (96), and seedborne + soilborne (95). Endophytic Bb did not effectively suppress plant pathogens that inhabited stems or were vascular pathogens. Across all survival strategies, endophytic Bb suppressed pathogens by 38% (351 studies, I^2 = 98.26%, P<0.05).

Plant pathogen saprophytic habitat (Fig. 3.23)

Endophytic Bb was highly effective and significant (I^2>50 and P<0.05) in suppressing pathogens with different saprophytic habitats, including the following: aphid and seed (64%), crop debris (44%), root (38%), soil (23%), and dead plants or soil (18%). Bb also suppressed pathogens when the saprophytic habitat was not reported (NK, 45%). Most studies included pathogens that were associated with plant roots (92), followed by those found in soil (77). Overall, endophytic Bb suppressed pathogens by 32% (351 studies, I^2 = 98.26%, P<0.05).

Plant pathogen survival spores and structures (Fig. 3.24)

Endophytic Bb was highly effective (high and significant heterogeneity) in suppressing pathogens with various survival propagules and structures, including virus particles (64%), oospores (57%), chlamydospores and oospores (40%), chlamydospores (37%), sclerotia (29%), chlamydospores and sclerotia (29%), microsclerotia and sclerotia (17%), and bacterial cells (15%). Most studies were on pathogens that had chlamydospores (86) as survival spores, followed by bacterial cells (73), sclerotia (60), and virus particles (32). Overall, endophytic Bb suppressed pathogens by 30% (351 studies, I^2 = 98.26%, P<0.05).

Plant factors

Plant species (Fig. 3.25)

In all studies, NK factors include studies in which no host plant was identified. Pathogens of several different plant hosts were suppressed effectively (I^2>50 and P<0.05) by endophytic Bb, including squash (64%), cabbage (47%), sweet pepper (46%), maize (39%), tomato (22%), and cotton (12%). The factor for NK (42%) involved pathogen suppression that did not include a

plant host. Most studies were cotton pathogens (98). Overall plant species, endophytic Bb suppressed pathogens by 37% (351 studies, I^2 = 98.26%, P<0.05).

Plant family (Fig. 3.26)

Endophytic Bb was highly effective and significant (I^2>50 and P<0.05) in suppressing pathogens affecting several plant families, including Cucurbitaceae (64%), Brassicaceae (53%), Poaceae (44%), Solanaceae (32%), and Malvaceae (12%). The NK factor (42%) did not involve a plant host. Most studies were in Malvaceae (99), and Solanaceae (90). Across all plant host families, endophytic Bb suppressed pathogens by 37% (351 studies, I^2 = 98.26%, P<0.05).

Plant taxonomic group (Fig. 3.27A)

Endophytic Bb provided effective and significant (I^2>50 and P<0.05) suppression of plant pathogens that affected plant species classified as monocot (42%), and eudicots (27%), as well as those that did not report taxonomic group (NK-Not known, 42%). Most studies were associated with eudicots (277), followed by NK (61), and monocots (13). Overall, pathogen suppression with endophytic Bb was 36% (351 studies, I^2 = 98.26%, P<0.05).

Plant life span (Fig. 3.27B)

Endophytic Bb effectively suppressed (I^2>50 and P<0.05) plant pathogens classified in different life span groups. Effective pathogen suppression was highest in annual plants (59%), followed by biennials (47%). Among studies that did not include a plant hose (NK), pathogen suppression was 42%, and it was 19% in perennial hosts. Plants in most studies were classified as perennials, (222), which had the smallest CI. Overall, endophytic Bb suppressed pathogens by 44%, for the factor plant life span (351 studies, I^2 = 98.26%, P<0.05).

Horticultural classification (Fig. 3.28A)

Several horticultural classification groups were found among the studies. Endophytic Bb provided effective and significant (I^2>50 and P<0.05) suppression of pathogens on plants categorized as grain crops (44%), vegetable (41%), fruits (21%), and fiber crops (12%). The NK factor included pathogen suppression without a host plant (42%). Most studies were vegetable (130) and fiber crops (98). Overall, endophytic Bb suppressed pathogens by 30% (351 studies, I^2 = 98.26%, P<0.05).

Horticultural use (Fig. 3.28B)

Endophytic Bb was highly effective and significant (I^2>50 and P<0.05) in suppression of pathogens based on the horticultural use of plant hosts. Suppression was 36% for pathogens of edible crops and 12% for industrial crops. Most studies were on edible crops (191). The NK factor included studies on pathogen suppression without a host plant (42%). Overall, endophytic Bb suppressed pathogens by 29% (351 studies, I^2 98.26%, P<0.05).

Experimental factors

Country where research was conducted (Fig. 3.29)

Research studies on suppression of plant pathogens by endophytic Bb isolates conducted in Jordan reported the highest level of effective suppression (I^2>50 and P<0.05) with 55%. This was followed by studies in India (50%), Colombia (48%), South Korea (42%), Nigeria (31%), Iran (28%), Mexico (24%), and USA (13%). The most studied included USA (170) and Jordan (92). Overall endophytic Bb suppressed pathogens by 35% (351 studies, I^2 = 98.26%, P<0.05).

Experimental conditions (Fig. 3.30)

Endophytic Bb provided effective and significant (I^2>50 and P<0.05) suppression of pathogens when the studies were conducted in protected environments, specifically with plants in glasshouses (43%), greenhouses (42%), and growth chambers (14%), as well as 42% for *in vitro* studies. Most studies were evaluated in protected environments [greenhouse (146) and growth chamber (96)]. Overall, endophytic Bb suppressed pathogens by 26% (351 studies, I^2 = 98.26%, P<0.05).

Incubation period after treatment with Bb (Fig. 3.31)

Reported incubation times ranged from 1 to 187 days. Treatment with endophytic Bb was highly effective (I^2>50 and P<0.05) for pathogen suppression based on incubation times after inoculation with Bb between 1 and 35 days, specifically 8 days (50%), 14 days (46%), 12 days (44%), 5 days (46%), 7 days (45%), 35 days (45%), 1 day (43%), 4 days (36%), 10 days (32%), 21 days (31%), 3-day (29%), 28 days (28%), 19 days (25%). The incubation period with the most studies was 19-24 days (54); however, almost all studies were evaluated within 35 days after inoculation. Over all incubation times, endophytic Bb suppressed pathogens by 30% (351 studies, I^2 = 98.26%, P<0.05).

Publication years (Fig. 3.32)

Publication years for articles on suppression of plant pathogens with endophytic Bb ranged from 2004 to 2019, which were divided into four periods. The most effective (I^2>50 and P<0.05) Bb treatments were in articles published from 2012–2015, while most studies (155) were in articles published during 2016-2019. Pathogen suppression in articles published during these time periods was 56% in 2012-2015, 33% in 2016-2019, 23% in 2008-2011, and 13% in 2004-

2007 (13%). Overall, endophytic Bb suppressed pathogens by 31% (351 studies, I^2 = 98.26%, P<0.05).

Journal (Fig. 3.33)

Articles with studies on effective (I^2>50 and P<0.05) endophytic Bb treatments for suppression of pathogens were published in several journals. The most effective studies with more than 30% suppression were published in the following: *Archives of Phytopathology* and *Plant Protection* (75%), *Mycoscience* (66%), *Biocontrol Science and Technology* (64%), *Indian Journal of Agricultural Sciences* (58%), Doctoral dissertation, Lincoln University (56%), *BioControl* (55%), *Biological Control* (47%), *Journal of Asia-Pacific Entomology* (38%), and *African Journal of Microbiology Research* (31%). Most studies were found in a doctoral dissertation, University of Tennessee, Knoxville (89). Overall, endophytic Bb suppressed pathogens by 35% (351 studies, I^2 = 98.26%, P<0.05).

Publication bias (PB) (Fig. 3.34)

In the study, publication bias was determined using CMA analysis. Several parameters, including Classic Fail-safe N, Orwin Fail-safe N, Kendall's tau b, and the Egger regression intercept were analyzed to confirm whether publication bias existed in the dataset, and no evidence was found that PB influenced the overall conclusions. Additionally, funnel plots indicated that all studies were dispersed symmetrically (Fig. 3.34), and Duval and Tweedie's Trim and Fill test illustrated that there was little change in Trim and Fill values. Overall, we concluded that there was no publication bias noted in the study; however, indirect, or unknown bias may always exist.

Discussion

Endophyte-based protection against plant pathogens has received more attention in the past two decades and has potential as an alternative dual strategy for pest and disease control to minimize pesticide usage in agriculture (Lacava and Azevedo, 2014). In this meta-analysis, we found that endophytic Bb had a significant impact on suppression of the activity of a variety of plant pathogens. These results are in agreement with reports on control of the plant pathogens *Rhizoctonia solani* and *Pythium myriotylum* in tomato and cotton, with reduced disease severity and disease symptoms expressed in plants treated with endophytic Bb (Ownley et al., 2004; Ownley et al., 2008; and Griffin, 2007). Endophytic Bb was effective against the downy mildew pathogen *Plasmopara viticola* in grape, with reduced disease severity and symptom expression in Bb-treated plants (Jaber, 2015) and controlled *R. solani* in tomato. (Bishop, 1999; Seth, 2001). Batson et al. (2000) reported season-long protection against *R. solani* in cotton treated with endophytic Bb. Jaber and Salem (2014) conducted a detailed study on the efficacy of Bb against *Zucchini yellow mosaic virus* in squash and found that disease severity and symptom expression were suppressed in Bb-inoculated plants. Further, they reported a reduction in multiplication and accumulation of virus in endophyte inoculated plants compared to other treatments. Similarly, Bb treatment resulted in reduction of plant diseases caused by *Fusarium* spp. (Orole and Adejumo, 2009). However, more research is needed to better understand the mechanisms underlying Bb's role as an endophyte or epiphyte and how it suppresses plant disease. More information is needed on the nature of Bb's co-existence with other endophytes in plants; whether there is any synergism among endophytes in disease or pathogen suppression; and how well Bb competes with other plant microbes for survival.

Additional questions include the following. How does endophytic Bb overcome or evade the plant defense system to reduce plant disease? Does Bb activate any defense genes in plants against plant pathogens? Do plants give any signals or triggers for Bb to become an endophyte and change from its parasitic mode on insects or saprophytic mode in soil, to an endophytic mode in plants? Does Bb produce any secondary metabolites as it lives as an endophyte? If it does, are these compounds beneficial or harmful to plants? Are these metabolites toxic to plant pathogens, and what mechanism is involved? Does the endophytic mode of Bb overlap with the suppressive plant pathogen mode in plants? If Bb is artificially applied to plants, becomes endophytic, and suppresses plant disease, is suppression solely caused by Bb, or are other fungi or bacteria already present in the plant responsible? Can we separate the mechanisms and microorganisms involved, or is there one, specific method? In a natural ecosystem, it will be difficult to monitor or separate plants colonized by Bb from non-colonized plants when Bb is present as an epiphyte or endophyte. Moreover, the detection and distinction between these lifestyles becomes difficult. Do plants consider entomopathogens as bodyguards or just mutualists? The answers to several of these questions are still unknown or unclear and further research is needed.

The suppressive effect of Bb on plant pathogens could be attributed to several mechanisms, including: (1) direct effect through antibiosis, competition, and parasitism, or by (2) indirect effect through induced systemic resistance and increased plant growth rate. Isolates of Bb produce several secondary metabolites (Vurro and Gressel, 2007), including antibiotics (Ownley et al., 2010), organic acids (Cheong, 2015; Bidochka and Khachatourians, 1991) and volatile organic compounds (Patocka, 2016). They also can produce chitinases and proteases

(Bidochka and Khachatourians, 1991. The fungus produces several insect virulence compounds, including bassianin, beauvericin, beauverolides or beauveriolides, tenellin, bassianolide (Strasser et al., 2000), oosporein, bassiacridin, bassiatin (Cheong, 2015), allobeauvericins, and alkaloids (Patocka, 2016). Most of the secondary metabolites produced by Bb have antibacterial, antifungal, or cytotoxic activity (Grove and Pople, 1980; Genthner et al., 1994; Lopez et al., 2006).

Beneficial organisms compete against phytopathogens for food resources such as carbon and nitrogen, as well as space (phyllosphere, rhizosphere, or intercellularly within the plant) (Ownley et al., 2010). When Bb and *B. brongniartii* were co-inoculated *in vitro* they competed for carbon resources (Canfora et al., 2017). Similarly, Tarasco et al. (2011) conducted an *in vitro assay* with two entomopathogens: Bb and the entomopathogenic nematode *Steinernema ichnusae.* These organisms were inoculated simultaneously in *Galleria mellonella* larvae. Simultaneous infection of the two organisms in host haemocoel led to competition for nutritional space and antagonism for survival. Fungal toxins negatively impacted reproduction and development of the nematode.

Mycoparasitism is the parasitism of one fungus by another. Varying degrees of host specificity are displayed by mycoparasites. Parasitism by Bb was reported for suppression of pythium damping-off in tomato (*Pythium myriotylum)* in dual culture assays where hyphae of Bb were coiled around hyphae of *P. myriotylum* (Ownley et al., 2010; Griffin, 2007).

Endophytic Bb acts as an endophyte in several crop species (including corn (Russo et al., 2019; Wagner and Lewis, 2000), cassava (Greenfield et al., 2016), cotton (Lopez, et al., 2014), coffee (Vega et al., 2010), tomato (Resquin-Romero et al., 2016), banana (Akello et al., 2008),

white jute (Biswas et al., 2013), and squash (Jaber and Salem, 2014). Furthermore, as an endophyte Bb can protect plants from different plant diseases (Ownley et al. 2004) and insect pests (Reddy et al., 2009). Since then many studies have been conducted to prove the endophytic nature of Bb by introducing it to plants artificially and examining its efficacy against different phytopathogens (Ownley et al. 2004; Ownley et al. 2008). The persistence level of Bb in the host plant and surrounding environment is an attribute that can contribute to long-term disease suppression (Sandhu et al., 2012). As a benefit, the endophyte is more protected from stresses of the external environment. Isolates of Bb have already been commercially developed as mycoinsecticides (Sandhu et al., 2012; Vega et al., 2009).

Colonization of plants by Bb can enhance plant defense mechanisms through induced systemic resistance (ISR), a plant-mediated biocontrol mechanism induced with colonization by nonpathogenic fungi or bacteria. In induced systemic resistance, the bioagent and the plant pathogen are not in physical contact with each another. Due to reaction of induced resistance in plants, defense-related genes are activated (Ownley et al. 2010).

Rathinam et al. (2014) demonstrated activation of ISR when a combination of two endophytes, Bb and *Bacillus subtilis* (Bs) were evaluated against fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*) in tomato under both greenhouse and field conditions. Defense compounds in tomato plants, including lipoxygenase (LOX), peroxidase (PO) and polyphenol oxidase (PPO), were activated up to 120 days in treated plants. Similarly, results of activation of defense pathways such as salicylic acid (SA) and jasmonic acid-ethylene (JA-ET) dependent response in *Arabidopsis* was noted in Bb-colonized plants (Cordier et al.,1998; Raad, 2016). Similar physiological changes have been reported for other endophytes. For example, Molitor

et al. (2011) proved that plant roots colonized with the basidiomycete fungus *Piriformospora indica,* were more resistant to the biotrophic leaf pathogen *Blumeria graminis* compared to non-*P. indica* plants. Activation of defense compounds has been reported in Bb-treated plants in other studies (Sonderby et al., 2010; Raad, 2016).

However, there are constraints to the use of Bb. Chemical pesticides have rapid action, whereas the Bb effect takes 1-3 weeks post application. In addition, the persistence and efficacy of Bb in the host population could vary among different plant species, thus host-specific application methods may need to be optimized to obtain long-term results. Furthermore, host specificity of Bb isolates may result in differences in efficacy, even among different cultivars of the same host plant species when challenged with a pathogen (Clark, 2006). Reasons for host specificity on insects could be due to genotypic variability among Bb isolates, as well as differences in pathogenesis based on their geographical region (Sandhu et al., 2012). Generally, Bb occurs as a facultative endophyte and is transmitted from plant to plant horizontally, rather than vertically. Therefore, special care must be taken during inoculation and monitoring of endophytic Bb in experiments because the fungus spreads easily and can contaminate external surfaces.

The large amount of literature that has accumulated recently on efficacy of endophytic Bb against plant pathogens, and the lack of a clear picture of Bb efficacy, lends this literature to evaluation with meta-analysis. In addition, most of literature on biocontrol efficacy has been non-quantitative (Hawkins et al., 1993). Therefore, meta-analysis was conducted to explain and validate findings, and illustrate conclusions drawn from different scientific studies conducted for two decades on endophytic Bb and its effect on phytopathogens. Meta-analysis enables

identification of overall influential and effective treatments and identification of differences between individual studies (Chandrasekaran et al., 2014; Biondi-Zoccai et al., 2006.), as well as variations and comparisons with each treatment among and between individual studies (Gurevitch and Hedges, 1999). A meta-analysis can also provide researchers with the best sample size needed for cost effectiveness and significant differences in treatments, as well as appropriate parameters and variables for future research (Russo, 2007). It also leads to a better understanding of sources of heterogeneity and its variability within and across studies (Haidich, 2010).

Although meta-analysis studies have been conducted on the efficacy of bioagents against plant pathogens, (Chandrasekaran et., 2016; Ojiambo and Scherm, 2006), there are few meta-analysis studies on endophytic fungus (Mayerhofer et al., 2013), and none on endophytic Bb protection against phytopathogens. Hence, this research could be a stepping stone for future researchers in exploring new strategies to better understand efficacy of endophytic Bb against plant pathogens.

Endophytic Bb could be influenced by several multitrophic interactions in the process of colonizing plants (Faeth and Bultman, 2002). In this study, several influential variables that influenced endophytic Bb in suppression of pathogens or disease were identified. Across all studies, isolates of Bb that had been commercialized performed better than native isolates in suppressing plant pathogens. This agrees with Jaber and Salem (2014) who reported that commercial isolate reduced disease caused by ZYMV better than native isolates. A commercial Bb product (Naturalis) reduced disease caused by *Fusarium oxysporum*, *F. culmorum* and *F. moniliforme in vitro* (dual-culture) and *in planta with s*weet pepper (Jaber and Alananbeh,

2018). Dara et al. (2016) evaluated the Bb isolate GHA against vascular wilt of cotton (*Fusarium oxysporum* f. sp. *vasinfectum* Race 4) and reported high disease reduction with the commercial Bb isolate. Although commercial isolates were more effective, native isolates were used more in research studies. This could be due to the abundance of Bb isolates in the natural environment, which are easy to isolate. In addition, researchers are always searching for improved isolates. However, wide-scale commercial application of biocontrols has decreased in recent years, due to inconsistent performance in field locations.

Isolates of Bb from different countries varied in performance against plant pathogens, and those from soil were more effective. The reason for improved performance of soil isolates is not clear. Bb occurs naturally in the soil as an insect parasite and is known to be highly host specific (Kaushal et al., 2016). Even though Bb soil isolates performed better, most studies used isolates from insects. The Bb isolates in this study came from insects classified in Coleoptera, Lepidoptera, Hemiptera and Diptera. More specifically, effective Bb isolates came from sorghum chafer, red spider mite, poppy stem gall wasp larva, maize stalk borer, black pine bark beetle, boll weevil, click beetle, red palm weevil and Colorado potato beetle.

Differences in efficacy of bioagents depend on how and when the microbe is applied, incubation period after application, and plant species, and application method plays a significant impact on microbial colonization of plants (Ojiambo and Scherm, 2006). In our results, application method played a key role in Bb efficacy against plant pathogens. Site of application also affected Bb efficacy. Bb on leaves gave the highest and most significant reduction in plant disease. Combinations of plant parts treated, or treatment of single sites, such as seed + root + soil, seed, roots, soil, seed + soil, stem, seedlings, plant base, and root +

seedling also gave good results. Foliar treatment was also the most common way to apply Bb, which was likely due to feasibility and less labor. Spray applications of Bb reduced plant disease more than root drenching and seed coating. Bb, applied as a foliar spray of conidia, colonized leaf tissue effectively and reduced plant disease caused by downy mildew (*Plasmopara viticola) in grapevine* (Jaber, 2015). *Zucchini yellow mosaic virus* in squash (Jaber and Salem, 2014). Fusarium crown and root rot in sweet pepper, caused by *F. culmorum*, *F. moniliforme*, and *F. oxysporum* applied as root drenches was reduced (Jaber and Alananbeh, 2018), while crown and root rot caused by *F. culmorum* in wheat was reduced when Bb was applied as a seed coating (Jaber, 2018). Bb applied as a topical spray of leaves in tobacco, wheat, corn, and soybean, resulted in the highest Bb colonization rate, compared to seed inoculation and root immersion (Russo et al.,2015). Resquin-Romero et al. (2016) suggested that when Bb was applied as a foliar spray, systemic and non-systemic colonization of the plant resulted, thereby improving treatment efficacy against plant disease. Endophyte colonization rate differs with crop species and application method (Russo et al., 2015).

The most effective and most studied concentration of Bb was 1 x 10^8 spores/ml. This concentration could become a standard for use in evaluation of Bb isolates against plant pathogens and disease. However, the most effective concentration of Bb may vary based on plant species and pathogen affecting the plant. In addition to concentration of Bb, other factors that influenced variability in pathogen suppression among studies were amount of Bb used and age of the plant when Bb was applied. A more uniform approach should be considered for evaluations.

Endophytic Bb was highly effective in suppressing different fungal diseases, such as white mold, cob rot, root rot, late blight of tomato, downy mildew, crown and root rot, tomato damping-off, fusarium wilt, gray mold, and viral disease (ZYMV). Bb was not effective against bacterial plant diseases and pathogens, but it did suppress *Bacillus cereus*, which is associated with plants and causes disease in humans. The pathogens that cause the fungal diseases were *S. sclerotiorum, F. verticillioides, Macrophoma theicola, Macrophoma* sp., *Plasmopara viticola*, *F. culmorum, F. oxysporum, F. moniliforme, Phytophthora infestans, Botrytis cinerea*, *F. lycopersici*, and *R. solani*.

Based on the taxonomic classification of plant pathogens, endophytic Bb was most effective against viral disease, followed by oomycetes (Chromista), and fungi, and least effective against bacteria. Endophytic Bb was highly effective against fungi in four taxonomic classes: Sordariomycete, Leotiomycete, Dothideomycete, and Agaricomycete; and six taxonomic orders: Peronosporales, Pleosporales, Helotiales, Hypocreales, Ceratobasidiales, and Botryosphaeriales. Endophytic Bb significantly reduced disease caused by an aphid- and seedborne virus, followed by stem inhabiting fungus, human pathogen, and seedborne and soilborne pathogens. While Bb was most effective against viral disease in plants, most research was on suppression of fungal and bacterial diseases with Bb. The reduction in viral disease could be caused by several mechanisms. Bb has inter- and intracellular movement both to the top and roots of the plant (Landa et al.,2013; Quesada-Moraga, et al.,2006; Wagner & Lewis,2000), which might interfere with or inhibit movement of virus particles and delay viral disease symptoms. In addition, Bb may have multiple mechanisms, which vary with phytopathogen species (Ownley et al., 2010; Vega et al., 2009).

Deng et al. (2019) evaluated Bb by inoculation into mosquito (*Aedes albopictus*) females that were blood-fed with or without Zika virus (ZIKV). ZIKV in Bb-treated mosquitoes was reduced by 3.6-fold in midguts, 12.3-fold in heads, and 7.8-fold in salivary glands compared to nontreated. Further, Bb infection reduced ZIKV dissemination rate (27%), transmission rate (38%), and population transmission (35%) compared to infected mosquitoes that were not treated with Bb. This suggests that Bb has potential in reducing the vector capacity of *A. albopictus,* which carries ZIKV, as well as other insect-vectored viruses.

Endophytic Bb was highly effective in suppressing fungal pathogens described as root inhabitants, soil inhabitants, and colonizers of crop debris. Bb was also effective on pathogens with a variety of spore types and resting structures, including oospores, chlamydospores, sclerotia, and microsclerotia. But information is lacking on whether Bb has any direct effect on these pathogen spores or their disease-causing mechanisms.

This meta-analysis included studies on endophytic Bb against pathogens of several plants. Bb had the most significant effects on squash, cabbage, sweet pepper, maize, tomato, and cotton. Based on plant families, endophytic Bb was most effective in protection for Cucurbitaceae, followed by Brassicaceae, Poaceae, Solanaceae, and Malvaceae, while the most studied included Malvaceae and Solanaceae. There was high heterogeneity among these studies. Our results confirm that there are differences in efficacy of plant protection with endophytic Bb among crops species. It has been reported that endophytic fungi that have a wide host range may have variable colonization patterns depending on host plant tissues (Sridhar, 2012). Cesaro et al. (2008) suggested that each plant species has specific associations with endophytic fungi. Endophytic abilities may affect how fungi respond to plants, plant host

specificity (Mayerhofer et al., 2013), habitat preference (Christensen, 1981; 1989, Christensen et al., 2008; Sieber and Grünig, 2006) and plant host preferentiality (Klironomos, 2003).

Endophytic Bb gave more protection against plant disease in monocots than in eudicots. Plant species may respond differently (positive, negative, neutral) to endophytic colonization or inoculation (Mayerhofer et al., 2013). In gymnosperms, spruce (*Picea*) showed a negative response to endophytic colonization (Sieber and Grünig 2006; Grünig et al., 2008b; Mandyam et al., 2010), while monocots, especially sedges and grasses, generally had positive responses to endophytic colonization (Sieber and Grünig 2006; Grünig et al., 2008b; Mandyam et al., 2010). In this meta-analysis, the studies on plant disease were conducted on angiosperms; there were no studies on gymnosperms in the extracted data.

Our data output confirmed that several plant factors were linked to the effect of Bb on plant pathogens, but there was also high variation among studies. Information is lacking on specific mechanisms involved in reduction of plant disease that occurs in the interaction between endophytic Bb, the plant host, and plant pathogens. A common theme in endophytic Bb and plant pathogens studies was that endophytic Bb successfully colonized the plant, followed by reduced disease symptom expression in plants, slower disease development, and increased plant growth.

Plant host-endophyte interactions and variation in endophyte colonization patterns can be influenced by environmental conditions (Sieber and Grünig, 2006). The endophyte-host plant relation may change over time from beneficial to neutral to mutualistic or pathogenic (Sieber and Grünig, 2006), based on changes in the plant host or prevailing environmental conditions. If abiotic and biotic conditions are unfavorable, the endophyte-plant relationship

may switch from mutualistic to pathogenic (Schulz et al., 1999; Sieber and Grünig, 2006; Grünig et al., 2008b; Mandyam et al., 2010; Kloepper et al., 2013; Hardoim et al., 2015). The mutualistic plant host-endophyte interaction could develop into balanced antagonism, while an imbalanced interaction could lead to plant disease development (Schulz et al., 1999; Mayerhofer et al., 2013). In some cases, habitat heterogeneities also cause endophyte ecological isolation, which could lead to development of cryptic species (Sieber and Grünig, 2006). Further research is essential to expand and explore knowledge on the ecology of plant endophyte research (Hardoim et al., 2015).

Endophytic Bb was highly effective in plant disease reduction when experiments were conducted in glasshouses, greenhouses, and growth chambers. Effective pathogen suppression was also found in *in vitro* studies, but experiments in outdoor environments and fields were less successful. It is well established that biocontrol microbes perform better under controlled-environment conditions than field conditions (Wei et al., 2011), and this is attributed to the feasibility of manipulating the environment (relative humidity, temperature, season of application) to favor microorganisms in protected environments (Paulitz and Belanger, 2001; Wei et al., 2011). Poor field performance could also be attributed to differences in delivery or application method, and competition with other microbes in the rhizosphere or phyllosphere in the field (Lugtenberg et al., 2001).

There were large differences in incubation periods (period of treatment with Bb) among the different studies in this analysis. This is partly related to differences in crop life cycle. Highly effective endophytic Bb treatment was noted when the incubation period was 8 days. Generally, effective periods ranged from less than 8 days and up to 35 days.

The most effective endophytic Bb treatment for pathogen suppression was when percentage colonization by Bb ranged between 0-50%, compared to 50-100%. Colonization range differed between and among studies. From our results, it appears that moderate colonization by Bb is ideal for pathogen suppression compared to higher percentages, but this may depend on plant species, plant host specificity, and the plant pathogen involved in the interaction. Colonization efficacy by the fungus may depend on the source of isolation, host species (Mayerhofer et al., 2013) or geographical region. Hart and Reader (2002) suggested that fungi have diverse colonization strategies and the pattern may differ taxonomically based on plant family. In some cases, fungi tend to preferentially colonize specific crop species. For example, Cesaro et al. (2008) demonstrated that *Glomus intraradices* preferentially colonized roots of *Solanum tuberosum*. The extent of endophytic colonization also depends on the inoculation method and the fungal isolate applied. Generally, the method used to confirm whether a microbe is an endophyte or not, is based on isolation of the microbe from plant tissues distant from the place of plant inoculation (El-Deeb et al., 2012), which shows that the endophytic microbe can move from one part of the plant to another (Posada et al., 2007).

It has been suggested that Bb can activate defense genes in plants against plant pathogens to “bodyguard” plants. We noted that both percent colonization and percent pathogen suppression differed among crop species in these studies. More research is needed to confirm whether Bb colonization efficacy is linked to reduction of pathogen development in the plant, which in turn leads to a reduction in plant disease.

Highly effective endophytic Bb treatments were published in articles from 2012-2015, followed by 2016-2019, 2008-2011, and 2004-2007, with more studies in 2016-2019. This meta-

analysis confirms that research on endophytic Bb/ plant / plant pathogen interaction studies has increased over the last few years. The variation in publication number each year could depend on journal editorial boards, journal where the studies were submitted or accepted, quality of data, and whether the research was interesting or significant.

In this meta-analysis, there were inconsistencies between and across studies and high heterogeneity, which is supported by previous meta-analysis reports on bioagent efficacy in the management of plant diseases (Ojiambo and Scherm, 2006). The inconsistencies in data could be due to wide usage of a single strain of microbe as well as indiscriminate use of a microorganism. Ojiambo and Scherm (2006) suggested that wider application of microorganisms without proper screening or without knowing whether the microbe is a good match for that host ecosystem is a problem. Chandrasekaran et al. (2014) illustrated that inconsistent endophytic efficacy results could be due to differential colonization abilities of a microorganism or competition with other microbes.

Bb is influenced by several biotic and abiotic factors, such as host species (insect or plant), host abundance, Bb application, delivery and timing, rate, and target region; and environmental conditions, such as moisture, temperature, season, rainfall, and soil factors (Kikankie et al., 2010). These could lead to inconsistent Bb colonization efficacy (Bugeme et al., 2008; Kessler et al., 2003; Inyang et al., 2000) and inconsistency in plant disease reduction or pathogen suppression.

In this paper, we present an historical overview of the research on endophytic Bb, which has led to our current understanding of this fungus as an endophyte, and its interactions with host plants and phytopathogens. It was our purpose to improve our understanding of current

concepts on endophytes, and to assess directions for new research. Based on this meta-analysis, it is apparent that more information is needed on the ecology of Bb as an endophyte, and that more standardization of methodologies and protocols are needed for future evaluations of Bb isolates.

In summary, the present meta-analysis of current studies confirmed that endophytic Bb can suppress plant pathogens and disease. The influential variables that impacted the efficacy of endophytic Bb should be studied to better understand this fungus, its endophytic mode in plants, and its effectiveness in control of various plant pathogen species and the diseases they cause. The results of this study could be a forward step toward quantitative analysis, and improved recommendations for future research and development of endophytic biocontrol organisms as commercial products for more effective plant disease management.

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Appendix 3

All tables and figures for Chapter 3 are in Appendix 3.

Table 3. Moderators that influenced plant disease reduction caused by endophytic Beauveria bassiana.

Category	Moderator	Factor
Endophytic fungus: *Beauveria bassiana* (Bb)	1. Bb isolate	ATCC 74040 (56), ATP01 (12), ATP05 (12), B2, 0.00 (3), Bb 11-98 (113), Bb 1205 (6), Bb 1215 (6), Bb 2R-3-3-1 (2), Bb 2R-4-5 (2),Bb 2R-4-7 (2), Bb 5-1 (2), Bb 6 (2), Bb California (21), Bb Egypt (1), Bb India (3), Bb Indonesia (1), Bb Korea (8), Bb Nigeria (12), Bb SDTu (2), Bb19 (6), Bb-MTCC (6), BG11 (3), EABb04/01-Tip (12), EPC8 (3), FRh2 (3), GHA (28), J12 (2), J18 (2), KJ24 (3), LAMFU ID 2772 (3), SD1 (1), SD12 (1), SD14 (1), SD15 (1), SD7 (1), SD8 (1), SD9 (1), TS12 (3), TS7 (3), Ufa-2 (1), Overall (351)
	2. General Bb isolate type	Commercial (84), native (267), Overall (351)
	3. Country of origin of Bb	Colombia (3), Egypt (1), Ethiopia (24), India (15), Indonesia (1), Iran (9), Italy (48), South Korea (27), New Zealand (10), Nigeria (12), NK – Not known (18), Russia (1), Spain (12), USA (170), Overall (351)
	4. General source of Bb isolate	Soil (10), plant (7), insect (240), NK-Not known (94), Overall (351)
	5. Specific source of Bb isolate	Soil (10), lawn daisy plant (3), maize (2), maize cob (2), maize stalk borer (12), black pine bark beetle (3), click beetle (113), Colorado beetle (1), sorghum chafer (12), boll weevil (84), red palm weevil (1), red spider mite (2), poppy stem gall wasp larva (12), NK – Not known (94), Overall (351)
	6. Method of application for Bb	Seed coating (62), seed + soil (4), seed + root dip + soil (6), soil (33), root dip (10), root drenching (107), root dip + foliar (11), stem injection (1), seedling injection (1), foliar spray (53), dual plate technique (44), radial growth inhibition assay (9), liquid-medium assays (1), solid-medium assay (1), agar disks (3), non-volatile culture filtrate (3), poison food technique (2), Overall (351)
	7. Site of Bb application on plants	Soil (15), seed (67), seed + soil (5), seed + roots + soil (2), roots (116), roots + seedling (11), plant base (21), stem (1), seedling (1), leaves (49),NK – Not known (63), Overall (351)
	8. Concentration of Bb	1 x 10^4 propagules/ml (1), 1 x 10^5 spores/ml (6), 1 x 10^5 CFU/ml (25), 1 x 10^6 spores/ml (16), 1 x 10^6 CFU (14), 1 x 10^7 spores/ml (47), 1 x 10^7 CFU (32), 1 x 10^8 spores/ml (56), 1 x 10^6-10^8 spore/ml (2), 1 x 10^9 spores/ml (6), 1 x 10^9 CFU (23), 1 x 10^{10} spores/ml (21), 1 x 10^{11} CFU (1), 1.4 x 10^5 conidia/seed (10), 1.5 x 10^7 spores/ml (1), 0.25mg conidia/seed (1), 0.5mg conidia/seed (2), 0.5 mg conidia/seed + 15 g dried prills/L soil) (1), 1 mg conidia/seed + 16.4 g mycelia/L soil (2), 1 mg conidia/seed (1), 1 mg conidia/seed + 15 g dried prills/L soil (1), 10 mg mycelia/seed (2), 2 g/seedling (1), 10 g/kg seed CFU (4), 15 g dried wheat bran with Bb + 250 ml deionized water/L soil) (2), 16.4 g mycelia (250 ml deionized water/L soil (2), 1 qt/ac (50 gal/30 min) liquid formulation (11), 2.5 qt/ac liquid formulation (9), 2% v/v (3), culture (1), filtrate (50% or 100%) (2), NK – Not known (45), Overall (351)
	9. Amount of Bb applied	0.2 µl CFU (54), 5 µl conidia (12), 0.2 ml conidia (1), 0.5 ml conidia (1), 1 ml conidia (58), 3 ml conidia (48), 8 ml conidia (2), 10 ml conidia (11), 15 ml conidia (36), 20 ml culture filtrate (3), 100 ml (21), 300 ml CFU (8), 500 ml CFU (2), 50 gals conidia (11), 0.25 mg conidia/seed (1), 0.5 mg conidia/seed +15 g dried prills (38 of Bb) (1), 0.5 mg conidia/seed (2), 0.6 mg conidia/seed (6), 1 mg conidia/seed (1), 1 mg conidia/seed +15 g dried prills (38 of Bb) (1), 1 mg conidia/seed + 16.4 g mycelia (2), 10 mg mycelia/seed (2), 2 g conidia/seed (1), 10 g/kg (4), 15 g dried wheat-bran pills + Bb) (2), 16.4 g mycelia (2), NK-Not known (58), Overall (351)
	10. Form of Bb application	Conidia suspension (178), conidia/seed (4), conidia/mycelia (2), conidia + dried prills (2), dried prills (2), CFU/ml (99), culture filtrate (3), mycelia (4), liquid formulation (9), NK-Not known (48), Overall (351)

"Table 3. Continued"

Category	Moderator	Factor
Endophytic fungus: *Beauveria bassiana* (Bb)	11. Colonization range (%) of Bb in plants	0-50% (44), 50-100% (141), NK-Not known (166), Overall (351)
	12. Plant age at which Bb was applied	Seed (72), right after planting seed (6), seedling stage (19), 5-6 true leaves (36), 5 days (9), 7 days (8), 8 days (1), 13 days (54), 14 days (34), 1-14 days after planting (3), 21 days (16), 25 days (3), 30 days (6), 152 days (21), NK- Not known (63), Overall (351)
Plant pathogen factors	13. Site of plant pathogen application	Soil (85), cotyledons (32), leaves (90), stem (2), root (44), plant base (21), NK- Not known (77), Overall (351)
	14. Specific plant disease	Charcoal rot (21), cob rot (3), crown and root rot (44), crown rot (3), root-rot disease (44), vascular streak dieback (VSD) (1), vascular wilt of cotton (9), fusarium wilt (27), pythium damping-off (16), tomato damping-off (9), downy mildew (16), powdery mildew (3), white mold (5), gray mold (25), black mold of cereals (3), purple blotch (3), blackleg or Phoma stem canker (3), branch canker pathogen (6), bacterial blight disease (63), late blight of tomato (2), maize leaf blight (2), potato late blight (1), *Zucchini yellow mosaic virus* (ZYMV) (32), NK – Not known (human pathogen) (10), Overall (351)
	15. Specific plant pathogen	*Fusarium oxysporum* (17), *Fusarium moniliforme* (14), *Fusarium lycopersici* (24), *Fusarium culmorum* (16), *Fusarium pallidoroseum* (3), *Fusarium verticillioides* (3), *Fusarium vasinfectum* Race 4 (9), *Macrophoma* sp. (4), *Macrophoma theicola* (2), *Macrophomina phaseolina* (21), *Pythium myriotylum* (16), *Rhizoctonia solani* (53), *Sclerotinia sclerotiorum* (5), *Cladosporium herbarum* (3), *Botrytis cinerea* (25), *Alternaria porri* (3), *Phytophthora infestans* (3), *Plasmopara viticola* (16), *Podosphaera aphanis* (3), *Setosphaeria turcica* (2), *Leptosphaeria maculans* (3), *Oncobasidium theobromae* (1), *Xanthomonas axonopodis* pv. *malvacearum* (63), *Bacillus cereus* (10), *Potyvirus* (32), Overall (351)
	16. Plant pathogen kingdom	Bacteria (73), Chromista (35), Fungi (211), Viruses and viroids (32), Overall (351)
	17. Plant pathogen phylum	Ascomycota (157), Basidiomycota (54), Pseudofungi (35), Proteobacteria (63), category: Viruses (32), NK – Not known (10), Overall (351)
	18. Plant pathogen taxonomic class	Agaricomycotina (54), Dothideomycetes (38), Leotiomycetes (33), Oomycetes (35), Sordariomycetes (86), Firmicutes (10), Gammaproteobacteria (63), Category: Viruses (32), Overall (351)
	19. Plant pathogen order	Botryosphaeriales (27), Capnodiales (3), Ceratobasidiales (54), Erysiphales (3), Helotiales (30), Hypocreales (86), Peronosporales (19), Pleosporales (8), Pythiales (16), Xanthomonadales (63), Bacillales (10), Category: Viruses (32), Overall (351)
	20. Plant pathogen family	Botryosphaeriaceae (27), Ceratobasidiaceae (54), Erysiphaceae (3), Leptosphaeriaceae (3), Mycosphaerellaceae (3), Nectriaceae (86), Peronosporaceae (19), Pleosporaceae (5), Pythiaceae (16), Sclerotiniaceae (30), Xanthomonadaceae (63), Bacillaceae (10), Potyviridae (32), Overall (351)
	21. Plant pathogen survival mechanism	Seedborne (96), seed + soilborne (95), soilborne (111), stem inhabiting pathogen (6), aphid + seedborne virus (32), vascular pathogen (1), human pathogen (10), Overall (351)
	22. Plant pathogen saprophytic habitat	Soil inhabitants (77), rhizosphere colonizers (3), root inhabitants (92), seedborne + aphids (3), crop debris (12), crop debris or seeds (63), dead plants or soil (42), NK-Not known (30), Overall (351)

"Table 3. Continued"

Category	Moderator	Factor
Plant pathogen factors	23. Plant pathogen survival spores and structures	Conidia (3), oospore (16), ascospores and pycnidia (conidia) (3), basidiospores (1), chlamydospores (86), chlamydospores + microsclerotia (3), chlamydospores + sclerotia, (25), chlamydospores + zoospores + oospores (16), chlamydospores + oospores (3), sclerotia (60), sclerotia + microsclerotia (27), cleistothecia (3), bacterial cells (73), virus particles (32), Overall (351)
Plant factors	24. Plant species	Arabidopsis (2), cabbage (6), cacao tree (1), cotton (98), date-palm (1), grape (16), maize (10), potato (1), squash (32), strawberry (32), sweet pepper (36), tomato (53), wheat (2), NK (61), Overall (351)
	25. Plant family	Arecaceae (1), Brassicaceae (8), Cucurbitaceae (32), Malvaceae (99), Poaceae (12), Rosaceae (32), Solanaceae (90), Vitaceae (16), NK – Not known (61), Overall (351)
	26. Plant taxonomic group	Eudicots (277), Monocots (13), NK (61), Overall (351)
	27. Plant life span	Annual (46), biennial (6), perennial (222), woody perennial vines (16), NK Not known (61), Overall (351)
	28. Horticultural classification	Vegetable (130), grain crop (12), fruits (48), beverage crop (1), fiber crop (98), evergreen trees (1), NK (61), Overall (351),
	29. Horticultural importance	Edibles (191), multipurpose (1), not edible (industrial crops) (98), NK Not known (61), Overall (351)
Experimental factors	30. Country in which research was conducted	Colombia (3), Egypt (1), India (15), Indonesia (1), Iran (9), Jordan (92), Korea (27), Mexico (18), New Zealand (2), Nigeria (12), Russia (1), USA (170), Overall (351)
	31. Experimental conditions	*In vitro* (64), glasshouse (8), greenhouse (146), growth chamber (96), outdoor environment (21), field (16), Overall (351)
	32. Incubation period after inoculation with plant pathogen	1 day (10), 3 days (8), 4 days (20), 5 days (6), 6 days (12), 7 days (22), 8 days (19), 9 days (6), 10 days (5), 10-12 days (2), 12 days (6), 14 days (23), 19 days (7), 21 days (40), 24 days (2), 19-24 days (54), 25 days (3), 28 days (45), 35 days (20), 42 days (13), 45 days (1), 49 days (3), 60 days (6), 91 days (1), 94 days (1), 96 days (1), 121 days (1), 124 days (1), 126 days (1), 155 days (1), 157 days (1), 182 days (2), 185 days (1), 187 days (1), NK-Not known (6), Overall (351)
	33. Publication years	2004-2007 (109), 2008-2011 (24), 2012-2015 (63), 2016-2019 (155), Overall (351)
	34. Journal	Acta Biologica Szegediensis (9), African Journal of Microbiology Research (12), Archives of Phytopathology and Plant Protection (3), Journal of Asia-Pacific Entomology (8), BioControl (19), Biocontrol Science and Technology (32), Biological Control (42), E-Journal of Entomology and Biologicals (20), Emerging Concepts in Plant Health Management (20), Entomologia Experimentalis et Applicata (1), Journal of Environmental Biology (18), Frontiers in Microbiology (2), Indian Journal of Agricultural Sciences (6), Journal of Invertebrate Pathology (12), Journal of Applied Biological Sciences (1), Mycoscience (12), Mycobiology (7), Pest Management Science (6), Plant Health (21), Planta (2), Scientific Journal of King Faisal University (1), Lincoln University Doctoral dissertation (8), University of Tennessee, Knoxville Doctoral dissertation (89), Overall (351)

* Figures in parenthesis are number of studies (n).

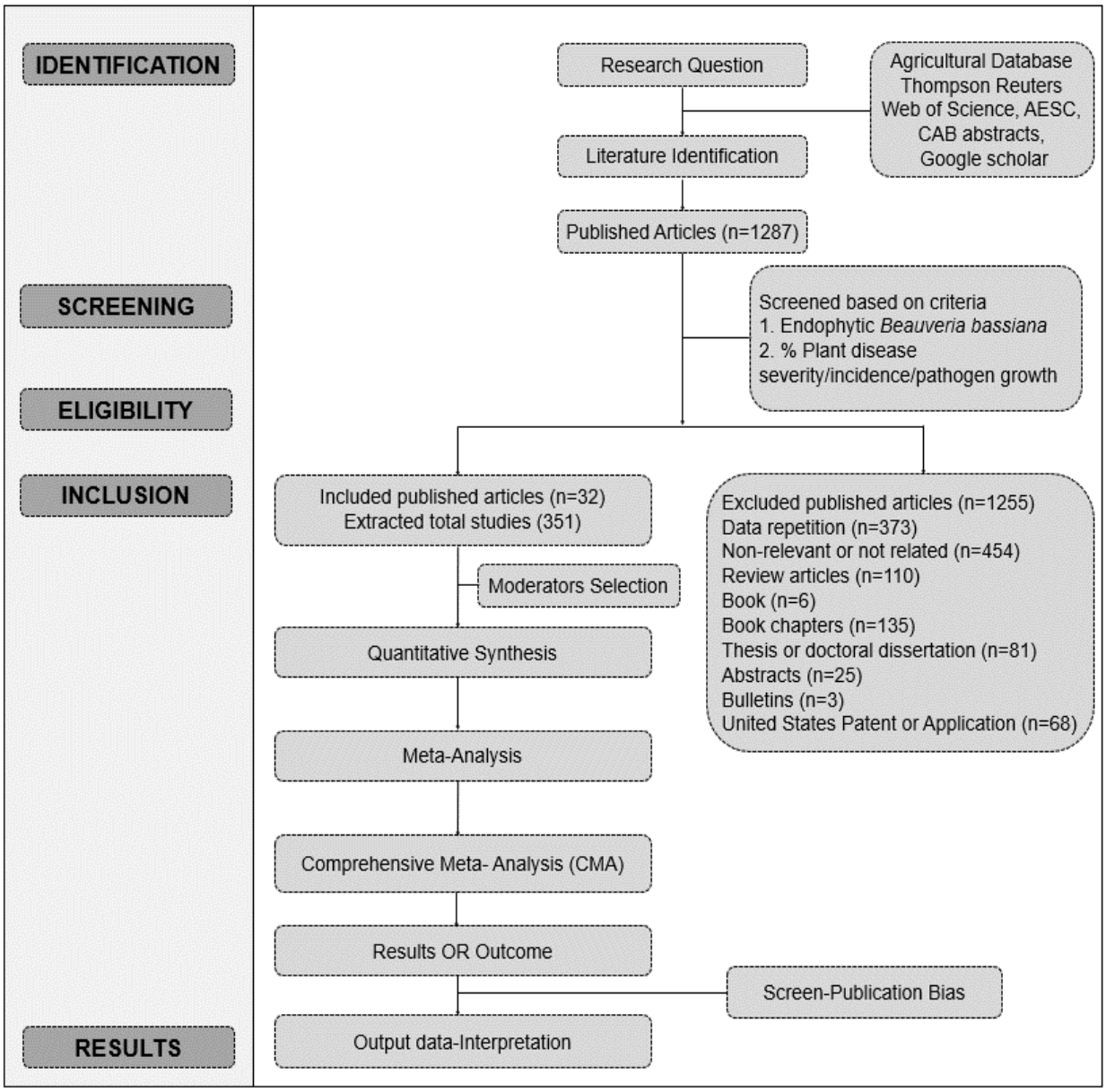
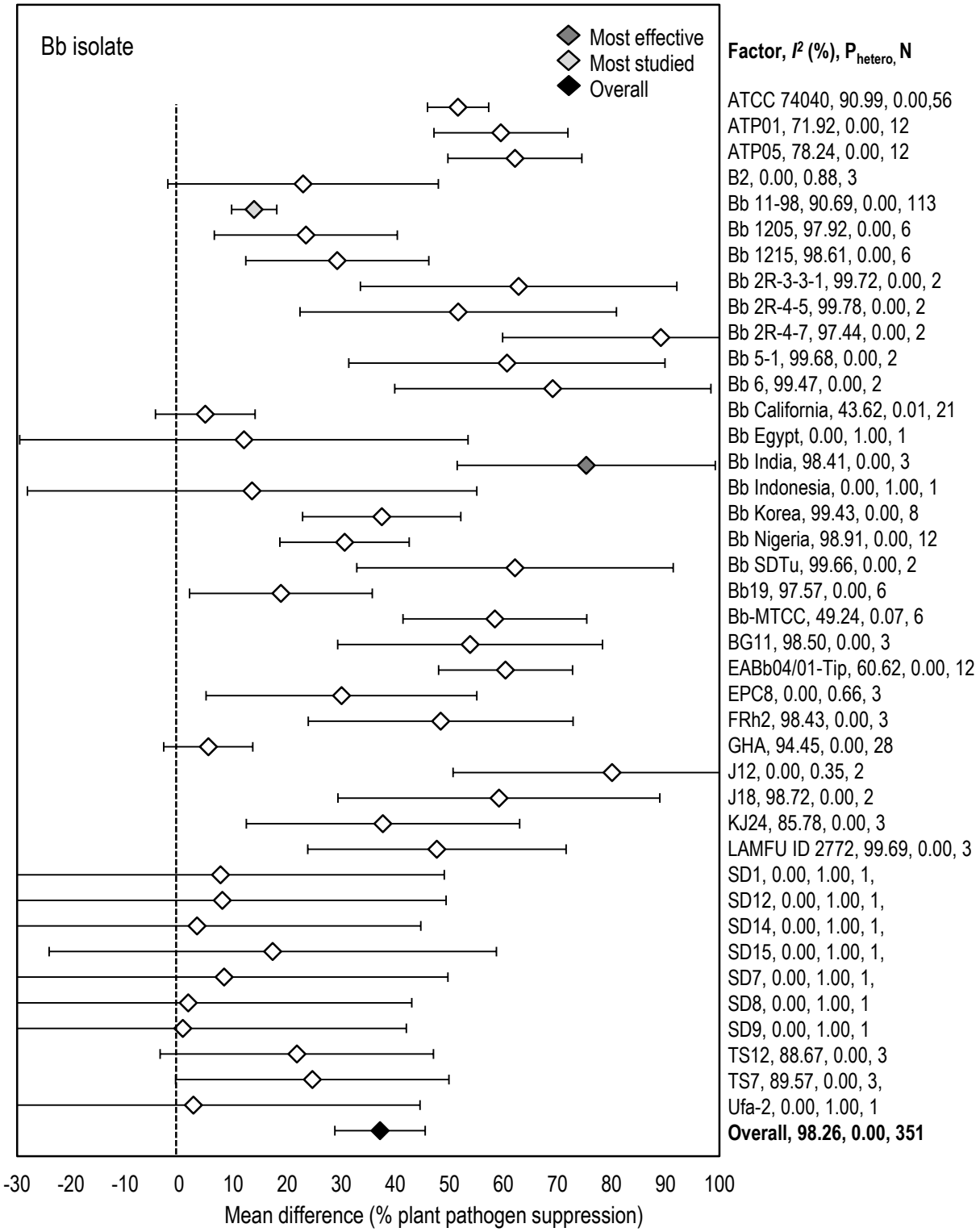


Figure 3.1. Schematic representation of meta-analysis procedure followed in the study[1].

[1] AESC = Agriculture & Environmental Science Database), CAB = International Comprehensive Database for Agriculture and Veterinary medicine).

Figure 3.2. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific Bb isolate.

Moderator: Comparison among studies on specific Bb isolates used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (Bb isolate) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.


Bb isolate
Most effective
Most studied
Overall
Factor, I^2 (%), P_{hetero}, N
ATCC 74040, 90.99, 0.00,56
ATP01, 71.92, 0.00, 12
ATP05, 78.24, 0.00, 12
B2, 0.00, 0.88, 3
Bb 11-98, 90.69, 0.00, 113
Bb 1205, 97.92, 0.00, 6
Bb 1215, 98.61, 0.00, 6
Bb 2R-3-3-1, 99.72, 0.00, 2
Bb 2R-4-5, 99.78, 0.00, 2
Bb 2R-4-7, 97.44, 0.00, 2
Bb 5-1, 99.68, 0.00, 2
Bb 6, 99.47, 0.00, 2
Bb California, 43.62, 0.01, 21
Bb Egypt, 0.00, 1.00, 1
Bb India, 98.41, 0.00, 3
Bb Indonesia, 0.00, 1.00, 1
Bb Korea, 99.43, 0.00, 8
Bb Nigeria, 98.91, 0.00, 12
Bb SDTu, 99.66, 0.00, 2
Bb19, 97.57, 0.00, 6
Bb-MTCC, 49.24, 0.07, 6
BG11, 98.50, 0.00, 3
EABb04/01-Tip, 60.62, 0.00, 12
EPC8, 0.00, 0.66, 3
FRh2, 98.43, 0.00, 3
GHA, 94.45, 0.00, 28
J12, 0.00, 0.35, 2
J18, 98.72, 0.00, 2
KJ24, 85.78, 0.00, 3
LAMFU ID 2772, 99.69, 0.00, 3
SD1, 0.00, 1.00, 1,
SD12, 0.00, 1.00, 1,
SD14, 0.00, 1.00, 1,
SD15, 0.00, 1.00, 1,
SD7, 0.00, 1.00, 1,
SD8, 0.00, 1.00, 1
SD9, 0.00, 1.00, 1
TS12, 88.67, 0.00, 3
TS7, 89.57, 0.00, 3,
Ufa-2, 0.00, 1.00, 1
Overall, 98.26, 0.00, 351
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant pathogen suppression)

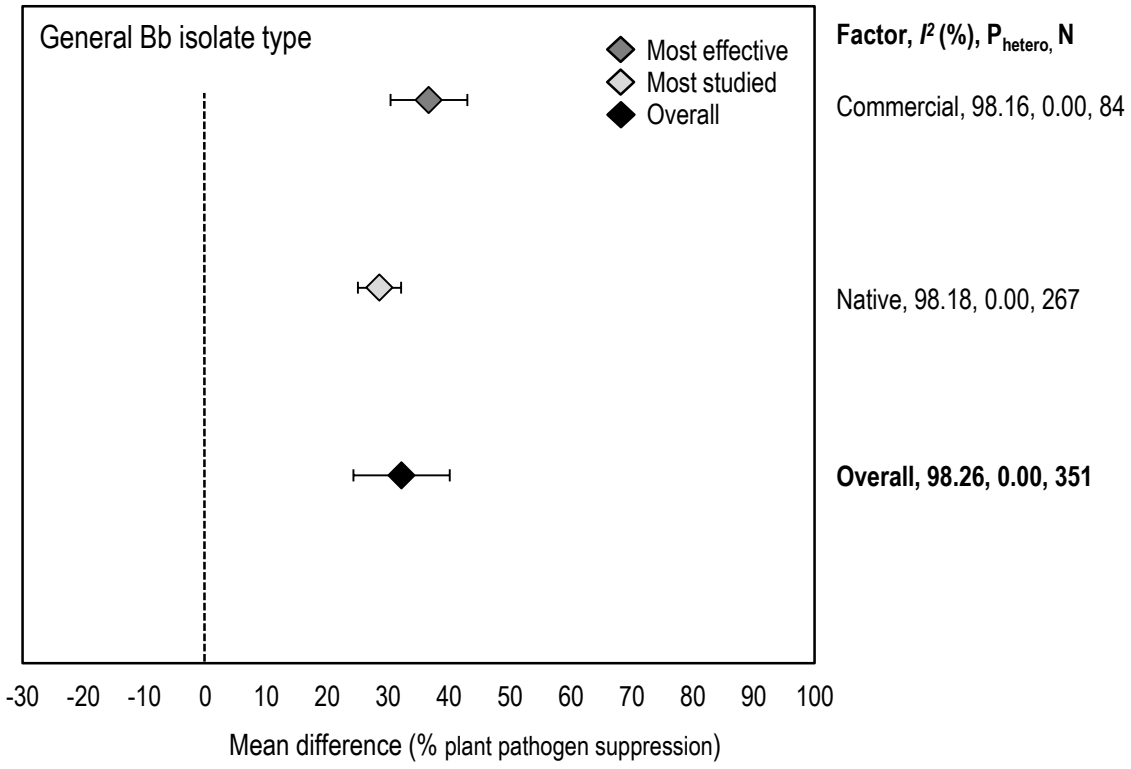


Figure 3.3. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on general Bb isolate type.

Moderator: Comparison among studies on general Bb isolate type used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (general Bb isolate type) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

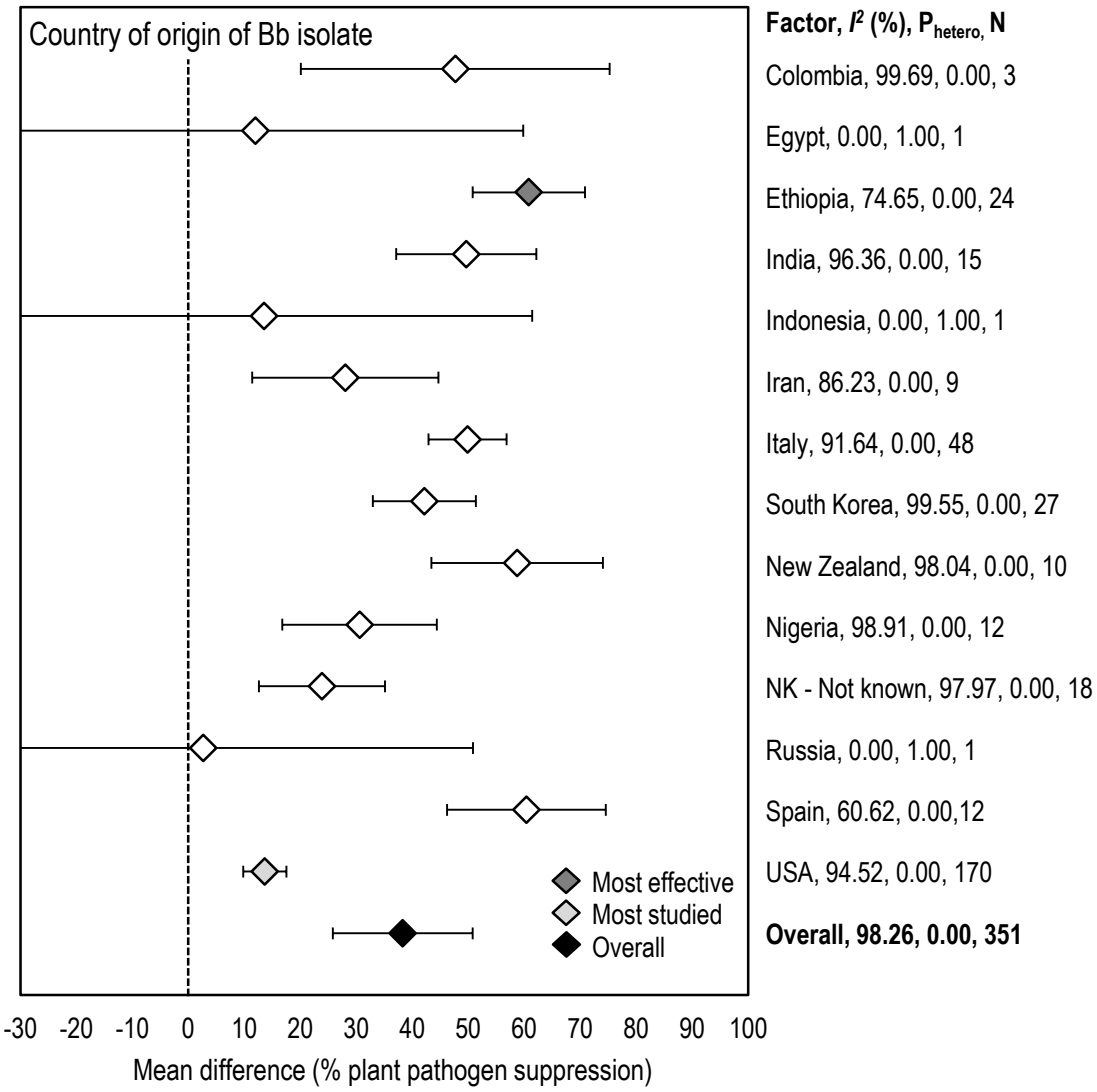


Figure 3.4. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on country of origin of Bb isolate.

Moderator: Comparison among studies on country of origin of Bb isolate used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (country of origin of Bb isolate) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

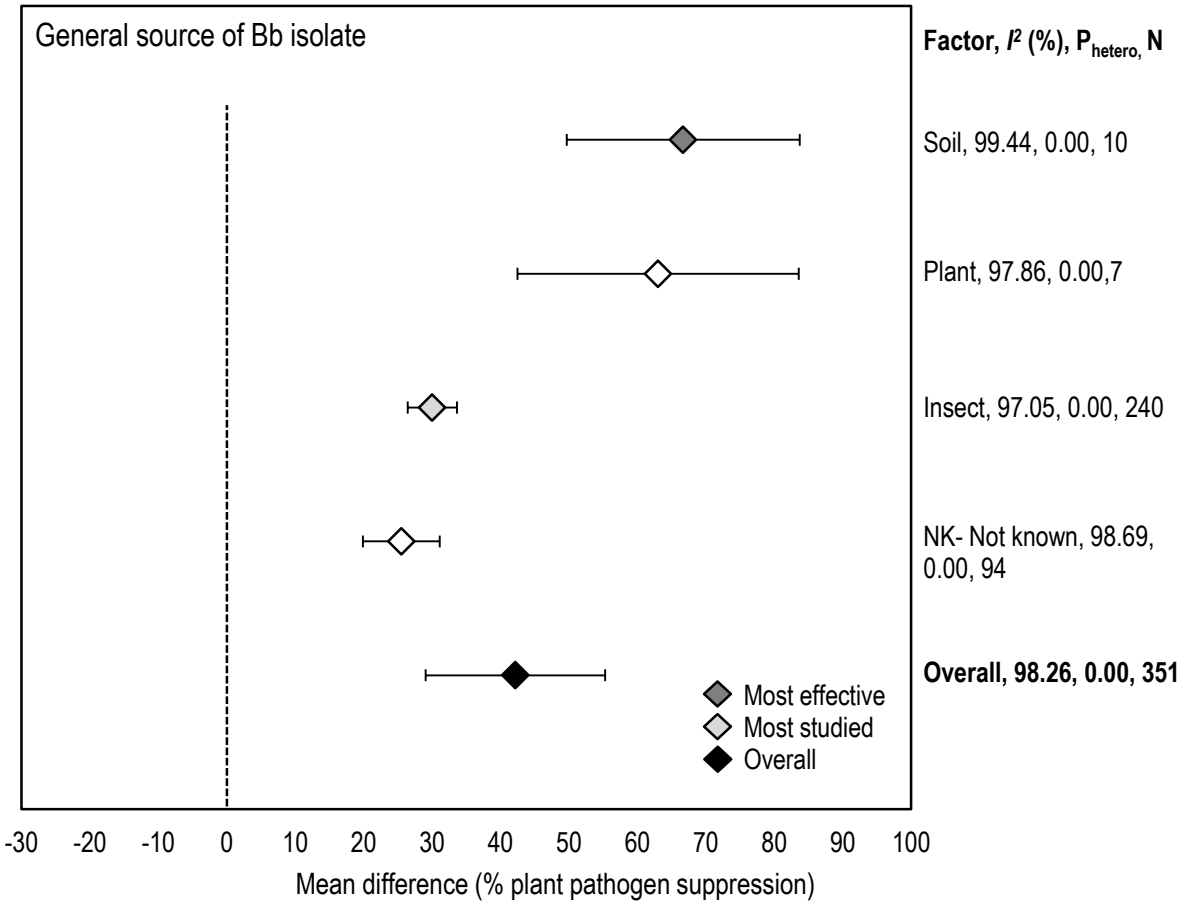


Figure 3.5. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on general source of Bb isolate.

Moderator: Comparison among studies on general source of Bb isolate used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (general source of Bb isolate) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

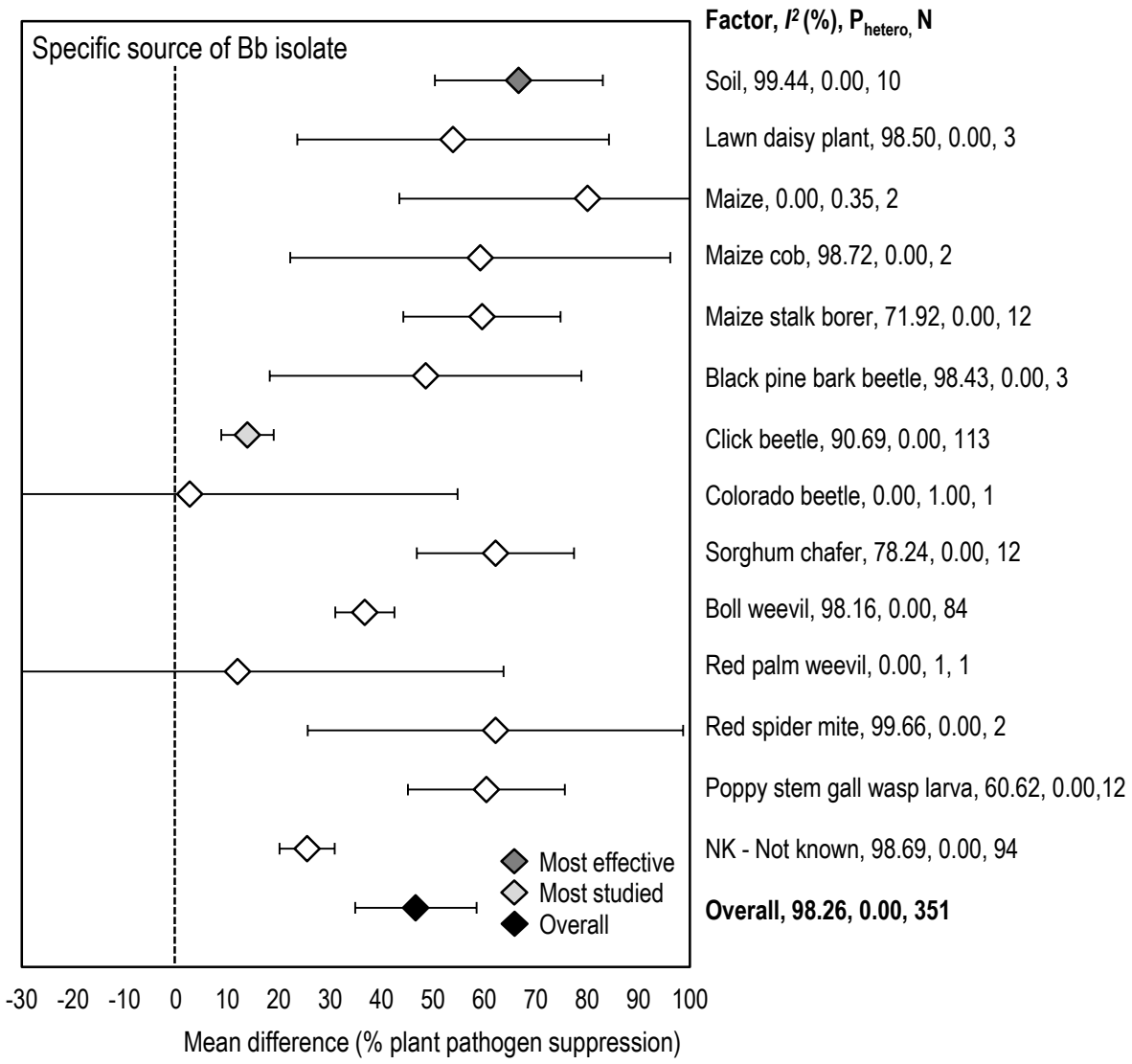
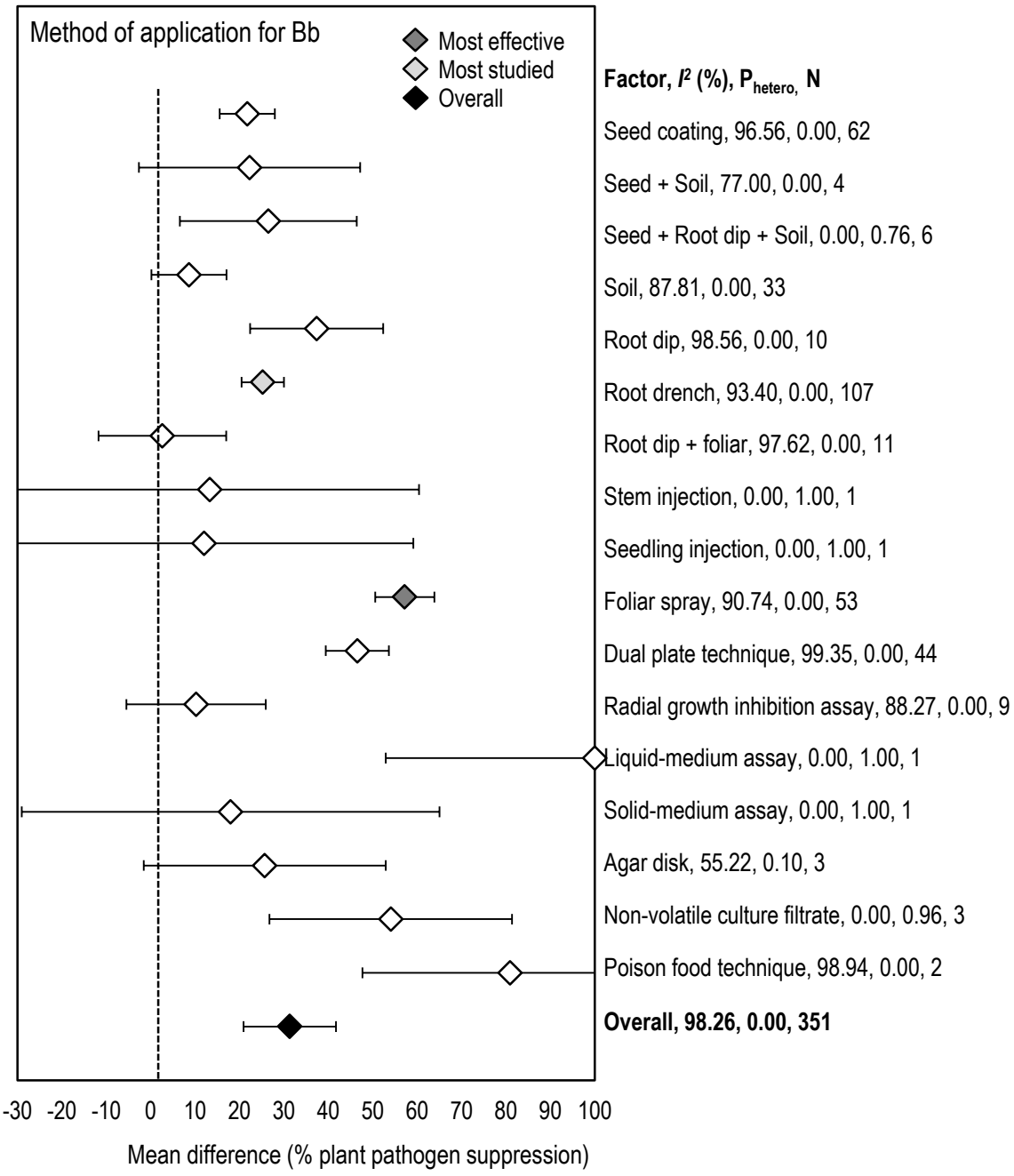


Figure 3.6. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific source of Bb isolate.

Moderator: Comparison among studies on specific source of Bb isolates used in plant pathogen studies for each level of moderator. Values on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (specific source of Bb isolate) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

Figure 3.7. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on method of Bb application.

Moderator: Comparison among studies on method of plant pathogen application used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (method of Bb application) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.


Method of application for Bb
Most effective
Most studied
Overall
Factor, I^2 (%), $P_{hetero,}$ N
Seed coating, 96.56, 0.00, 62
Seed + Soil, 77.00, 0.00, 4
Seed + Root dip + Soil, 0.00, 0.76, 6
Soil, 87.81, 0.00, 33
Root dip, 98.56, 0.00, 10
Root drench, 93.40, 0.00, 107
Root dip + foliar, 97.62, 0.00, 11
Stem injection, 0.00, 1.00, 1
Seedling injection, 0.00, 1.00, 1
Foliar spray, 90.74, 0.00, 53
Dual plate technique, 99.35, 0.00, 44
Radial growth inhibition assay, 88.27, 0.00, 9
Liquid-medium assay, 0.00, 1.00, 1
Solid-medium assay, 0.00, 1.00, 1
Agar disk, 55.22, 0.10, 3
Non-volatile culture filtrate, 0.00, 0.96, 3
Poison food technique, 98.94, 0.00, 2
Overall, 98.26, 0.00, 351
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant pathogen suppression)

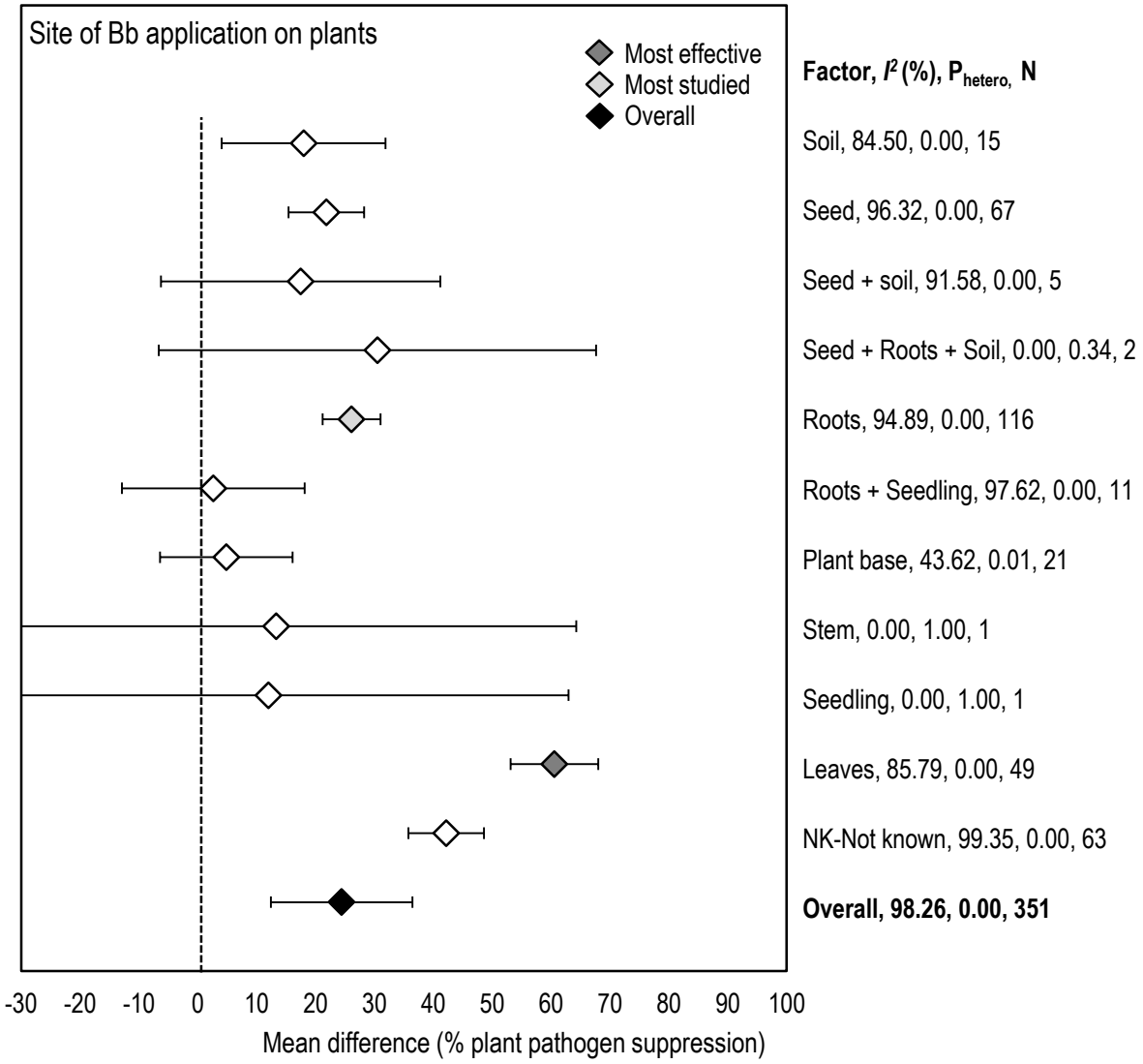


Figure 3.8. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on site of Bb application on plants.

Moderator: Comparison among studies on site of Bb application on plants in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (site of Bb application on plants) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

Figure 3.9. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on concentration of Bb applied.

Moderator: Comparison among studies on concentration of Bb applied in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (concentration of Bb applied) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

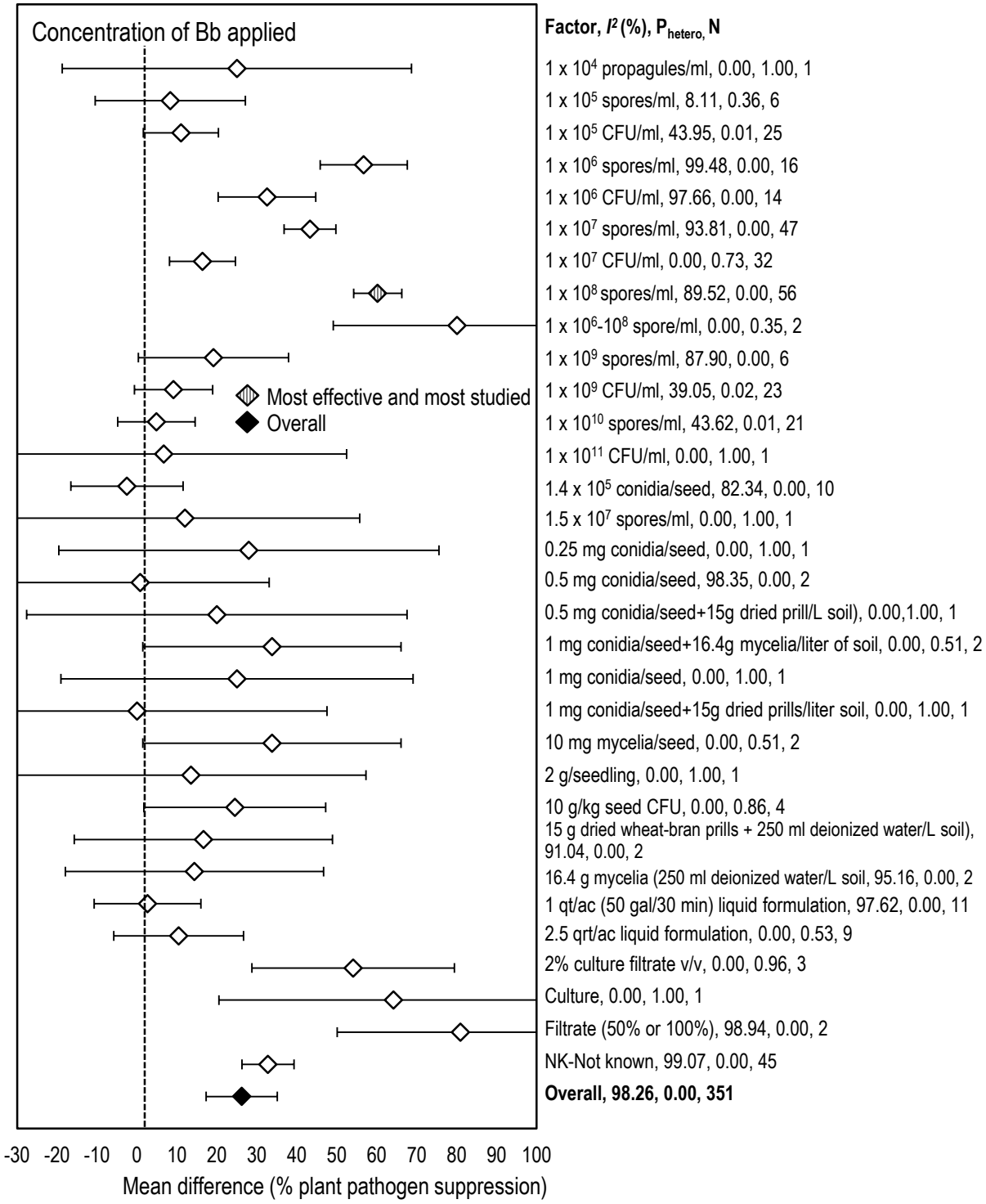
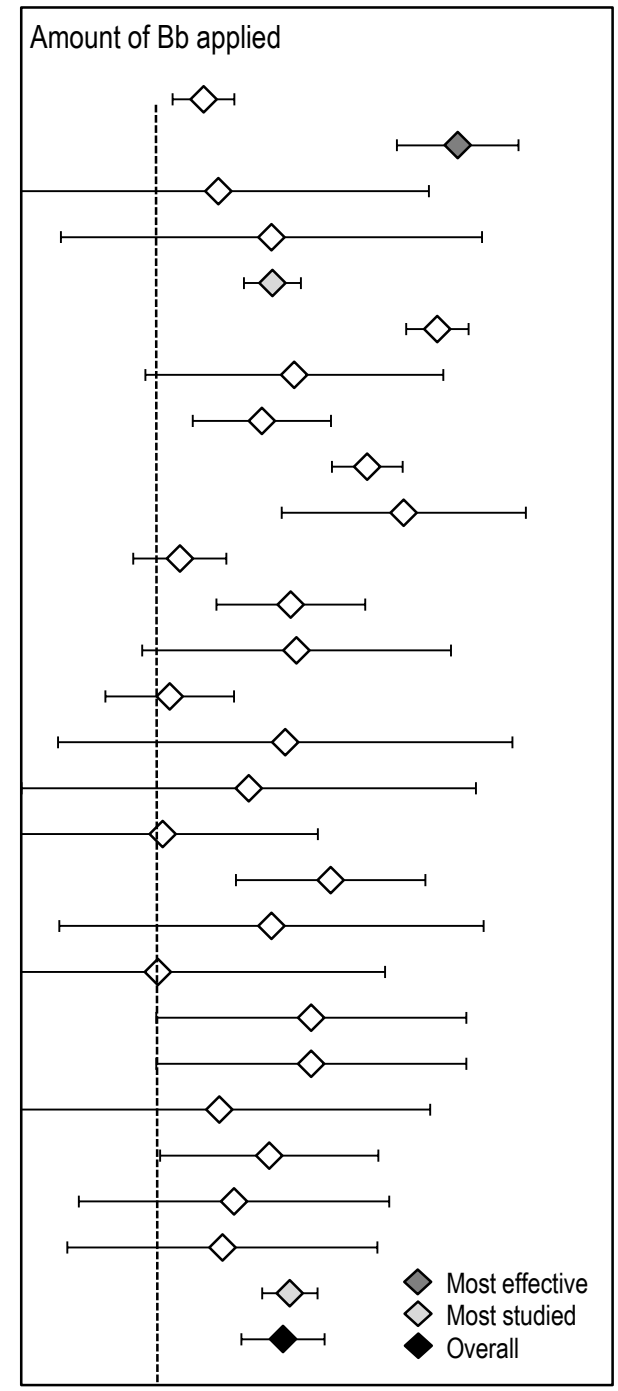

Concentration of Bb applied
Most effective and most studied
Overall
Factor, I^2 (%), P_{hetero}, N
1 x 10^4 propagules/ml, 0.00, 1.00, 1
1 x 10^5 spores/ml, 8.11, 0.36, 6
1 x 10^5 CFU/ml, 43.95, 0.01, 25
1 x 10^6 spores/ml, 99.48, 0.00, 16
1 x 10^6 CFU/ml, 97.66, 0.00, 14
1 x 10^7 spores/ml, 93.81, 0.00, 47
1 x 10^7 CFU/ml, 0.00, 0.73, 32
1 x 10^8 spores/ml, 89.52, 0.00, 56
1 x 10^6-10^8 spore/ml, 0.00, 0.35, 2
1 x 10^9 spores/ml, 87.90, 0.00, 6
1 x 10^9 CFU/ml, 39.05, 0.02, 23
1 x 10^{10} spores/ml, 43.62, 0.01, 21
1 x 10^{11} CFU/ml, 0.00, 1.00, 1
1.4 x 10^5 conidia/seed, 82.34, 0.00, 10
1.5 x 10^7 spores/ml, 0.00, 1.00, 1
0.25 mg conidia/seed, 0.00, 1.00, 1
0.5 mg conidia/seed, 98.35, 0.00, 2
0.5 mg conidia/seed+15g dried prill/L soil), 0.00,1.00, 1
1 mg conidia/seed+16.4g mycelia/liter of soil, 0.00, 0.51, 2
1 mg conidia/seed, 0.00, 1.00, 1
1 mg conidia/seed+15g dried prills/liter soil, 0.00, 1.00, 1
10 mg mycelia/seed, 0.00, 0.51, 2
2 g/seedling, 0.00, 1.00, 1
10 g/kg seed CFU, 0.00, 0.86, 4
15 g dried wheat-bran prills + 250 ml deionized water/L soil), 91.04, 0.00, 2
16.4 g mycelia (250 ml deionized water/L soil, 95.16, 0.00, 2
1 qt/ac (50 gal/30 min) liquid formulation, 97.62, 0.00, 11
2.5 qrt/ac liquid formulation, 0.00, 0.53, 9
2% culture filtrate v/v, 0.00, 0.96, 3
Culture, 0.00, 1.00, 1
Filtrate (50% or 100%), 98.94, 0.00, 2
NK-Not known, 99.07, 0.00, 45
Overall, 98.26, 0.00, 351
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant pathogen suppression)

Figure 3.10. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on amount of Bb applied.

Moderator: Comparison among studies on amount of Bb applied in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (amount of Bb applied) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.



Factor, I^2 (%), $P_{hetero,}$ $_{\mu}$N

0.2 µl CFU, 0.00, 0.77, 54

5 µl conidia, 99.43, 0.00, 12

0.2 ml conidia, 0.00, 1.00, 1

0.5 ml conidia, 0.00, 1.00, 1

1 ml conidia, 96.21, 0.00, 58

3 ml conidia, 67.21, 0.00, 48

8 ml conidia, 96.00, 0.00, 2

10 ml conidia, 91.07, 0.00, 11

15 ml conidia, 92.43, 0.00, 36

20 ml culture filtrate, 0.00, 0.96, 3

100 ml, 43.62, 0.01, 21

300 ml CFU, 98.72, 0.00, 8

500 ml CFU, 0.00, 0.34, 2

50 gals conidia, 97.62, 0.00, 11

0.25 mg conidia/seed, 0.00, 1.00, 1

0.5 mg conidia/seed + 15 g dried wheat-bran prills colonized by Bb in potting mix, 0.00, 1.00, 1

0.5 mg conidia/seed, 98.35, 0.00, 2

0.6 mg conidia/seed, 0.00, 0.45, 6

1 mg conidia/seed, 0.00, 1.00, 1

1 mg conidia/seed +15 g dried wheat-bran prills colonized by Bb in potting mix, 0.00, 1.00, 1

1 mg conidia/seed +16.4 g mycelia, 0.00, 0.51, 2

10 mg mycelia/seed, 0.00, 0.51, 2

2 g conidia/seed, 0.00, 1.00, 1

10 g/kg, 0.00, 0.86, 4

15 g dried wheat bran prills colonized by Bb to potting mix, 91.04, 0.00, 2

16.4 g mycelia, 95.16, 0.00, 2

NK-Not known, 99.10, 0.00, 58

Overall, 98.26, 0.00, 351

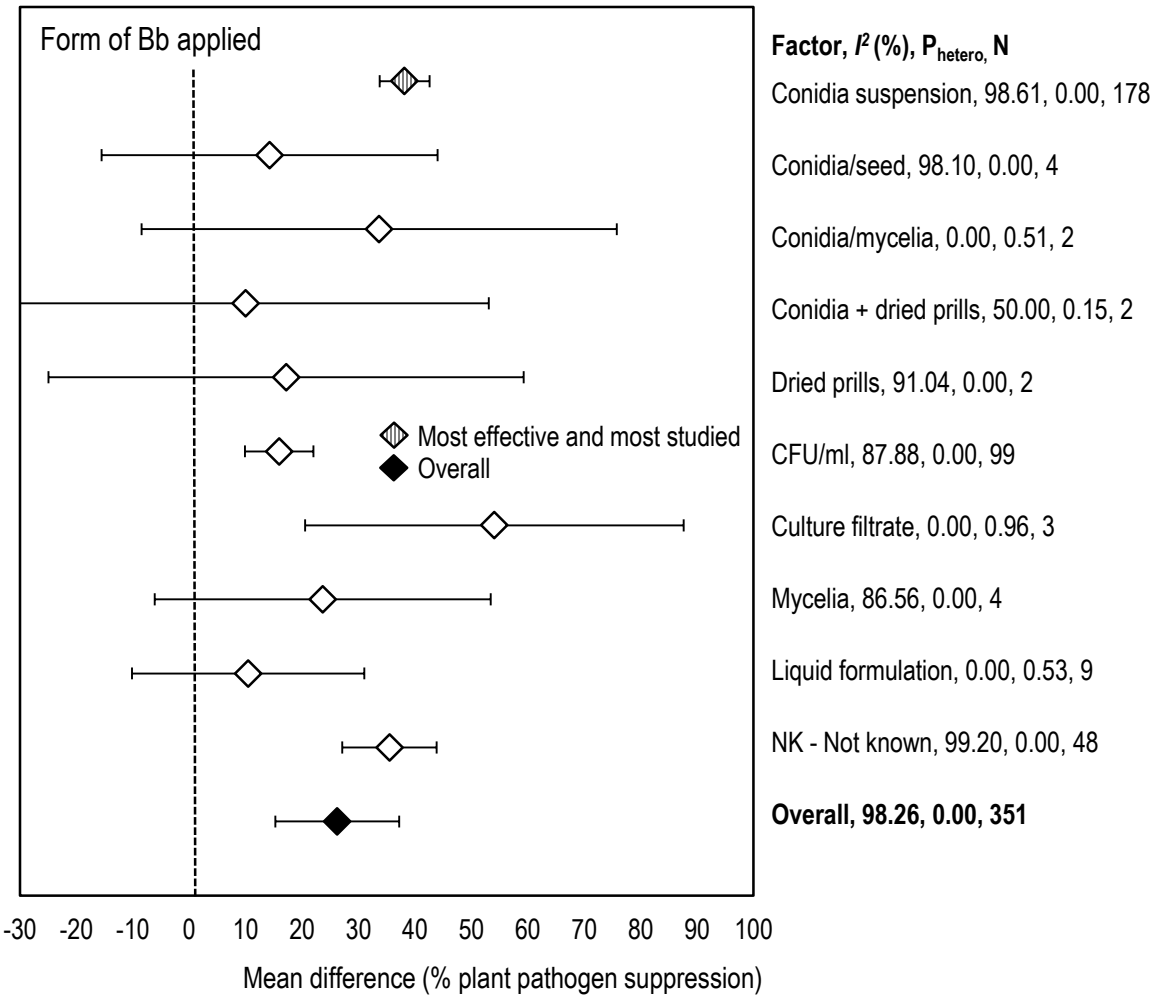


Figure 3.11. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on form of Bb applied.

Moderator: Comparison among studies on form of Bb applied in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (form of Bb applied) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

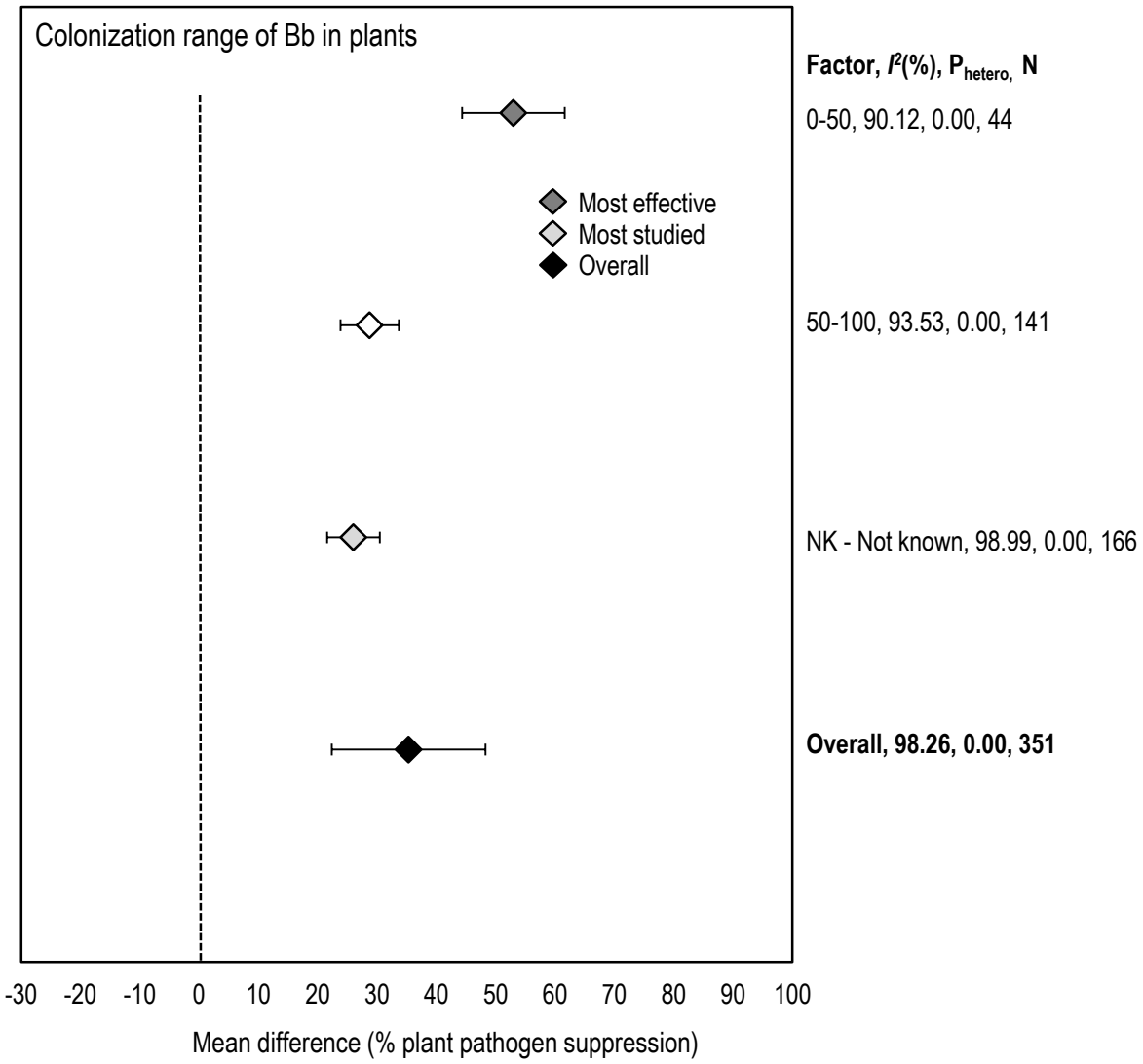


Figure 3.12. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on colonization range of Bb in plants.

Moderator: Comparison among studies on colonization range of Bb in plants in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (colonization range of Bb in plants) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

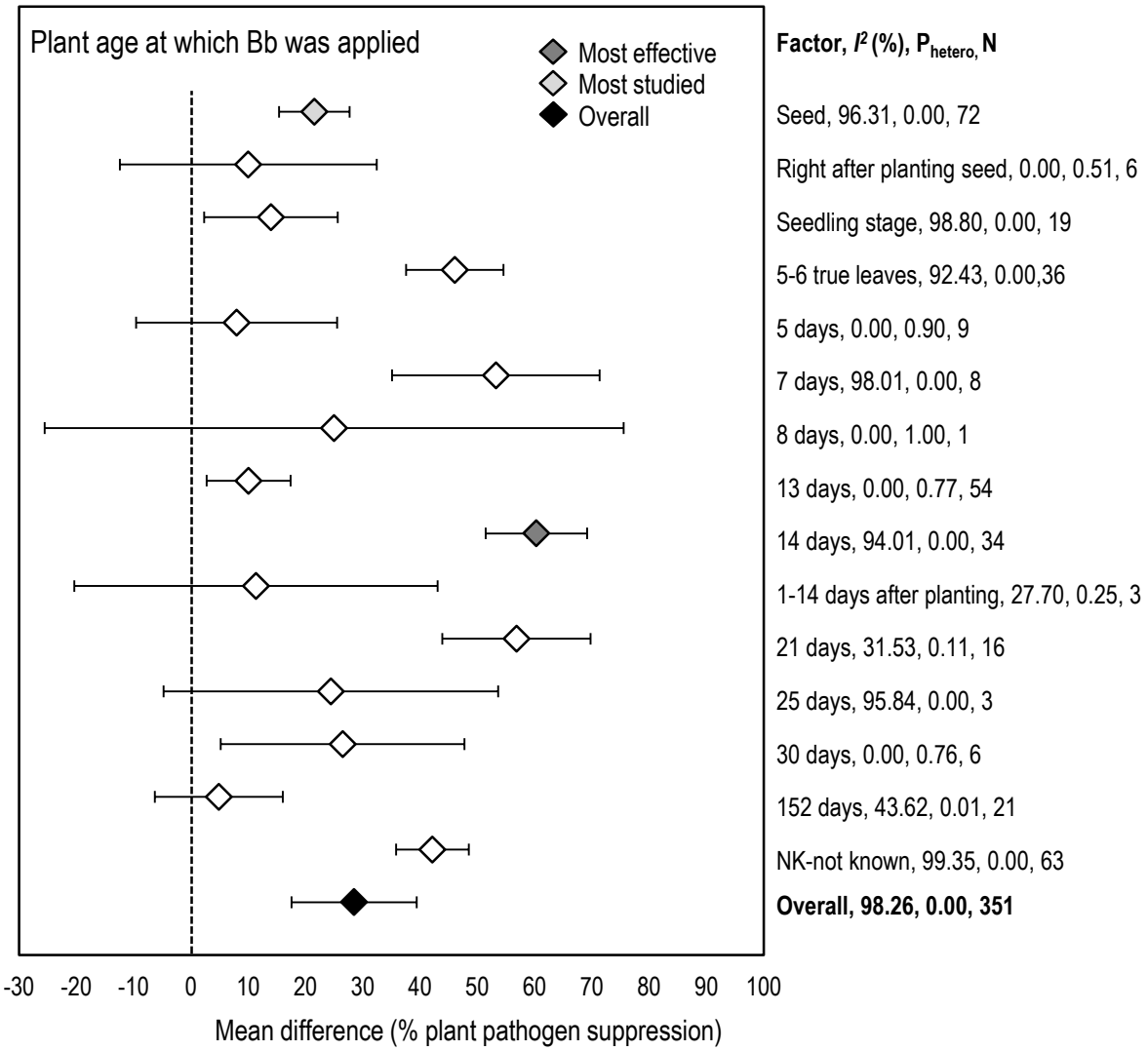


Figure 3.13. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant age at which Bb was applied.

Moderator: Comparison among studies on plant age at which Bb was applied in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant age at which Bb was applied) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

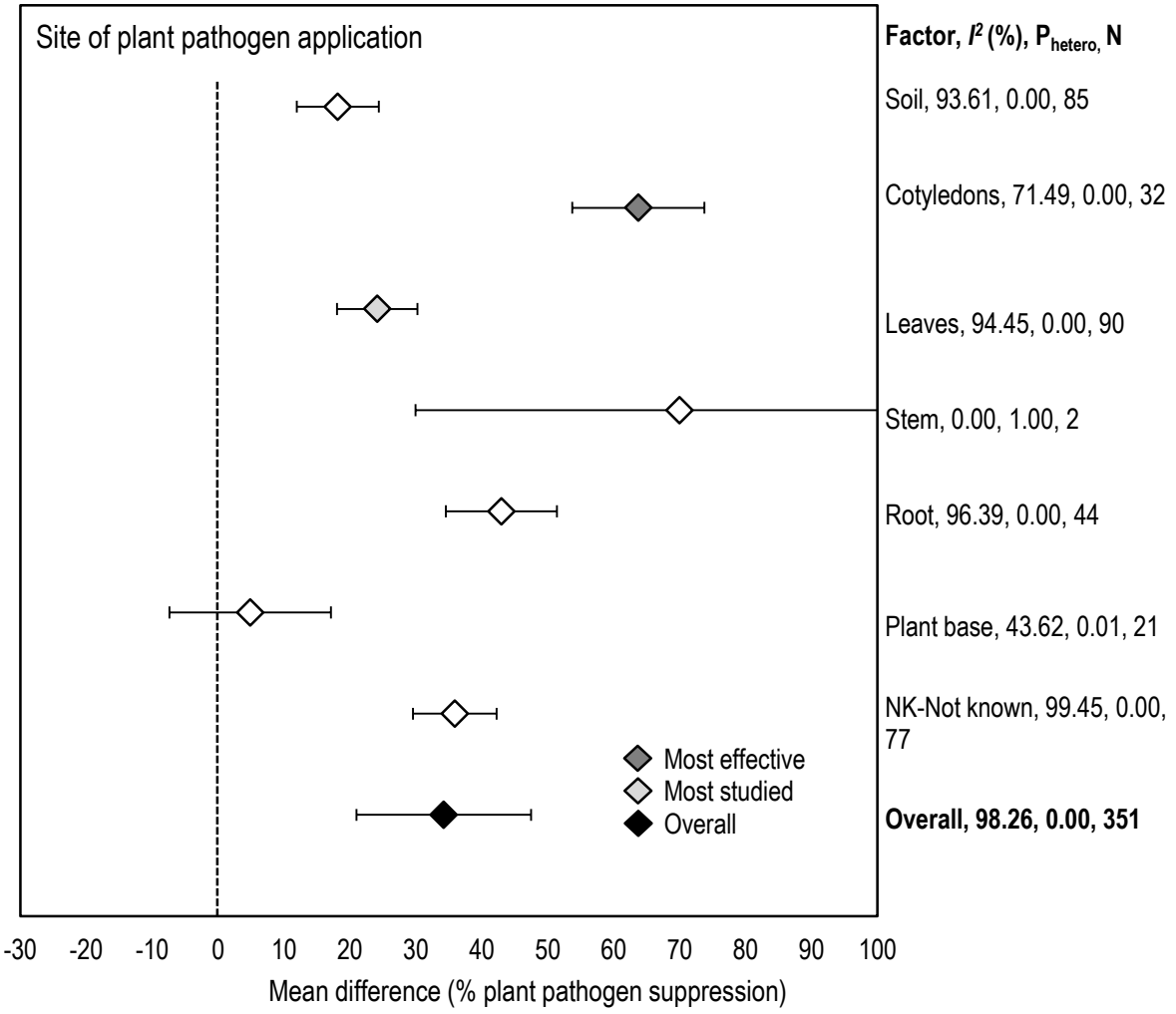


Figure 3.14. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on site of plant pathogen application.

Moderator: Comparison among studies on site of plant pathogen application used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (site of plant pathogen application) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

Figure 3.15. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific plant disease.

Moderator: Comparison among studies on specific Bb isolates used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (specific plant disease) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

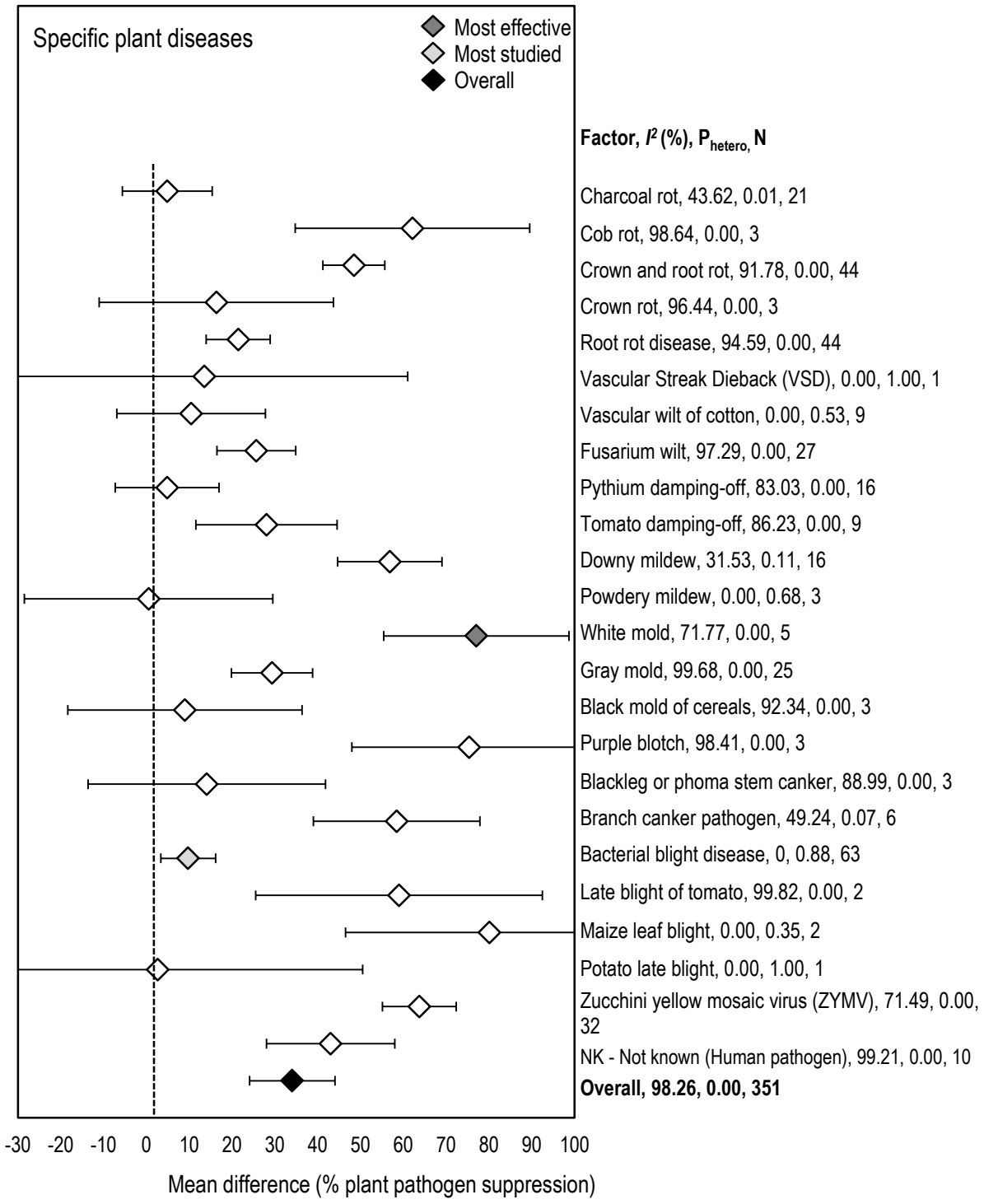
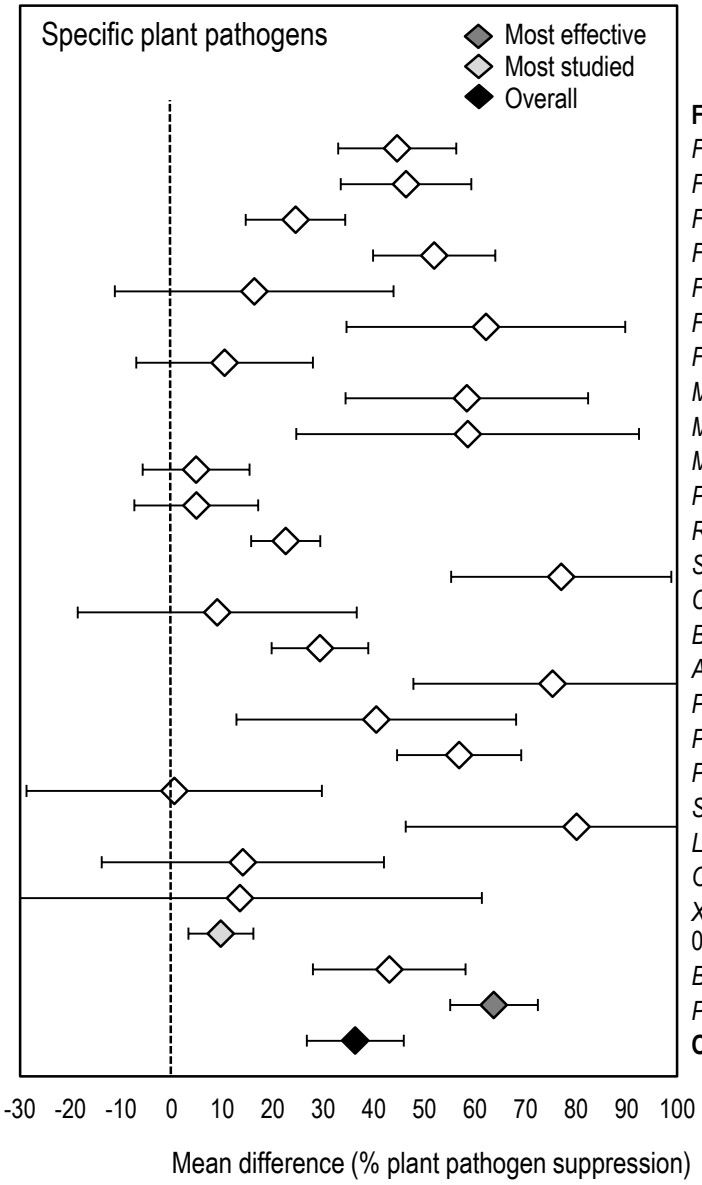

Specific plant diseases
Most effective
Most studied
Overall
Factor, I^2 (%), $P_{hetero,}$ N
Charcoal rot, 43.62, 0.01, 21
Cob rot, 98.64, 0.00, 3
Crown and root rot, 91.78, 0.00, 44
Crown rot, 96.44, 0.00, 3
Root rot disease, 94.59, 0.00, 44
Vascular Streak Dieback (VSD), 0.00, 1.00, 1
Vascular wilt of cotton, 0.00, 0.53, 9
Fusarium wilt, 97.29, 0.00, 27
Pythium damping-off, 83.03, 0.00, 16
Tomato damping-off, 86.23, 0.00, 9
Downy mildew, 31.53, 0.11, 16
Powdery mildew, 0.00, 0.68, 3
White mold, 71.77, 0.00, 5
Gray mold, 99.68, 0.00, 25
Black mold of cereals, 92.34, 0.00, 3
Purple blotch, 98.41, 0.00, 3
Blackleg or phoma stem canker, 88.99, 0.00, 3
Branch canker pathogen, 49.24, 0.07, 6
Bacterial blight disease, 0, 0.88, 63
Late blight of tomato, 99.82, 0.00, 2
Maize leaf blight, 0.00, 0.35, 2
Potato late blight, 0.00, 1.00, 1
Zucchini yellow mosaic virus (ZYMV), 71.49, 0.00, 32
NK - Not known (Human pathogen), 99.21, 0.00, 10
Overall, 98.26, 0.00, 351
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant pathogen suppression)

Figure 3.16. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on specific plant pathogen.

Moderator: Comparison among studies on specific plant pathogen used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (specific plant pathogen) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.



Factors, I^2(%), $P_{hetero,}$ N

Fusarium oxysporum, 94.60, 0.00, 17
Fusarium moniliforme, 90.67, 0.00, 14
Fusarium lycopersici, 97.28, 0.00, 24
Fusarium culmorum, 90.71, 0.00, 16
Fusarium pallidoroseum, 96.44, 0.00, 3
Fusarium verticillioides, 98.64, 0.00, 3
Fusarium vasinfectum Race 4, 0.00, 0.53, 9
Macrophoma sp, 57.45, 0.07, 4
Macrophoma theicola, 64.24, 0.09, 2
Macrophomina phaseolina, 43.62, 0.01, 21
Pythium myriotylum, 83.03, 0.00, 16
Rhizoctonia solani, 93.90, 0.00, 53
Sclerotinia sclerotiorum, 71.77, 0.00, 5
Cladosporium herbarum, 92.34, 0.00, 3
Botrytis cinerea, 99.68, 0.00, 25
Alternaria porri, 98.41, 0.00, 3
Phytophthora infestans, 99.71, 0.00, 3
Plasmopara viticola, 31.53, 0.11, 16
Podosphaera aphanis, 0.00, 0.68, 3
Setosphaeria turcica, 0.00, 0.35, 2
Leptosphaeria maculans, 88.99, 0.00, 3
Oncobasidium theobromae, 0.00, 1.00, 1
Xanthomonas axonopodis pv. *malvacearum,* 0.00, 0.88, 63
Bacillus cereus, 99.21, 0.00, 10
Potyvirus, 71.49, 0.00, 32
Overall, 98.26, 0.00, 351

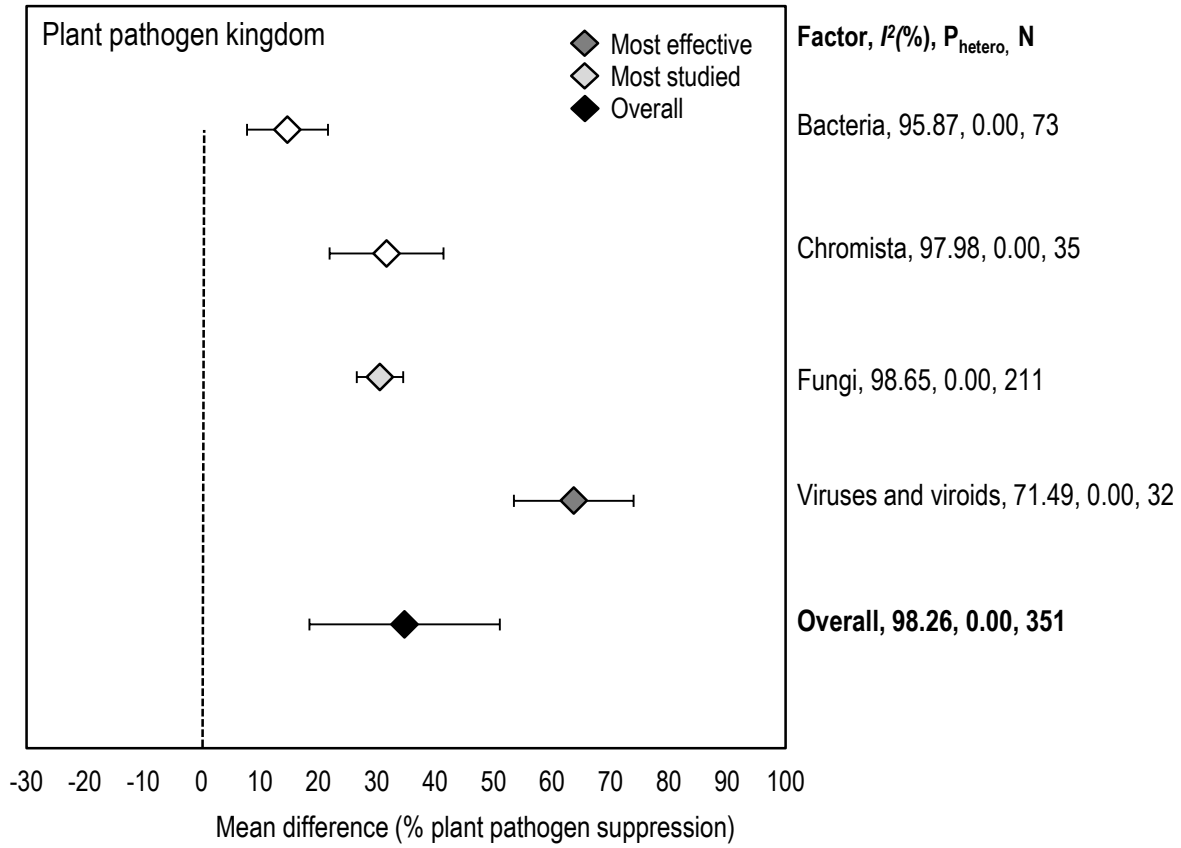


Figure 3.17. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant pathogen kingdom.

Moderator: Comparison among studies on plant pathogen kingdom in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant pathogen kingdom) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

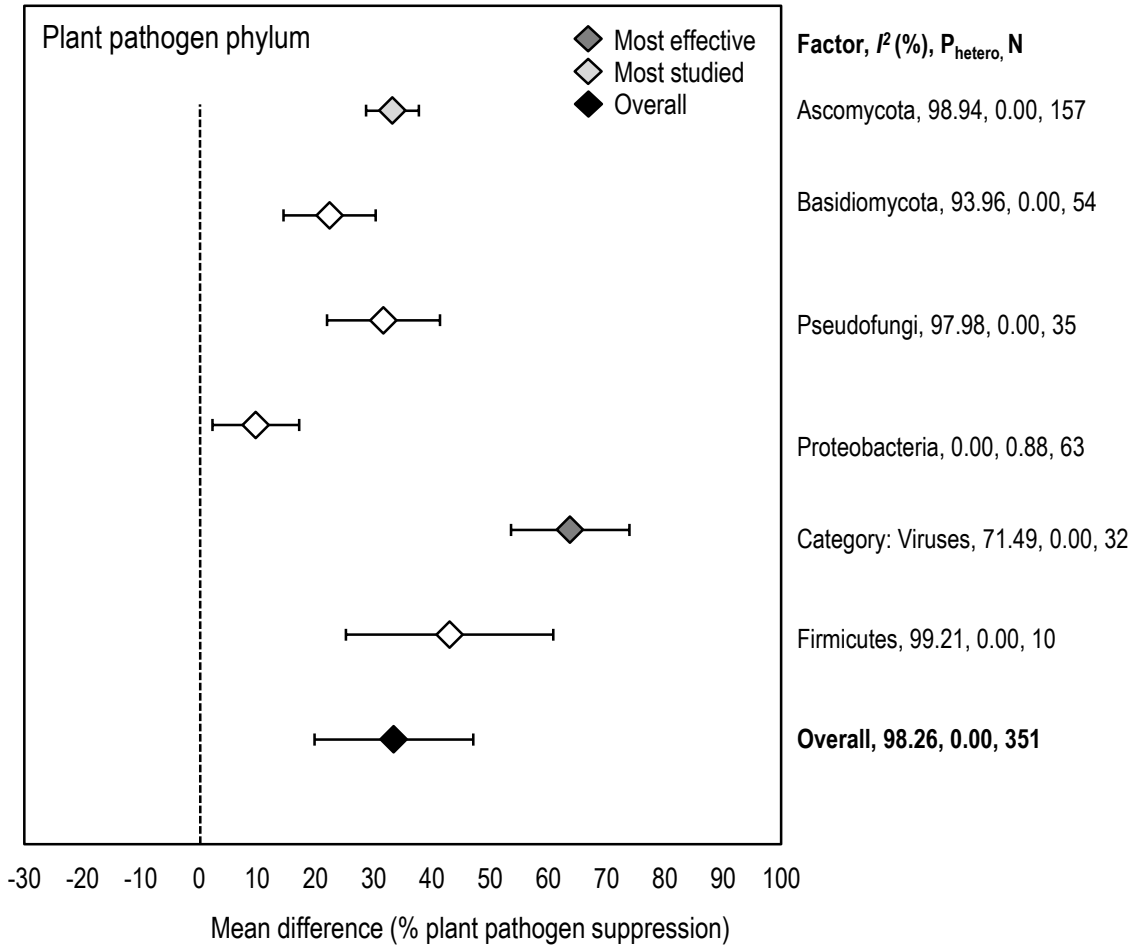


Figure 3.18. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant pathogen phylum.

Moderator: Comparison among studies on plant pathogen phylum in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant pathogen phylum) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

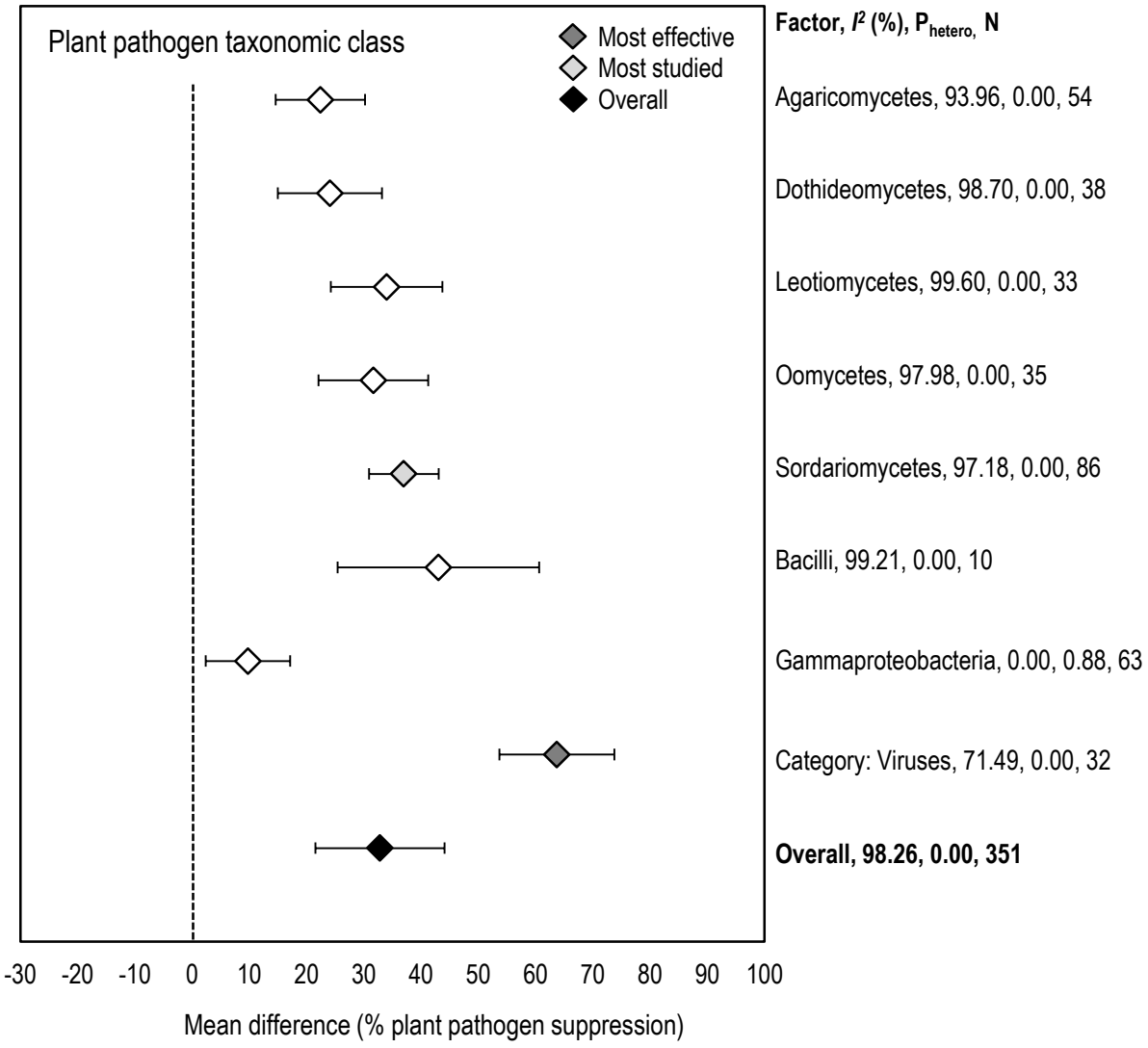


Figure 3.19. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B bassiana (Bb) analyzed with meta-analysis based on plant pathogen taxonomic class.

Moderator: Comparison among studies on plant pathogen taxonomic class used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant pathogen taxonomic class) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

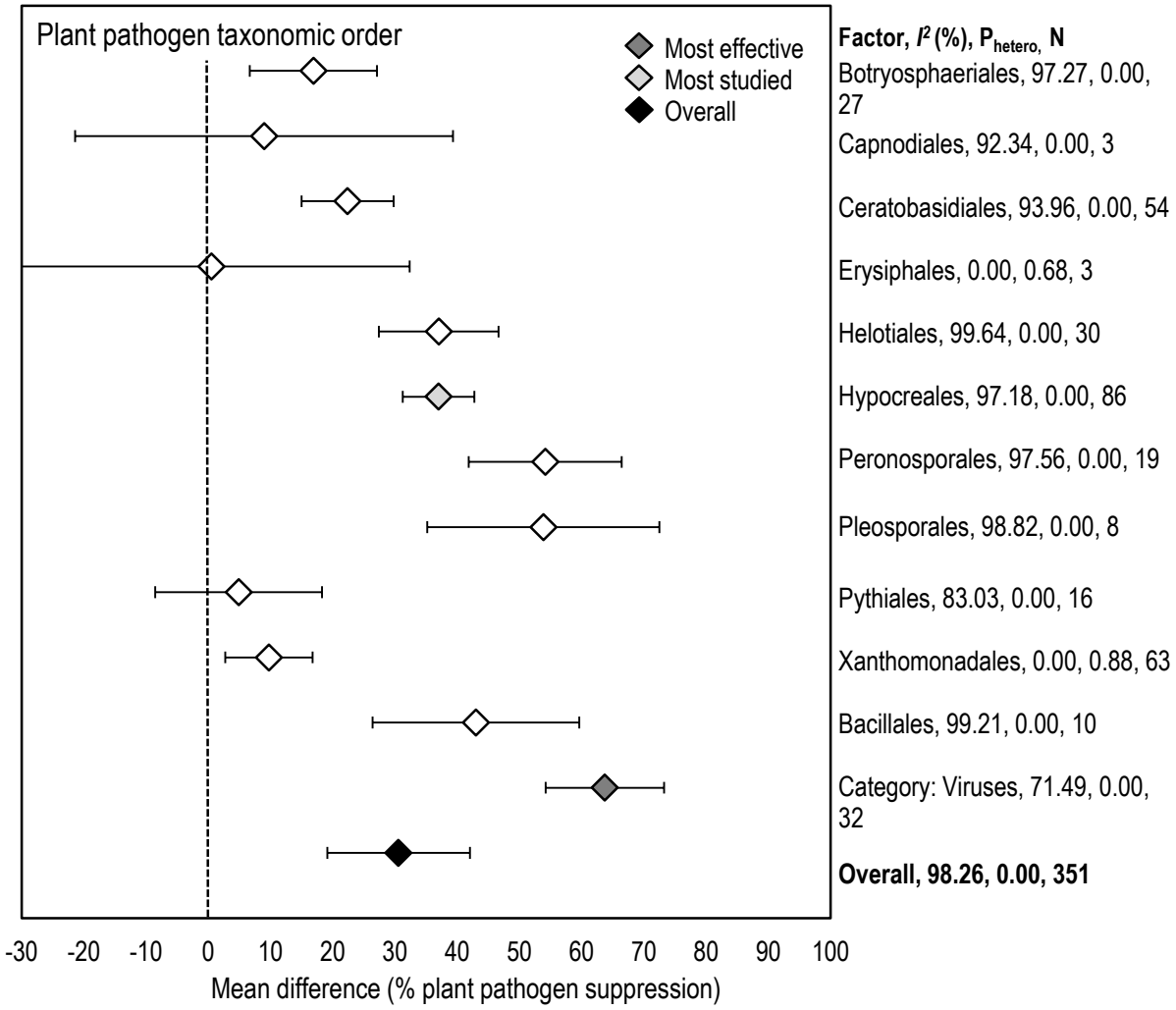


Figure 3.20. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant pathogen taxonomic order.

Moderator: Comparison among studies on specific Bb isolates used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant pathogen taxonomic order) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

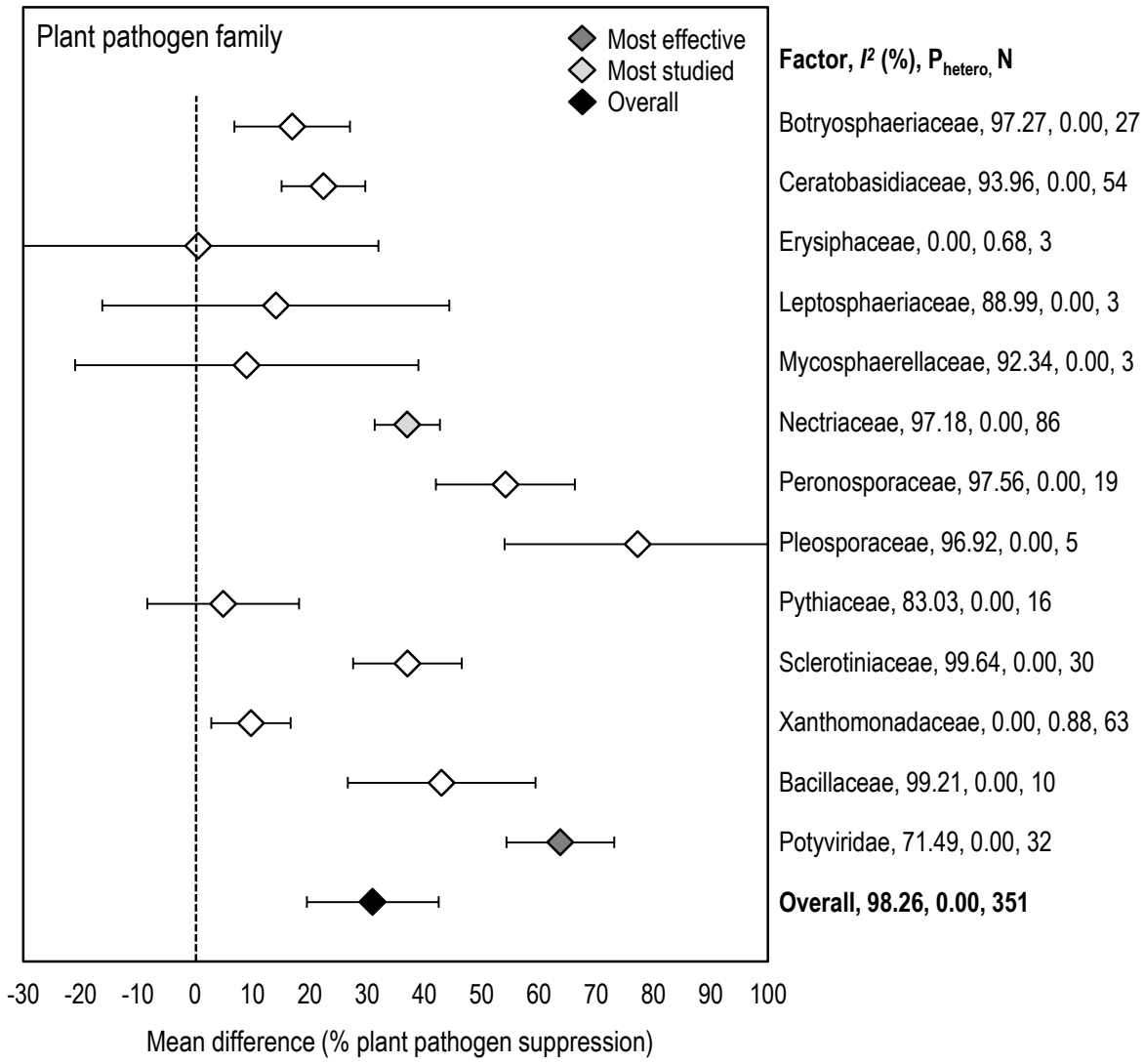


Figure 3.21. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant pathogen taxonomic family.

Moderator: Comparison among studies on plant pathogen taxonomic family used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant pathogen taxonomic family) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

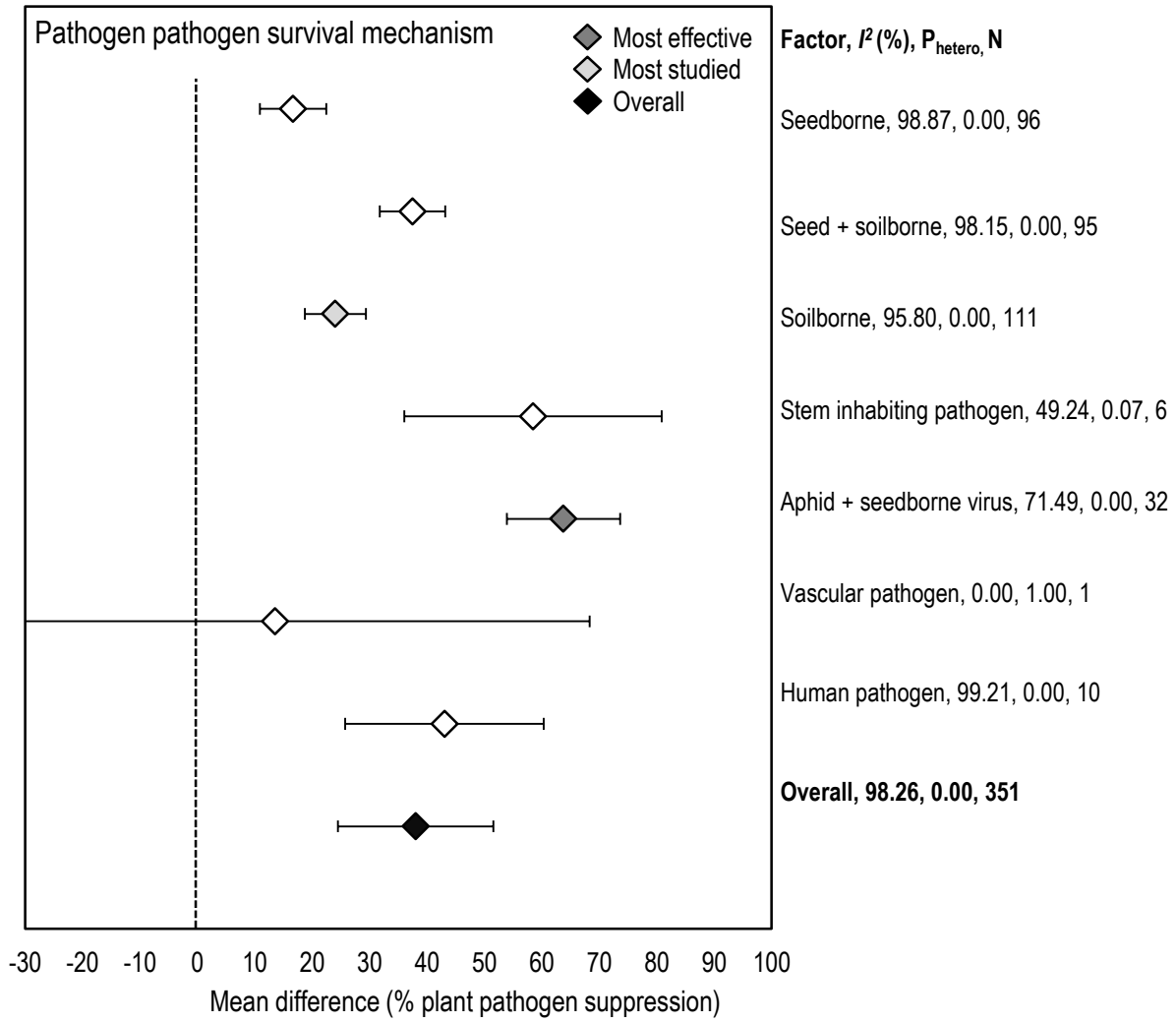


Figure 3.22. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on survival mechanism of plant pathogens.

Moderator: Comparison among studies on survival mechanism of plant pathogens used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant pathogen survival mechanism) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

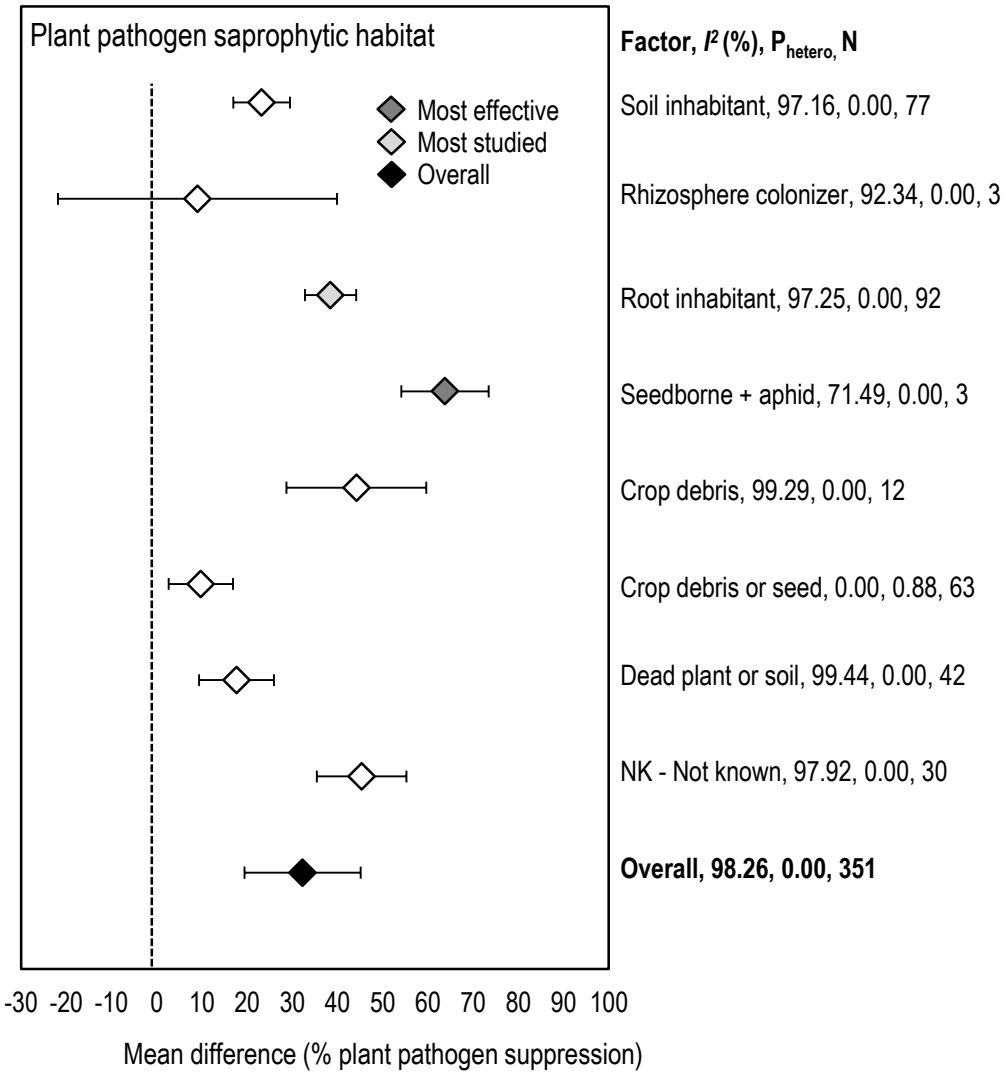
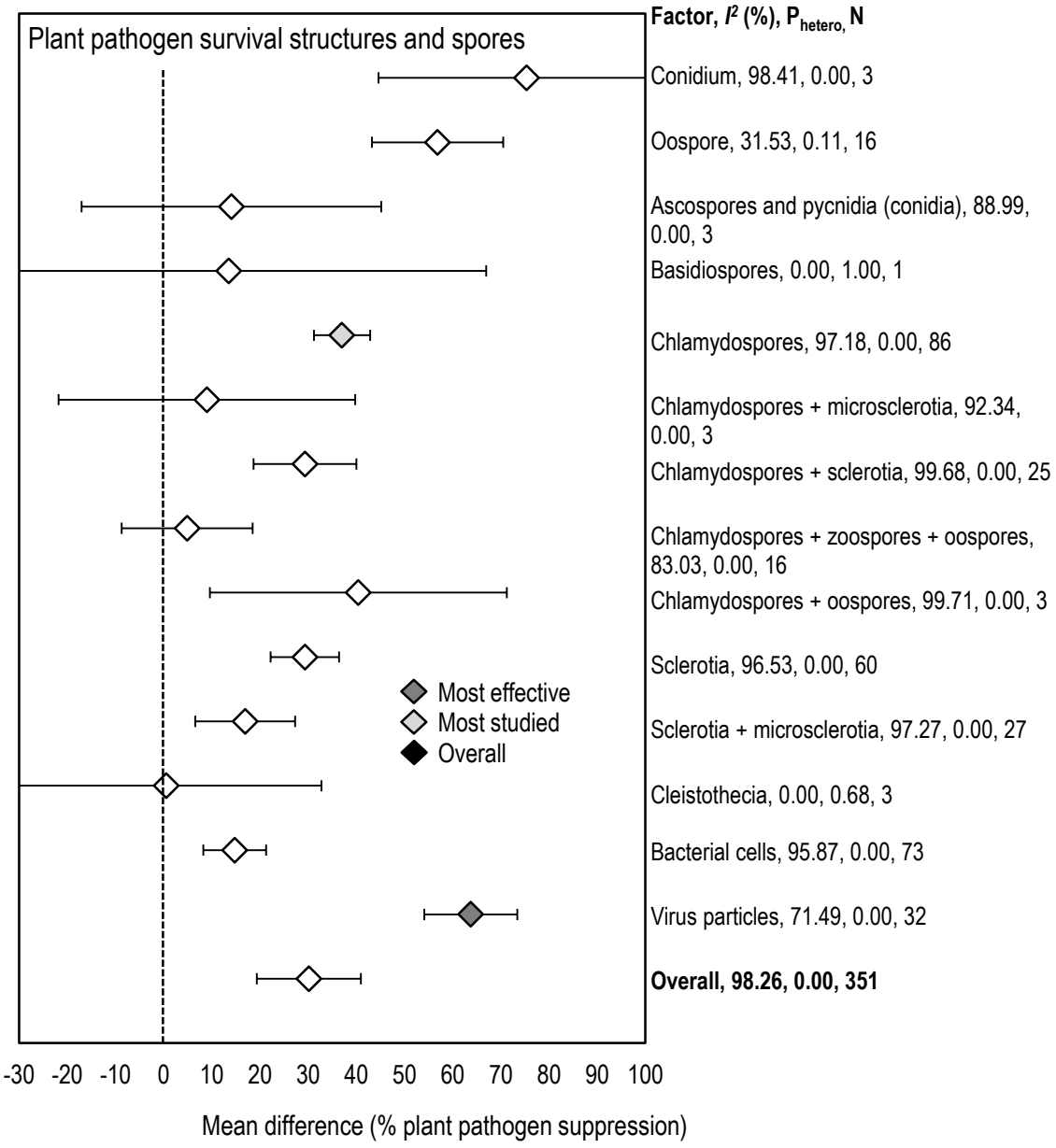


Figure 3.23. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant pathogen saprophytic lifestyle.

Moderator: Comparison among studies on specific Bb isolates used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant pathogen saprophytic lifestyle) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

Figure 3.24. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant pathogen resting stage.

Moderator: Comparison among studies on plant pathogen resting stage used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant pathogen resting stage) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.


Plant pathogen survival structures and spores
Factor, I^2 (%), P_{hetero}, N
Conidium, 98.41, 0.00, 3
Oospore, 31.53, 0.11, 16
Ascospores and pycnidia (conidia), 88.99, 0.00, 3
Basidiospores, 0.00, 1.00, 1
Chlamydospores, 97.18, 0.00, 86
Chlamydospores + microsclerotia, 92.34, 0.00, 3
Chlamydospores + sclerotia, 99.68, 0.00, 25
Chlamydospores + zoospores + oospores, 83.03, 0.00, 16
Chlamydospores + oospores, 99.71, 0.00, 3
Sclerotia, 96.53, 0.00, 60
Sclerotia + microsclerotia, 97.27, 0.00, 27
Cleistothecia, 0.00, 0.68, 3
Bacterial cells, 95.87, 0.00, 73
Virus particles, 71.49, 0.00, 32
Overall, 98.26, 0.00, 351
Most effective
Most studied
Overall
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant pathogen suppression)

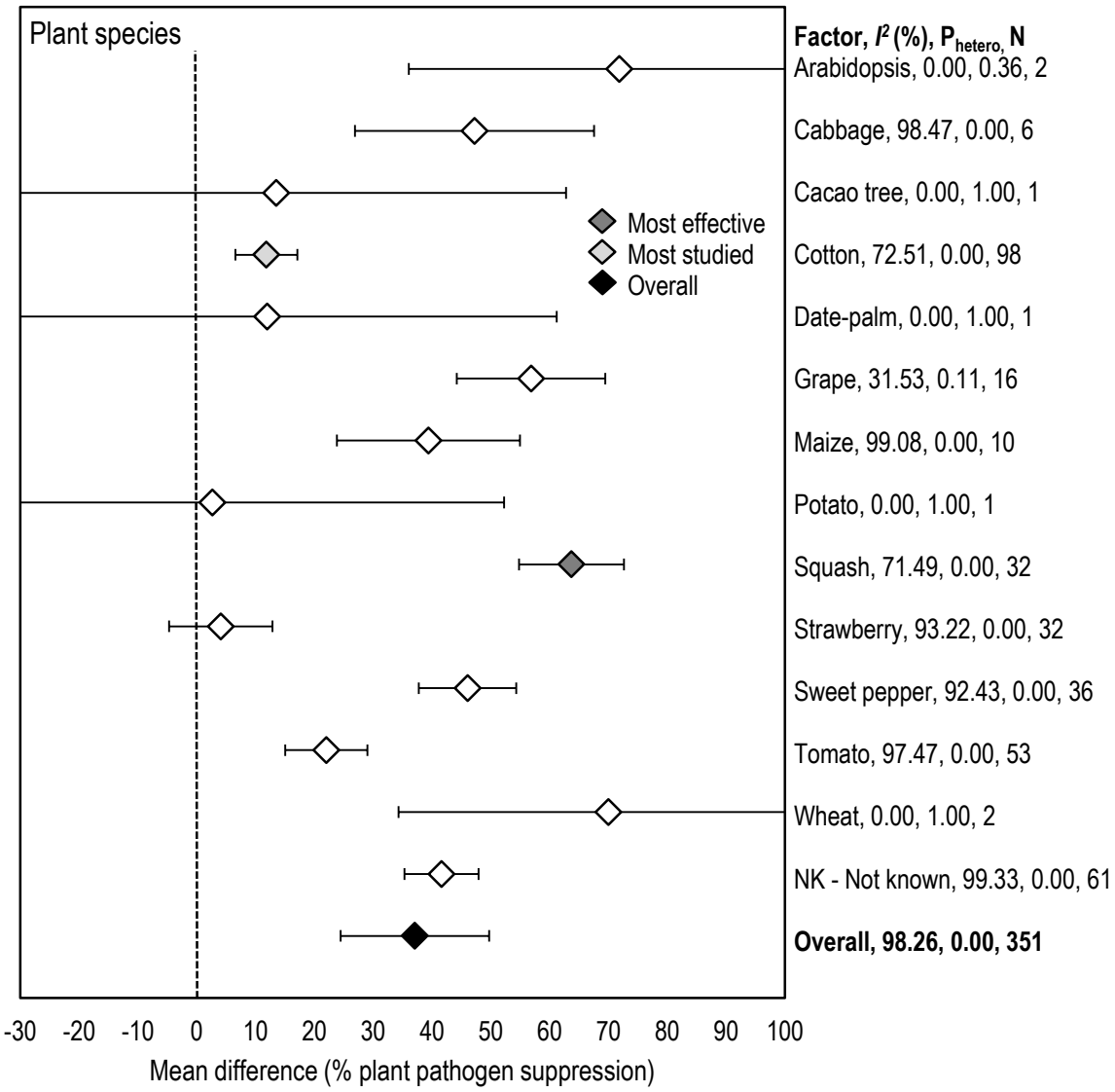


Figure 3.25. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant species.

Moderator: Comparison among studies on plant species used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant species) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

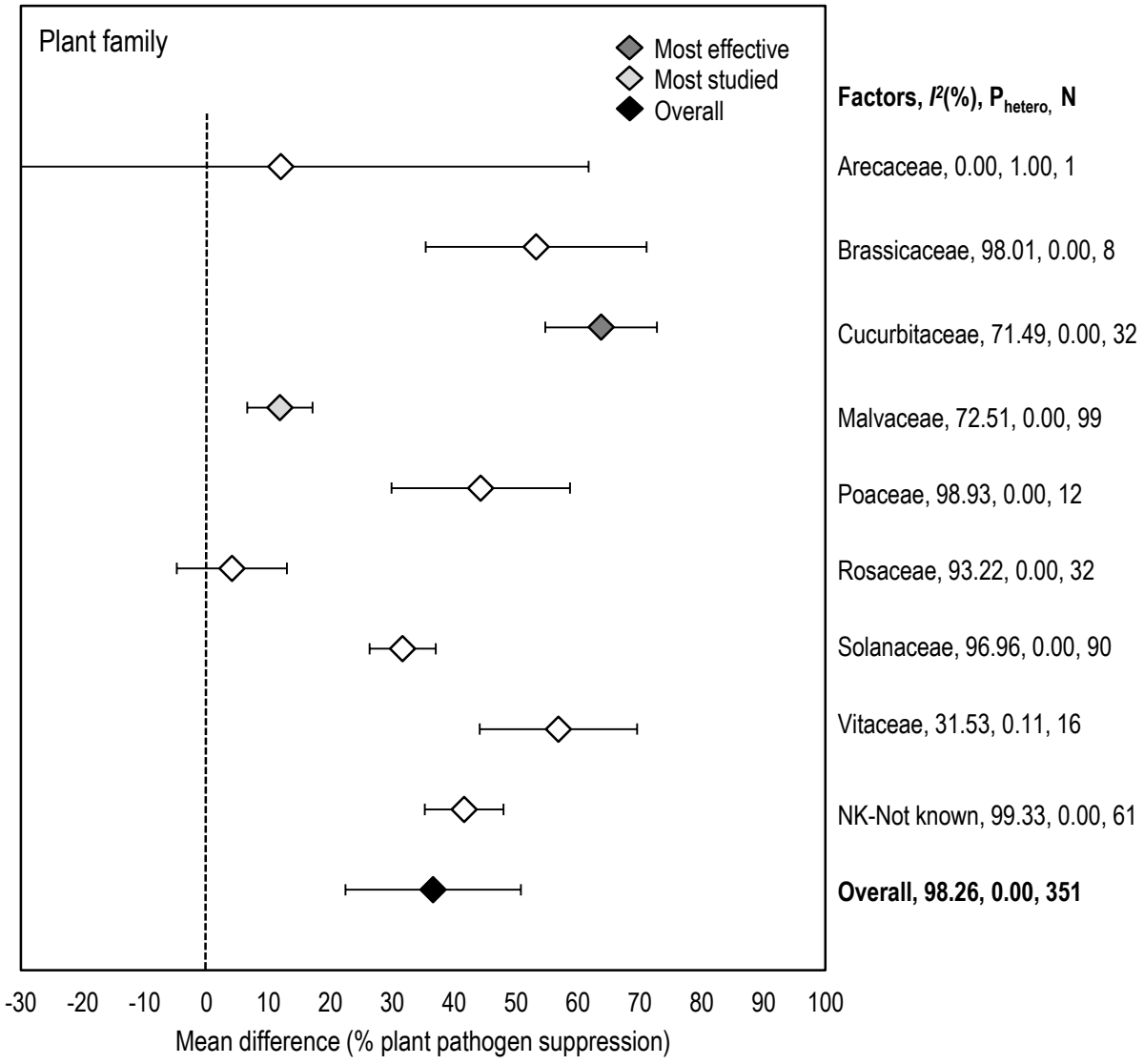


Figure 3.26. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on plant family.

Moderator: Comparison among studies on plant family used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (plant family) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

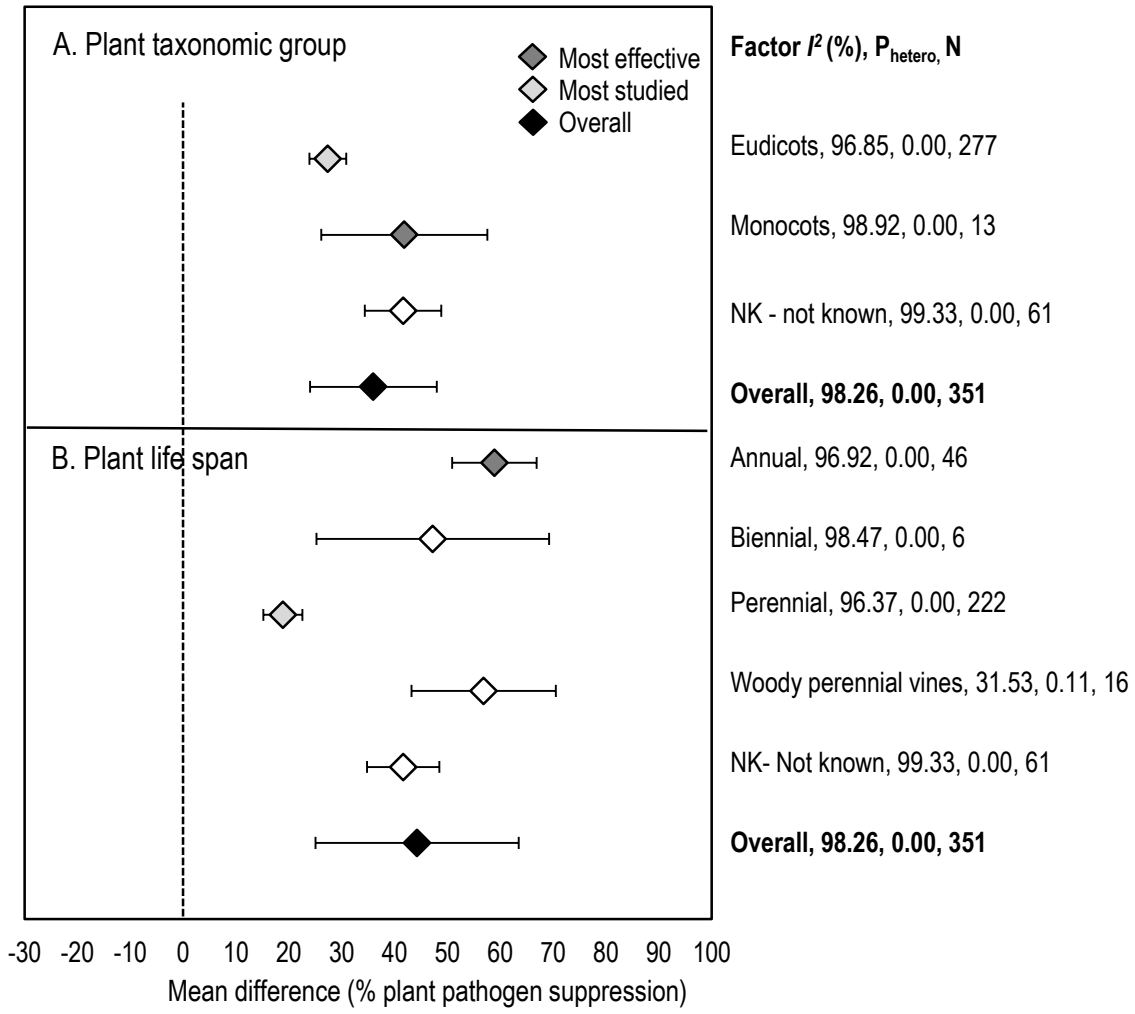
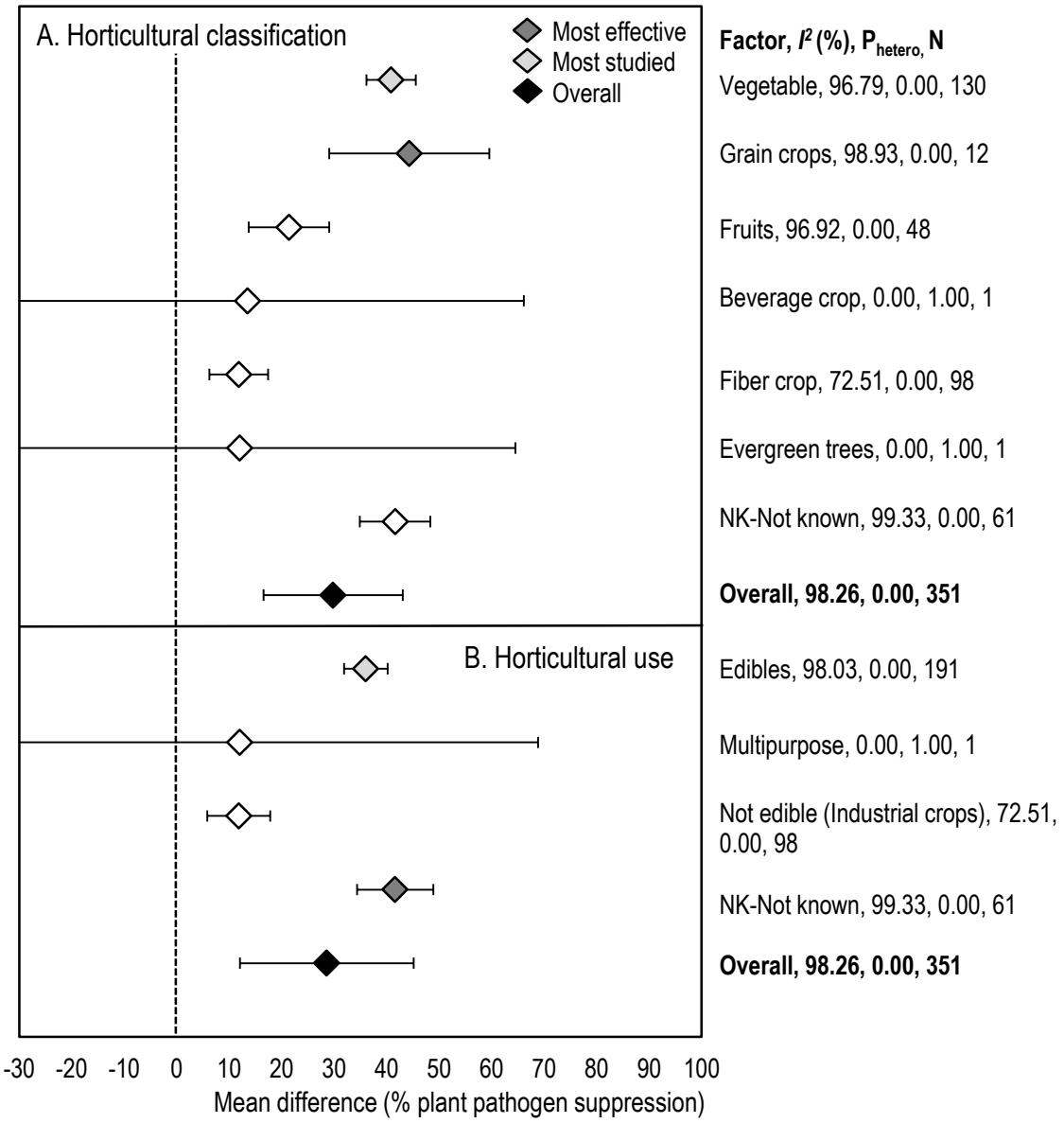


Figure 3.27. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on A) plant taxonomic group, and B) plant life span.

Moderator: Comparison among studies on plant taxonomic group (A), and plant life span (B) in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor [plant taxonomic group (A), and plant life span (B)] listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

Figure 3.28. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on A) horticultural classification and B) horticultural use.

Moderator: Comparison among studies on A) horticultural classification and B) horticultural importance of plants used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor [(A) horticultural classification and (B) horticultural importance] listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.



Factor, I^2 (%), P_{hetero}, N

Vegetable, 96.79, 0.00, 130

Grain crops, 98.93, 0.00, 12

Fruits, 96.92, 0.00, 48

Beverage crop, 0.00, 1.00, 1

Fiber crop, 72.51, 0.00, 98

Evergreen trees, 0.00, 1.00, 1

NK-Not known, 99.33, 0.00, 61

Overall, 98.26, 0.00, 351

Edibles, 98.03, 0.00, 191

Multipurpose, 0.00, 1.00, 1

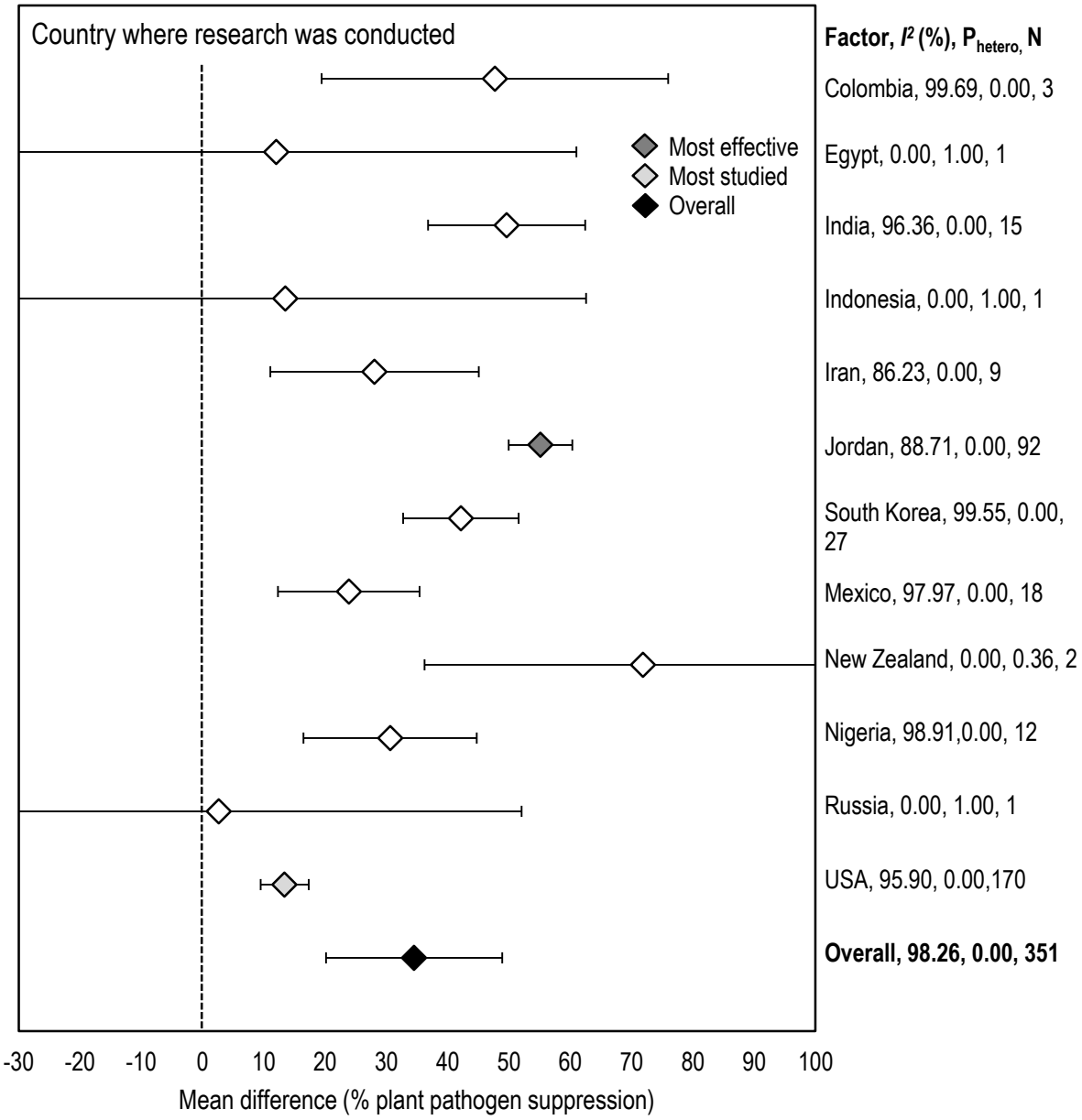
Not edible (Industrial crops), 72.51, 0.00, 98

NK-Not known, 99.33, 0.00, 61

Overall, 98.26, 0.00, 351

Figure 3.29. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on country where research was conducted.

Moderator: Comparison among studies on country where research was conducted in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (country where research was conducted) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.


Country where research was conducted
Most effective
Most studied
Overall
Factor, I^2 (%), P_{hetero}, N
Colombia, 99.69, 0.00, 3
Egypt, 0.00, 1.00, 1
India, 96.36, 0.00, 15
Indonesia, 0.00, 1.00, 1
Iran, 86.23, 0.00, 9
Jordan, 88.71, 0.00, 92
South Korea, 99.55, 0.00, 27
Mexico, 97.97, 0.00, 18
New Zealand, 0.00, 0.36, 2
Nigeria, 98.91,0.00, 12
Russia, 0.00, 1.00, 1
USA, 95.90, 0.00,170
Overall, 98.26, 0.00, 351
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant pathogen suppression)

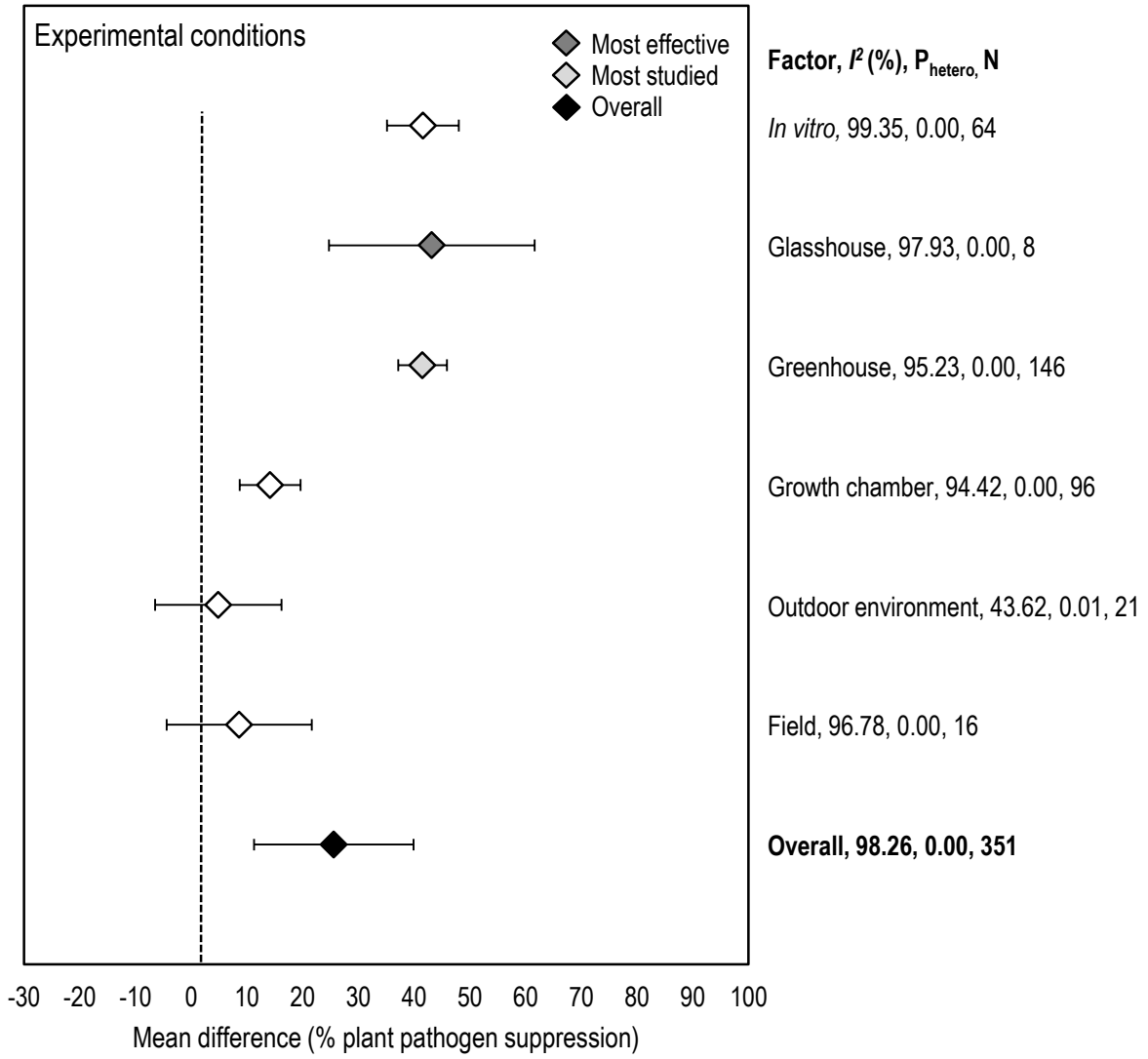
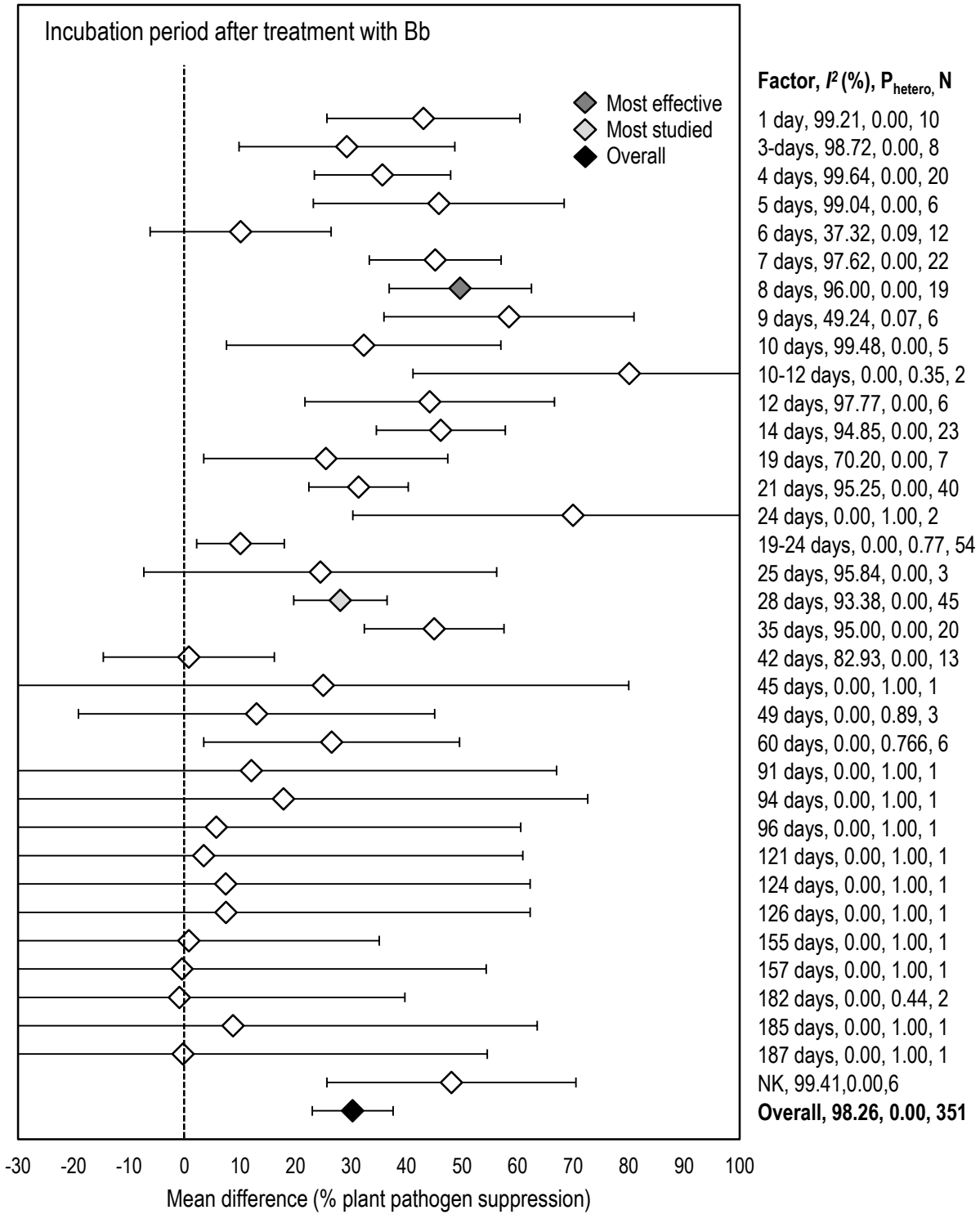


Figure 3.30. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on experimental conditions.

Moderator: Comparison among studies on experimental conditions used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (experimental conditions) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

Figure 3.31. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on incubation period after inoculation with Bb.

Moderator: Comparison among studies on incubation period after inoculation with Bb in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (incubation period after inoculation with Bb) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.


Incubation period after treatment with Bb
Most effective
Most studied
Overall
Factor, I^2 (%), P_{hetero}, N
1 day, 99.21, 0.00, 10
3-days, 98.72, 0.00, 8
4 days, 99.64, 0.00, 20
5 days, 99.04, 0.00, 6
6 days, 37.32, 0.09, 12
7 days, 97.62, 0.00, 22
8 days, 96.00, 0.00, 19
9 days, 49.24, 0.07, 6
10 days, 99.48, 0.00, 5
10-12 days, 0.00, 0.35, 2
12 days, 97.77, 0.00, 6
14 days, 94.85, 0.00, 23
19 days, 70.20, 0.00, 7
21 days, 95.25, 0.00, 40
24 days, 0.00, 1.00, 2
19-24 days, 0.00, 0.77, 54
25 days, 95.84, 0.00, 3
28 days, 93.38, 0.00, 45
35 days, 95.00, 0.00, 20
42 days, 82.93, 0.00, 13
45 days, 0.00, 1.00, 1
49 days, 0.00, 0.89, 3
60 days, 0.00, 0.766, 6
91 days, 0.00, 1.00, 1
94 days, 0.00, 1.00, 1
96 days, 0.00, 1.00, 1
121 days, 0.00, 1.00, 1
124 days, 0.00, 1.00, 1
126 days, 0.00, 1.00, 1
155 days, 0.00, 1.00, 1
157 days, 0.00, 1.00, 1
182 days, 0.00, 0.44, 2
185 days, 0.00, 1.00, 1
187 days, 0.00, 1.00, 1
NK, 99.41,0.00,6
Overall, 98.26, 0.00, 351
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100
Mean difference (% plant pathogen suppression)

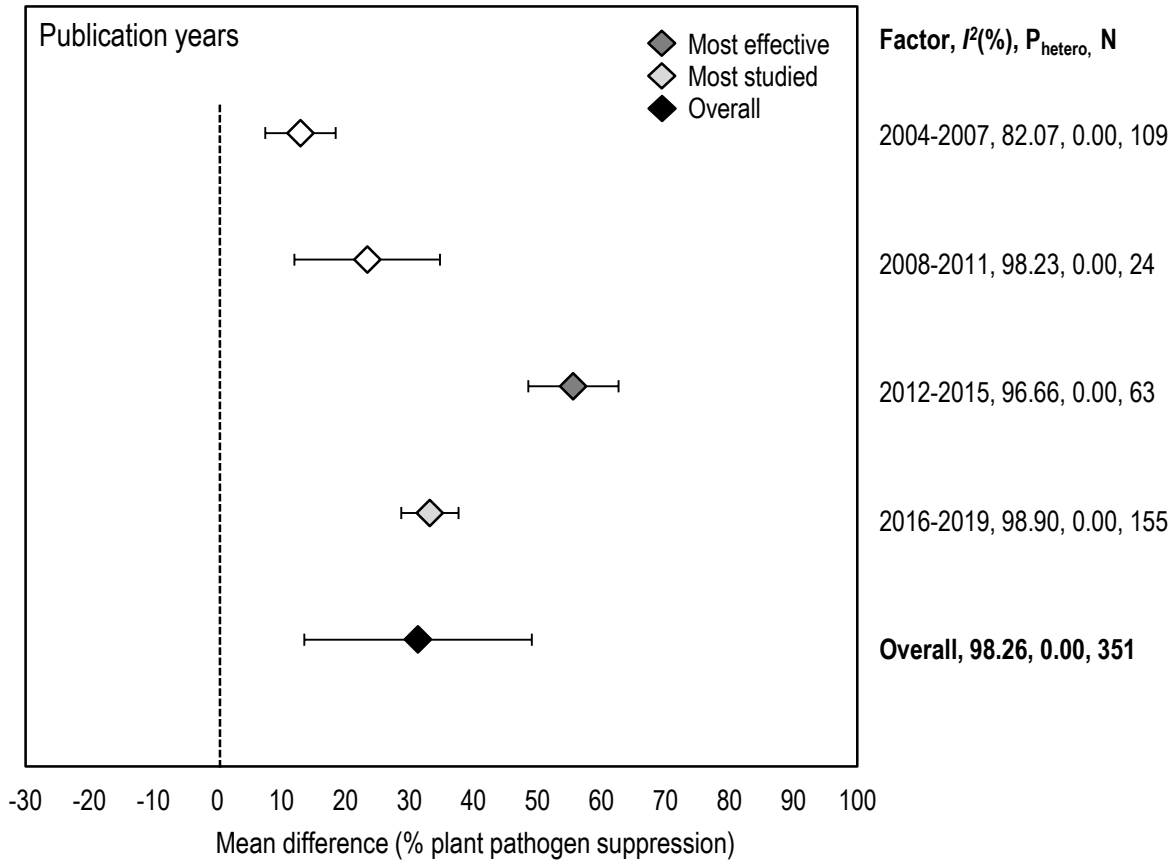
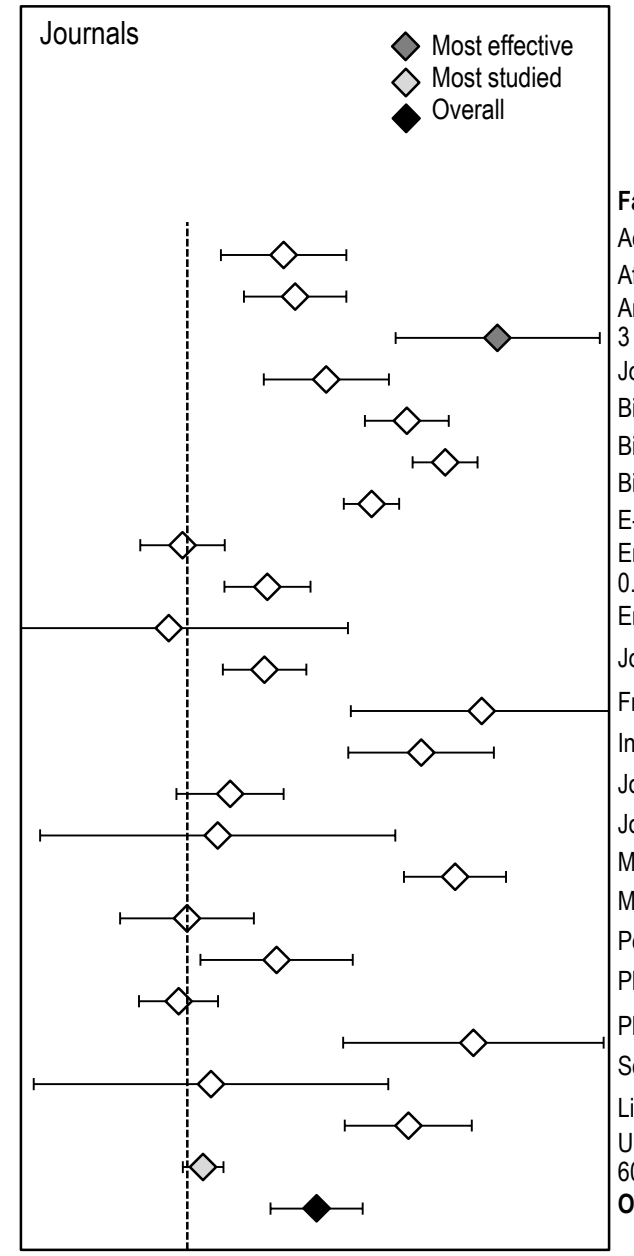


Figure 3.32. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on period of publication years for journal articles.

Moderator: Comparison among studies on period of publication years for journal articles used in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (period of publication years for journal articles) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.

Figure 3.33. Mean difference and 95% confidence intervals of percent plant pathogen suppression caused by endophytic B. bassiana (Bb) analyzed with meta-analysis based on journal where article was published.

Moderator: Comparison among studies on journal where article was published in plant disease and pathogen studies for each level of moderator. Values presented on the horizontal axis are mean difference in percent plant pathogen suppression (percent control – percent treatment with endophytic Bb). For each factor (journal where article was published) listed on the right, values are I^2 (percent heterogeneity due to true variation among moderator levels), P_{hetero} (test null hypothesis that all studies share a common effect size; when P>0.05 there is no significance; if P<0.05 there are significant differences at the moderator level), and N = number of studies. Values with $I^2 = 0$, or confidence interval values <0 or >100, are non-significant and have no Bb effect on percent pathogen suppression.



Factor, I^2(%), $P_{hetero,}$ N

Acta Biologica Szegediensis, 86.23, 0.00, 9

African Journal of Microbiology Research, 98.91, 0.00, 12

Archives of Phytopathology & Plant Protection, 98.41, 0.00, 3

Journal of Asia-Pacific Entomology, 99.43, 0.00, 8

BioControl, 97.42, 0.00, 19

Biocontrol Science and Technology, 71.49, 0.00, 32

Biological Control, 91.77, 0.00, 42

E-Journal of Entomology and Biologicals, 95.59, 0.00, 20

Emerging Concepts in Plant Health Management, 94.35, 0.00, 20

Entomologia Experimentalis et Applicata, 0.00, 1.00, 1

Journal of Environmental Biology, 97.97, 0.00, 18

Frontiers in Microbiology, 0.00, 0.36, 2

Indian Journal of Agricultural Sciences, 49.24, 0.07, 6

Journal of Invertebrate Pathology, 95.97, 0.00, 12

Journal of Applied Biological Sciences, 0.00, 1.00, 1

Mycoscience, 99.43, 0.00, 12

Mycobiology, 81.18, 0.00, 7

Pest Management Science, 0.00, 0.76, 6

Plant Health, 43.62, 0.01, 21

Planta, 0.00, 1.00, 2

Scientific Journal of King Faisal University, 0.00, 1.00, 1

Lincoln University Doctoral dissertation, 98.45, 0.00, 8

University of Tennessee, Knoxville Doctoral dissertation, 60.15, 0.00, 89

Overall, 98.26, 0.00, 351

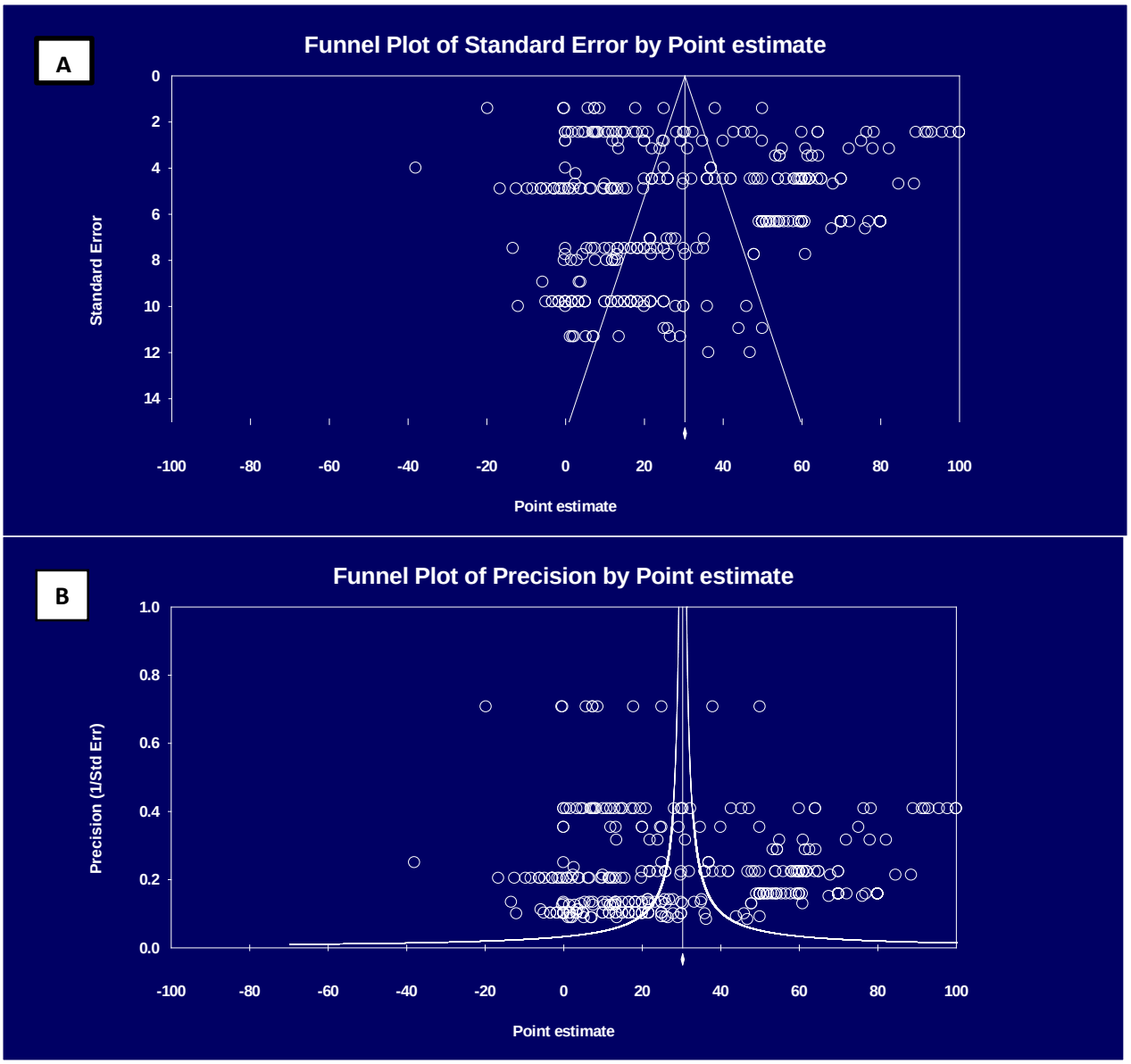


Figure 3.34. Publication bias represented in the form of funnel plots for (A) Standard error by point estimate, and (B) Precision by point estimate.

The horizontal axis shows point estimate or mean differences (%) at 95% confidence intervals for endophytic *B. bassiana* (Bb) effect on percent plant pathogen suppression analyzed with meta-analysis extracted from different research studies. The vertical axis represents (A) Standard error or (B) Precision (1/Standard error). The circles represent each individual study (total = 351).

CHAPTER 4

ASSESS IMPACT OF ENDOPHYTIC *BEAUVERIA BASSIANA* ON ROOT-KNOT NEMATODE IN GREENHOUSE-GROWN TOMATO PLANTS, AND EFFECT OF *B. BASSIANA* CONIDIA ON ROOT-KNOT NEMATODE HATCH AND JUVENILE SURVIVAL IN *IN VITRO* ASSAYS

Abstract

Beauveria bassiana (Bb) has been reported as an endophyte of several plant species. It has also been shown to protect host plants against insect pests and plant pathogens, but much less is known about its activity against plant-parasitic nematodes. To determine the effect of *B. bassiana* isolate 11-98 on *Meloidogyne incognita* (southern root-knot nematode), *in vitro* and *in vivo* bioassays were conducted. An *in vitro* assay was performed with different ratios of *B. bassiana* conidia, root knot nematode (RKN) eggs, and water, and the mixtures were incubated for short and long time periods (day 1 to day 22). Data collected included percentages of hatched and unhatched RKN eggs, and percentages of mobile and immobile nematodes. Treatments were: 1) Control – Bb conidia: RKN egg ratio = 0.00; 2) ratio = 0.42; 3) ratio = 1.27; 4) ratio = 2.1; 5) ratio = 2.93; and 6) ratio = 3.78. *In vitro* assay results showed that with increased incubation time, there was a trend toward increased percentages of egg hatch, and mobile and immobile nematodes, and a decreased percentage of unhatched eggs. However, overall there was no significance noted among treatments except for a few incubation times. A gnotobiotic assay (repeated once) was conducted to confirm that Bb 11-98 could establish as an endophyte in 'Rutgers' tomato, and to compare colonization patterns of Bb 11-98 in 'Mountain Spring' tomato. In the gnotobiotic assay, percent Bb colonization was greater in stems and leaves compared to roots in both tomato cultivars. Bb colonization was also equal to or higher in 'Rutgers' than in 'Mountain Spring'. A greenhouse experiment was conducted with four treatments 1) Bb seed coat alone; 2) Bb seed coat + RKN; 3) RKN alone; 4) Control – no Bb or RKN. In addition to seed coating with Bb, Bb was applied as a soil drench (to Bb treatments) and RKN was inoculated to soil at 5 wk after seed sowing. Data was collected at 30 DAT (days after

treatment with nematode or Bb soil drench) and 60 DAT, and the experiment was repeated once. At 30 DAT and 60 DAT, shoot height (cm), shoot weight (g), root weight (g), and root galling index were evaluated. Exclusively at 60 DAT, fruit weight (g), fruit number, flower number, root system length (cm) and egg count per root system were evaluated. There were no differences among treatments for shoot height, shoot weight, and root weight at both incubation periods in both trials, except for root weight at 60 DAT in both trials. Similarly, there were no significant differences in fruit weight, fruit number, flower number, and root system length at 60 DAT in both trials, except for root system length in trial 2. There was significance among treatments for root galling and egg count/root system in both trials and both incubation times. Interestingly, higher root galling and egg count/root were recorded in RKN + Bb treatments followed by RKN alone, suggesting that Bb may stimulate nematode activity. Further, an *in vitro* nematode assay was conducted to evaluate the egg hatching rate in two treatments (RKN and RKN + Bb) with eggs extracted from greenhouse nematode studies. The experiment was conducted in a 96-well plate with two trials and 6 to 10 replicates per trial. Data were collected on percentages of hatched and unhatched eggs, and immobile and mobile nematodes. In the assay, in all treatments, as incubation time increased, there was a trend towards increased percent egg hatch, and immobile and mobile nematodes, and decreased percent unhatched RKN eggs. Overall, differences between treatments were not significant for percent egg hatch or unhatched eggs, except day 4 of trial 1. However, at most time points, there was a significant difference in percent mobile and immobile nematodes in trial 1, but not in trial 2. In trial 1, percent mobile RKN were greater with Bb treatment than RKN alone, and percent immobile RKN were greater with RKN alone than with Bb treatment, again suggesting a

stimulatory role for Bb. Viable conidia in the egg extraction water were confirmed by plating aliquots of suspension from RKN + Bb treatments onto Beauveria Selective Medium, followed by observation of Bb colonies. In conclusion, results suggest that Bb 11-98 does not inhibit southern root-knot nematode on 'Rutgers' tomato, and in fact, may have a stimulatory effect on RKN activity, as evidenced by increased root galling and egg counts. More research is needed to investigate the possible reasons and triggering factor responsible for increased RKN populations in the presence of endophytic Bb in 'Rutgers' tomato.

Introduction

The plant rhizosphere is dominated by fungi, bacteria, nematodes, and other organisms that frequently encounter one another (Ingham, 1988). Plant-parasitic nematodes are soft-bodied, slender round worms that feed on plant roots (Lambert and Bekal, 2002; Hol and Cook, 2005), and cause interruptions in nutrient and water flow, leading to severe crop damage and yield losses (Sasser and Freckman, 1987; Nicol et al., 2011; Schouteden et al., 2015; Bernard et al., 2017). The mechanical wounds on roots made by nematodes favor entry of secondary pathogens such as fungi, bacteria, and viruses.

More than 4,100 species of plant parasitic nematodes have been identified (Decraemer and Hunt, 2006) and economic losses due to crop damage have been estimated at $80 to $118 billion dollars per year (Nicol et al., 2011; Sasser and Freckman, 1987). Of all identified nematodes, only 15% cause significant economic crop losses (Koenning et al.,1999; Bernard et al., 2017). Genera of economic importance in the U.S. are *Meloidogyne*, *Heterodera*, *Pratylenchus*, *Hoplolaimus*, *Xiphinema* and *Rotylenchulus.* (Koenning et al., 1999; Bernard et al.,

2017). *Meloidogyne incognita*, the southern root-knot nematode (RKN), has been reported as the most devastating threat to agricultural crop production (Postnikova et al., 2015).

Meloidogyne incognita is cosmopolitan in distribution. It is a sedentary nematode that establishes a permanent feeding site within the plant host root to obtain nutrients and complete its lifecycle (Koenning et al., 1999; Bernard et al., 2017). Moreover, *M. incognita* has a wide host range (Bernard et al., 2017) and can cause plant disease complexes with fungi and bacteria (Shalini et al., 2014). This nematode causes significant crop yield loses, and is a major threat to global food security (Bernard et al., 2017).

Nematicides are often used to control plant parasitic nematodes, but they can cause enormous environmental pollution, hence we need an alternative, sustainable, ecofriendly management strategy to control plant-parasitic nematodes and reduce the pollution impact of nematicides. Identification and implementation of endophyte-host resistance towards plant-parasitic nematodes could be a potential approach (Bernard et al., 2017).

Recently, there have been several studies on plant protection against diseases with *Beauveria bassiana* (Balsamo) Vuillemin (Bb). *Beauveria bassiana* is an entomopathogenic fungus (Nirupama, 2014) that can act as an endophyte in plants (Bing and Lewis, 1991). Since the report of Bing and Lewis (1991) on the endophytic nature of Bb in maize, several other studies have reported on the efficacy of this fungus against insects and pathogens after artificial introduction to plants. Akutse et al. (2013) evaluated the endophytic nature of Bb on *Vicia faba* and *Phaseolus vulgaris*, and found that it successfully colonized both plant species. Posada et al. (2007) inoculated coffee seedlings with Bb using different methods, and isolated the fungus from distant parts after inoculation, further confirming Bb as endophyte. They also detailed

endophyte movement in the plant. Rondot and Reineke (2013) attempted to establish Bb in grape, by spraying a spore suspension on the grape leaf surface, and obtained successful colonization. Other researchers have demonstrated the endophytic ability of Bb in other plants, including potato, tomato (Leckie, 2002; Ownley et al., 2008; Gurulingappa et al., 2010), snap bean (Ownley et al., 2008), tobacco and soybean (Russo et al., 2015), wheat (Gurulingappa et al., 2010; Russo et al., 2015), common bean (Parsa et al., 2013), banana (Akello et al., 2007), bean, corn, pumpkin (Gurulingappa et al., 2010), sorghum (Tefera and Vidal, 2009), and cowpea (Maketon et al., 2013). Bb has been reported as an endophyte in jimsonweed and cocklebur (Jones 1994), cotton (Jones 1994; Ownley et al., 2008; Gurulingappa et al., 2010), cocoa (Evans et al., 2003; Posada and Vega, 2005), American hornbeam (Bills and Polishook, 1991), date palm (Gomez- Vidal et al., 2006; Arab and El-Deeb, 2012), jute (Biswas et al., 2011), opium poppy (Quesada - Moraga et al., 2006), and pine (Ganley and Newcombe, 2005; Brownbridge et al., 2012).

As an endophyte, Bb not only protects the host plant against insect pests (Rondot and Reineke, 2013), it can provide protection against plant diseases (Ownley et al., 2010). However, little is known about the effectiveness of endophytic Bb against plant-parasitic nematodes. Bb produces various secondary metabolites such as beauvericin, bassianin, bassianolides, bassiacridin, beauverolides, and oosporein (Strasse et al., 2000), which have antifungal and antibacterial activities. Further investigation is needed to determine if these secondary metabolites have potential nematicidal properties. This approach offers exciting possibilities for developing sustainable plant protection strategies utilizing an entomopathogenic fungus for management of nematode diseases (Zhao et al., 2013; Liu et al., 2008).

In the current paper, the potential of reducing egg hatch and root galling of root-knot nematodes on tomato was investigated with *in vitro* and greenhouse assays. An isolate of Bb (11-98), demonstrated to be an endophyte and reduce plant disease in 'Mountain Spring' tomato was used. The specific objectives were the following.

1. Determine and compare the ability of Bb 11-98 to endophytically colonize two tomato cultivars, Rutgers and Mountain Spring. *Hypothesis*: there will be differences in the colonization patterns of the two cultivars.

2. Evaluate the effect of Bb conidia on the health of nematode eggs and juveniles over long and short incubation times. *Hypothesis*: The percentage egg hatch and mobile nematodes will be lower with Bb treatment than in endophyte-free controls.

3. Determine the impact of endophytic Bb in tomato roots on root galling and egg number per root system caused by *M. incognita* in greenhouse experiments. *Hypothesis*: Root galling and egg number per root will be significantly lower on tomato plants colonized by endophytic Bb, compared to endophyte-free control tomato plants.

The overall goal of this research is to develop a comprehensive plant protection program against plant-parasitic nematodes through use of a novel fungal plant-endophytic association with Bb.

Materials and Methods

Endophytic Bb and culture media

An endophytic, entomopathogenic isolate of Bb was used in this study for all experiments. Bb isolate 11-98 is in the fungal collection of Dr. Bonnie Ownley, University of Tennessee, Knoxville. For these experiments, the fungus was grown on Sabouraud dextrose agar (SDA)

(Difco, Sparks, MD) or Beauveria selective medium (BSM; Doberski and Tribe, 1980). The latter was used for confirmation of the fungus from plant tissue. BSM was prepared with glucose (32 g), neopeptone (8 g), agar (12 g) and crystal violet (0.008 g) in 800 ml of deionized water. The medium was autoclaved for 45 min, cooled, and cycloheximide (0.2 g/4 ml) and chloramphenicol (0.4 g/4 ml) were added before pouring the plates. SDA was prepared according to manufacturer's instructions. The fungus was grown on SDA and incubated at 25^{o}C for approximately for 3 to 4 weeks for production of conidia. Dry conidia were harvested from the surface of BSM culture plates with a camel-hair brush, and stored in glass vials in a desiccator and refrigerated (~4^{o}C) until needed for experiments.

Tomato cultivars

Tomato seed was purchased from Park Seed, Greenwood, SC. The seed germination rate for each lot of seed was 85% for 'Rutgers' and 88% for 'Mountain Spring'.

Meloidogyne incognita

The culture of southern root-knot nematode (RKN) used in all experiments was obtained from Dr. Ernest Bernard (University of Tennessee, Knoxville), and was maintained on 'Rutgers' tomato plants in the greenhouse.

Gnotobiotic colonization assay with Bb 11-98 and two tomato cultivars

A gnotobiotic assay was performed to confirm the endophytic ability of *B. bassiana* 11-98 in Rutgers and Mountain Spring tomato cultivars.

Seed treatment

Beauveria bassiana 11-98 was applied to 'Rutgers' and 'Mountain Spring' tomato seeds as follows. A methyl cellulose solution was prepared with 2 g methyl cellulose (Sigma Chemical, St.

Louis, MO) dissolved in 100 ml hot, autoclaved, deionized water in a 250-ml beaker. The solution was stirred on a hot plate stirrer until a uniform, cloudy suspension was obtained, and then the solution was placed in an ice bath on a stirrer (15-20 min) until the solution was clear. The mass of dry Bb 11-98 conidia used was based on the number of seeds to be treated and the desired concentration of conidia per seed. Conidia were suspended in sterile 2% methyl cellulose solution containing 0.02% Tween 20 (USB, Cleveland, OH). Seeds were coated with Bb 11-98 based on a ratio of 2 g seed: 1 ml 2% methyl cellulose + Bb. Seeds were stirred until the coating of conidia appeared uniform and had started to dry. Coated seeds were spread on aluminum foil and air-dried in a biological safety cabinet for approximately 3 h. Dry, coated seeds were placed in a glass vial for storage at 4^{o}C with a desiccant until needed. A replicated sample of treated seed was used to determine the density of Bb conidia per seed by standard dilution plating; 10 seed were used per cultivar. Dilutions were plated onto BSM. The number of conidia per seed was ~log 7.

Gnotobiotic assay

The experiment was replicated and repeated once. The treatments were Bb-treated seed and control seed treated with 2% methyl cellulose sticker only, for both cultivars. There were 17 replicate seeds for Trial 1 and 14 replicate seeds for Trial 2 of each treatment for each tomato cultivar. Culture tubes (24-mm outside diameter and 15-cm length) were filled with 20 cm^3 vermiculite (Palmetto Vermiculite Co., Medium A-2, Woodruff, SC) and 20 ml of deionized water. Tubes were sealed with plastic caps, sterilized by autoclaving for 1 h on each of two consecutive days, cooled, and then transferred to a biosafety cabinet. Culture tubes were uncapped and one seed was placed approximately 0.5 cm below the surface of the vermiculite

for each treatment/cultivar combination. Culture tubes were completely randomized within the tube rack. Tubes were recapped and placed in a growth chamber (Baxter Scientific Products, Deerfield IL) with continuous light for 72 h at 25^{o}C. The tubes were then transferred to a walk-in growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) at 25^{o}C with a 18/6 light-dark regimen. Planted seeds were maintained for 21 days and percent germination was recorded.

Isolation of Bb from tomato seedlings

After seedlings reached the 2 true-leaf stage (21 days after planting), endophyte presence was determined with a cultural dependent method. Seven seedlings of each treatment/cultivar combination were randomly selected from each trial and removed from tubes. Roots were rinsed free of vermiculite, and each plant was wrapped in a moistened paper towel and labeled. Each seedling was surface-sterilized with 95% ethanol for 1 min, followed by 10% bleach (Clorox) for 1 min, and sterile deionized water for 1 min, and then placed on sterile paper towels to remove excess moisture.

Surface-sterilized tomato seedlings were aseptically cut into 1-cm sections (leaf, stem, and root) and placed on BSM. Five pieces of each sample (leaf, root, stem) were placed equidistant from each other on BSM, plates were sealed with Parafilm, and incubated in darkness at 25°C. Plates were observed daily for emergence of the endophytic fungus from the plant sections. Plant pieces exhibiting presence of Bb were transferred to new plates containing fresh BSM. Presence or absence of fungal growth was recorded for each plant piece and colonies were examined microscopically for conidiophore and conidial characteristics. Percent colonization was calculated according to the formula of Akutse et al. (2013), where

$$\text{Percent colonization } = \left(\frac{\text{Number of sections exhibiting fungal growth}}{\text{Total number of pieces plated}}\right) x100$$

In addition to microscopic observation of the fungus, identification of Bb was confirmed with a cultivation independent method. Genomic DNA was extracted from 7-day-old cultures of the fungus isolated from samples of treated 'Rutgers' and Mountain Spring' tomato seedlings in the gnotobiotic assay using Phire Plant Direct PCR Master Mix (ThermoFisher Scientific, Waltham, MA) and ITS primers 1 and 2. The presence of a PCR product was confirmed with gel electrophoresis and SYBR Safe DNA gel stain (ThermoFisher Scientific). For sequencing, the PCR amplicons were cleaned of excess primer and unincorporated nucleotides with ExoSAP-IT (ThermoFisher Scientific) following the manufacturer's directions. Samples were sequenced at the University of Tennessee, Molecular Biology Resource Facility. Sequences obtained were submitted to the NCBI Nucleotide Blast alignment tool and identification of Bb was verified by comparisons with known Bb sequences in the nucleotide database.

Statistical analysis: The gnotobiotic assay was arranged in a completely randomized design in the growth chamber. For analysis of colonization data, the study was a 2 × 3 factorial with two cultivars and three tissue types. Data was analyzed for significance with mixed model ANOVA in SAS 9.4. Least significant difference (LSD) was used to determine significant mean differences ($P \leq 0.05$).

***In vitro* Bb conidia and RKN egg bioassay**

The *in vitro* Bb conidia + RKN egg assay had six treatments with different concentrations of Bb conidia paired with a standard number of RKN eggs in a set volume of fluid. This scheme resulted in six different ratios of Bb conidia to RKN eggs. The concentration of a solution of Bb

conidia was estimated with a hemocytometer as 1.26 x 10^7 conidia/ml. A concentration of log 7 was confirmed with standard dilution plating onto BSM, followed by colony counts. The ratio of Bb conidia to RKN eggs was determined as follows.

$$Ratio = (\frac{\text{Number of Bb conidia}}{\text{Number of RKN eggs}})/\text{Total volume (ml) of water in bioassay}$$

The six treatment ratios of Bb conidia to RKN eggs in the bioassay included: 1) 0.00 (untreated control), 2) 0.42, 3) 1.27, 4) 2.1, 5) 2.93, and 6) 3.78. The specific components of each treatment are given in Table 4.

Data collection

From Day 1 to Day 22, data was collected daily on the number of hatched and unhatched eggs, and the number of immobile and mobile nematodes. Percentages were calculated for each measured variable.

Statistical analysis

The experiment was arranged in a randomized complete block design in a 96-well polystyrene plate (Corning Glass Works, Corning NY), and one trial was conducted. Data were transformed as needed to ensure normality and equal variance, and analyzed for significance with mixed model ANOVA with SAS 9.4. Least significant difference (LSD) was used to determine significant mean differences ($P \leq 0.10$).

Greenhouse assay with endophytic Bb, tomato, and RKN

The experiment consisted of four treatments: 1) Bb seed coat alone, 2) Bb seed coat + RKN, 3) RKN alone, and 4) Control (no Bb seed coat + no RKN). 'Rutgers' tomato seeds (30 seeds per

treatment) were surface sterilized with 95% ethanol (1 min), followed by 10% bleach (Clorox) (1 min) and sterile deionized water (1 min), and placed on sterile paper towels to remove excess moisture. For treatments with Bb, seeds were treated with Bb in 2% methyl cellulose as described previously to achieve log 7 conidia per seed. Colony counts on seed were confirmed on BSM. Treatments without Bb were coated with the methyl cellulose sticker.

Endophyte-treated tomato seeds and control seeds were sown in plug trays with potting mix (Sunshine #1 Natural & Organic, Sun Gro Horticulture, Agawan, MA). The trays were covered with a polyethylene cover until the seeds germinated. Germination rate was recorded daily and seedlings were watered as needed.

Two weeks after planting Trial 1, seedlings were fertilized with fish fertilizer (half-strength Alaska Fish Emulsion Fertilizer Concentrate 5-1-1, Pennington, Madison, GA). For Trial 2, fish fertilizer was applied twice (2 weeks after planting and 1 week later). At 3 weeks after sowing, seedlings were transferred into individual pots (3.78-L size), filled with sand (All Purpose Sand, Quikrete, Atlanta, GA) and potting soil (Sunshine #1 Natural & Organic) at a ratio of 2:1. Fertilizer (5 ml; Osmocote Smart-Release Plant Food Plus Outdoor and Indoor, Scotts Company, Marysville, OH) was added to each pot near the root zone of the seedling at transplant. Fish fertilizer was applied at 1 week and 2 weeks after seedling transplant. Osmocote (5 ml) was applied at 2 weeks after transplant also.

Nematode inoculation

Five weeks after sowing seed, 10,000 RKN eggs in an aqueous suspension were inoculated near the root zone of each seedling and evenly distributed by creating four holes around the root zone, and adding 2,500 RKN eggs per hole. The RKN egg concentration was 500 eggs/ml and 20

ml were added to each hole for a total of =10,000 RKN eggs/plant in all RKN treatments. Control plants received no RKN. In addition, pots that received Bb as a seed treatment, were drenched with 100 ml of Bb conidial suspension near the root zone of each plant 1-2 days before RKN inoculation. Concentrations of Bb in the drench solution were log 8/ml for Trial 1 and log 7/ml for Trial 2.

Thirty days after RKN inoculation, root systems from half the plants were carefully separated from the soil. The number of galls/root system were rated based on a standard rating scale of 1 to 10 (Zeck, 1971). Growth parameters, including shoot height (cm), shoot weight (g), and root weight (g) were measured. Sixty days after nematode inoculation, the remaining root systems were harvested and the number of galls/root system were rated. Shoot height (cm), shoot weight (g), root weight (g), fruit weight (g), fruit number, flower number, and root system length (cm) were assessed.

Egg count was determined for all nematode treatments (RKN and RKN + Bb). Eggs were extracted by the following procedure: roots were chopped into small sections (1-2 cm) and placed in 400 ml of 20% bleach (Clorox) in 1,500 ml tap water for 4 min. The chopped root suspension was manually shaken, and then passed through a No. 200 sieve (74 µm) to catch debris, and a No. 500 sieve (25 µm) to catch eggs. The RKN egg suspension collected on the No. 500 sieve was poured into a bottle and water was added to 500 ml. Extracted eggs were counted by pouring 1 ml of egg suspension into a counting dish. Two 1-ml samples were assessed from each egg suspension from each plant and counts were averaged. The egg hatch rate from egg suspensions extracted from each plant with an RKN treatment were determined in an *in vitro* assay.

In vitro egg hatch assay from greenhouse plants: To evaluate the hatch rate from eggs extracted from greenhouse nematode treatments (RKN and RKN + Bb) samples were placed in a 96-well plate. Each well received 100 µl of egg suspension. Data was collected on number and percentages of hatched and unhatched eggs, and immobile and mobile RKN.

Statistical analysis

The *in vitro* assay with egg suspensions from greenhouse plants was arranged as a randomized complete block with six replicates for Trial 1 and incubation for up to 6 days. For Trial 2, there were four replicates, and plates were incubated up to 10 days. Different concentrations of RKN eggs were present in each treatment replicate because the eggs were taken directly from plant root systems in the greenhouse experiment.

Determination of Bb from RKN eggs

The egg suspensions (500 µl) extracted from greenhouse treatments RKN and RKN + Bb were inoculated onto BSM. The presence or absence of endophytic Bb growth from eggs was observed and recorded.

Statistical analysis

The greenhouse experiment was designed as a randomized complete block. Each treatment was replicated 10 times for Trial 1 and 6 times for Trial 2. The data was transformed as needed and analyzed for significance with mixed model ANOVA (SAS 9.4). Least significant difference (LSD) was used to determine significant mean differences ($P \leq 0.05$).

Results

Gnotobiotic colonization assay with Bb 11-98 and two tomato cultivars

The assay was conducted to confirm that Bb 11-98 was endophytic in 'Rutgers' tomato, and to compare the extent of colonization with 'Mountain Spring', which has been used in multiple studies with endophytic Bb (Ownley et al., 2008). 'Rutgers' is susceptible to RKN and is commonly used in studies with this nematode.

Seed germination

Germination rate was recorded for all treatments. In Trial 1, for seed treated with Bb, germination was 94% for 'Mountain Spring' and 82% for 'Rutgers'. For control seed coated with methyl cellulose, germination was 82% for 'Rutgers' and 65% for 'Mountain Spring'. In Trial 2, the germination rate was 86% in Bb-treated seed for 'Rutgers' and 96% in 'Mountain Spring'. For methyl cellulose control seed, the germination rate was 96% in 'Rutgers' and 76% in 'Mountain Spring'.

Bb colonization of tomato seedlings

At 10-15 days after plating plant samples on BSM, white mycelial growth was noted in most seedling samples (leaf, stem, and roots) grown from Bb-coated seeds, while no fungal growth was observed in non-endophyte treated control plants. Across both cultivars, there were significant differences in colonization of roots, leaves and stem samples ($P<0.0001$) in both the trials. Across both tomato cultivars, the colonization rate of Bb was higher in stems and leaves from Bb treatments than in roots (Fig. 4.1). Percent colonization was higher in 'Rutgers' than in 'Mountain Spring' in Trial 1, but not different in Trial 2 (Fig. 4.2). For Trial 1, percent colonization of stems (93%) was higher than leaves (60%), and significantly higher than roots

(40%) for ‘Rutgers’. The colonization rate of ‘Rutgers’ stems was significantly higher than leaf, stem, or root of ‘Mountain Spring’. Colonization rates of ‘Mountain Spring’ leaf, stem, and root samples were not different. Patterns of colonization were similar in Trial 2. However, there were no differences in the colonization rates of leaf and stem samples for the two cultivars, and colonization was higher in roots for ‘Mountain Spring’ than ‘Rutgers’ (Fig 5.2). Because colonization patterns were similar for the two cultivars, there was no significant interaction between cultivar and plant tissue type in either trial. Over all tissues, colonization was greater in ‘Rutgers’ in trial 1 (P=0.002), but differences were not significant in trial 2 (P=0.17).

Identity of the fungus growing from leaf, stem and root samples was confirmed by culturing isolates on BSM in pure culture, followed by microscopic observations of conidia and conidiophores, as well as observations on cultural characteristics. Molecular identification was performed on isolates obtained in Trial 1. The fungus recovered from plant tissue had 100% ITS region sequence identity with isolates of *B. bassiana* in the NCBI nucleotide database.

In vitro Bb conidia and RKN egg bioassay

Separate analyses were run on data collected at day 1, 5, 10, 15, 20, and 22 for the *in vitro* assay with different ratios of Bb conidia and RKN eggs. The assay was performed to investigate whether Bb conidia had an antagonistic effect on RKN. Generally, there were differences between treatments only on day 1 or day 2 of the assay.

The percentage unhatched eggs differed among treatments only on day 5 (P=0.08) of the assay. On day 5, the highest rate of unhatched eggs was in the 3.78 ratio treatment, with the highest proportion of conidia to eggs. The lowest rates of unhatched eggs were in the control (no conidia) and the 2.1 ratio treatment. The percentage of unhatched eggs decreased

as the percentage of hatched eggs increased over 22 days. Like unhatched eggs, the percent of hatched eggs only differed (P=0.08) on day 5 (Fig. 4.4). On day 5, the highest percent egg hatch was recorded in the 2.1 ratio treatment, and the least egg hatch was in the 3.78 ratio treatment. There were no significant differences among the treatments for percent mobile nematodes during the 22-day incubation period for days tested (Fig. 4.5).

Over the 22-day assay, the percent of immobile nematodes increased. Significant differences were found for percent immobile nematodes on day 10 (P=0.05) and day 20 (P=0.04) (Fig. 4.6). On day 10, the highest percentage of immobile nematode was in treatment 2.93 ratio, which differed from the 0.42 and 1.27 ratio treatments, which had lower rates of immobile RKN. On day 20, the highest percentage of immobile nematodes was recorded in the 0.42 ratio treatment, which differed from two higher ratio treatments (2.93 and 3.78) and the control (0.00 ratio). No parasitism of root-knot nematode eggs by Bb was observed in any of the treatments. However, fungal mycelia were observed in a few treatment replicates.

Greenhouse assay with endophytic Bb, tomato, and RKN

Germination rate of 'Rutgers' seed and colony counts of Bb on seed: Germination rate was recorded in both endophytic coated and methyl cellulose control seed up to day 12 (Trial 1) and day 15 (Trial 2). In Trial 1, 'Rutgers' seed germination was 92% for Bb-treated and control. In Trial 2, germination was 100% for the methyl cellulose control and 76% in Bb-treated. The population of Bb on 'Rutgers' seed was log 7 for both trials.

In the greenhouse assay, growth of tomato plants treated with Bb and RKN were compared with three controls: Bb only, RKN only, and no treatment. There were no differences among treatments for shoot height (Fig. 4.7), shoot weight (Fig. 4.8), and root weight (Fig. 4.9)

at 30 days after treatment (DAT) in Trial 1. However, root galling (Fig. 4.10) was significant ($P<0.0001$) because there were no galls on the control and the Bb only treatment. Both treatments with RKN were not different. In Trial 2, there were no differences in shoot height (Fig. 4.7), and root weight (Fig. 4.9) but there were differences in shoot weight ($P=0.09$) (Fig. 4.8). The control had greater shoot weight than Bb only or RKN + Bb treatment. Galling index was higher ($P<0.0001$) on RKN + Bb treatment than on RKN alone, and no galls were found on the control and Bb only treatment (Fig. 4.10).

At 60 DAT, there were no differences in shoot height (Fig. 4.7) or shoot weight (Fig. 4.8), in Trial 1, but root weight ($P<0.0001$) was greater with the RKN + Bb treatment and least in the control (Fig. 4.9). Root galling also differed ($P<0.0001$) with a higher galling index in the RKN + Bb treatment than RKN along. No galling was found for treatments that did not receive RKN. In Trial 2, at 60 DAT, there were no differences among treatments in shoot height (Fig. 4.7) and shoot weight (Fig. 4.8). However, root weight of the RKN + Bb treatment was significantly greater ($P=0.015$) than the control or Bb alone (Fig. 4.9), but did not differ from RKN alone. Root galling (Fig. 4.10) was greater ($P<0.0001$) with RKN + Bb than RKN alone. No galls occurred on plants that did not receive RKN.

Fruit weight (Fig 4.11) was measured at 60 DAT, and there were no significant differences among treatments in either trial. However, fruit number varied ($P=0.07$) in Trial 1, but not in Trial 2 (Fig. 4.12). Fruit number was lowest in RKN + Bb, and highest in Bb only and RKN only. Flower number did not differ in either trial (Fig. 4.13), and root system length did not differ among treatments in Trial 1, but was significantly ($P=0.025$) shorter for treatments with RKN (RKN only and RKN + Bb) than for the control or Bb alone in Trial 2 (Fig. 4.14).

There were significant differences in egg count per root system (Fig 4.15) among treatments for both trials (P<0.0001, P=0.0002) at 60 DAT incubation period. In Trial 1, the egg count from RKN + Bb was significantly higher than RKN alone, but in Trial 2, the difference was due to treatments with and without RKN added. The highest egg count was in RKN + Bb in both trials, followed by RKN alone. There were no egg counts for control and Bb only.

In vitro egg hatch assay from greenhouse plants: The egg hatch rate from eggs extracted from greenhouse nematode treatments (RKN + Bb and RKN alone) were evaluated in an *in vitro* assay for up to 6 (Trial 1) and 10 (Trial 2) days. Data was collected daily on the number of hatched and unhatched eggs, and mobile and immobile nematodes, and percentages were calculated. Different concentrations of nematode eggs were present in each treatment replicate because the eggs were directly taken from root systems of plants in the greenhouse experiment.

Over the 6 days of Trial 1, and 10 days of Trial 2, percent egg hatch (Fig. 4.16, Fig. 4.17) was only significant in day 4, Trial 1 (P=0.013), where the hatch rate was higher in RKN + Bb than in RKN alone. Although this was the only data point with significance, the same trend was found for all other days in both trials. Similarly, the percent unhatched eggs (Fig. 4.18, Fig. 4.19) had significant differences only for day 4, Trial 1, with percent unhatched being greater for RKN alone than for RKN + Bb.

The percent of immobile nematodes increased over time for both trials (Fig 4.18). Significant differences among treatments were significant for incubation days 3 (P=0.04), 4 (P=0.01), 5 (0.004), and 6 (0.01) in Trial 1, with higher percent immobile nematodes for RKN

alone than for RKN + Bb. The percent immobile RKN was higher for RKN + Bb, than RKN alone in Trial 2, but differences were not significant.

Like the results with immobile nematodes, there was an overall increase in percent mobile nematodes over time (Fig 5.19). In trial 1, treatments differed for days 3 (P=0.01), 4, (P=0.0006), 5, (P=0.05) and 6 (P=0.02) with more mobile RKN in RKN + Bb, than in RKN alone. Treatments were not different in Trial 2.

The presence of Bb in the RKN egg suspension extracted from tomato roots in the greenhouse experiment was confirmed by growth of the fungus on BSM following inoculation of 500 µl of egg suspension.

Discussion

Results of the gnotobiotic assay confirmed that Bb was endophytic in 'Rutgers' tomato. This was important for the current study because 'Rutgers' is very susceptible to RKN, and was the cultivar of choice for the greenhouse studies. Colonization patterns of 'Rutgers' by Bb were similar to those of 'Mountain Spring', which has been used in other studies on endophytic Bb. Overall, the extent of colonization may be greater in 'Rutgers' than 'Mountain Spring'. Differences in endophyte colonization of specific plants or cultivars could be due to differences in plant genes related to resistance or susceptibility, presence of specific plant chemicals that enhance or inhibit the growth of Bb, or growth of other microorganisms that compete with the endophyte (Greenfield et al., 2016). More studies are needed on the preference of endophytes for their plant hosts.

Our research findings indicate that Bb colonization was higher in stems and leaves compared to roots in both tomato cultivars (Rutgers and Mountain Spring). Bb was applied as a

seed treatment, enabling the fungus to colonize the radicle and hypocotyl as they emerged from the seed. From the hypocotyl, the fungus had an early opportunity to colonize cotyledons, stem, and true leaves, as well as roots developing from the radicle. Competition from soil microorganisms may be greater for colonization of roots than colonization of parts above the ground. It has been suggested that fungal colonization is more likely to occur on plant parts where it was applied directly first, than to distant plant parts (Akello et al., 2007, Akello et al., 2009, Tefera and Vidal, 2009). Methods used in isolation of endophytes to confirm their presence could also play a role in where they are found on or in the plant. Isolation of an endophyte from plant tissues distant from the place of plant inoculation (El-Deeb et al., 2012) shows that the endophyte can move from one part of the plant to another (Posada et al., 2007), and demonstrates a pattern of systemic colonization (Tefera and Vidal, 2009).

In this study, a systemic colonization pattern of tomato, with significant differences in percent colonization among plant tissues was noted. These results are in agreement with studies that have observed that Bb can establish as an endophyte throughout the entire plant, especially after seed treatment (Akutse et al., 2013, Ownley et al., 2008, Quesada-Moraga et al., 2009). Contrary to results of the current study, there are research reports that Bb does not systemically colonize some plants or plant parts. This may be related to specific Bb isolates used or plant tissue type (Wearn et al., 2012, Yan et al., 2015).

The differential colonization rate of Bb that we noted in tissues of the two tomato cultivars agree with Biswas et al. (2012), who demonstrated that when Bb was artificially applied to jute, higher percent colonization in leaves was achieved (56%) compared to stems (13%) and capsules (42%). Similarly, Russo et al. (2015) demonstrated that when Bb was

inoculated by different methods, at different times, onto different crops, colonization rates varied. For example, the highest colonization rates were achieved with foliar spray with 100% in tobacco leaves at 7 days, 40% wheat leaves at 14 days, 7.8% corn seedling leaves at 7 days, and 24% for soybean seedling leaves at 7 days. Variation in the way the Bb colonizes various plant tissues could be related to its opportunistic endophytic fungal lifestyle (Vidal and Jaber, 2015; McKinnon et al., 2017; Landa et al. 2013). Further studies are needed to understand the nature of differential colonization patterns by Bb.

The lowest colonization by Bb was in roots in the current study, which contrasts with studies by Greenfield et al., 2016. They reported that soil drenches of Bb conidia around stem cuttings of cassava allowed Bb to successfully colonize cassava roots, and no colonization was noted in stems and leaves. Colonization was higher when plants were sampled at 7 to 9 days post-inoculation (84%) compared to 47-49 days (40%). Recently it was reported that *Beauveria* spp. are more prone to colonize the rhizosphere of plants experiencing insect herbivory. McKinnon et al., (2018) suggested that this could be an adaptive strategy of these fungi to increase the potential encounter of susceptible insect hosts. Bb is a ubiquitous terrestrial entomopathogenic fungus that infects several insect species (Vega, 2008).

Patterns of colonization and establishment, penetration, and growth of Bb into plant tissues were first detailed by Wagner and Lewis (2000). Conidia of Bb germinate on plant tissues and form a germ tube, followed by gradual development of hyphae and entry into plant tissue, either directly through natural openings or via enzymes. The fungus might apply mechanical pressure through the epidermal cell walls for entry, but no appressoria have been observed (Wagner and Lewis, 2000; Quesada-Moraga et al., 2006). In the case of insects, the Bb

infection processes starts with adhesion of conidia, followed by germination, and appressorium formation, which depends on the nature of the insect cuticle (Butt et al.,1995).

Some studies with entomopathogenic Bb have reported failure of the fungus to establish as an endophyte. Gurulingappa et al. (2010) reported that direct application of Bb inoculum to soil resulted in no fungus establishment in leaves, stems, or roots of wheat and cotton. In contrast high Bb post- recovery was reported in coffee plants when Bb was injected directly (Posada et al., 2007). Variability in reports of plant colonization with Bb highlight that fungus-host plant interactions play an essential role in endophyte establishment. Differential Bb colonization of plants may depend on crop species type, host specificity, inoculation method used, or fungal isolate used (Mayerhofer et al., 2013) or geographical location or soil conditions prevailing when the fungus is applied.

The *in vitro* assay was performed to investigate whether the presence of Bb conidia had an antagonistic effect on root-knot nematode. Based on the results of the current study, we reject our hypothesis that the percentages of egg hatch and mobile nematodes will be lower with Bb treatment than in endophyte-free controls. We found no significant differences among treatments with different ratios of Bb conidia to RKN eggs for percent mobile nematodes over a 22-day incubation period. We found weak evidence for differences in egg hatch at one time point where the highest ratio of conidia to eggs had lower egg hatch than eggs without Bb. But this was not consistent throughout the incubation period. The number of dead nematodes increased at longer incubations for all treatments and no parasitism of eggs was observed in any of the treatments. These results suggest that endophytic Bb isolate 11-98 is not a good candidate for biocontrol of RKN.

Other researchers have reported antagonism of Bb to nematodes. Ekanayake and Jayasundara (1994) demonstrated that beauvericin, a secondary metabolite produced by Bb had weak nematicidal activity against root-knot nematode. Chen et al. (1996) found that Bb showed less nematode egg parasitism than other fungi, but Sun et al. (2006) conducted an in vitro assay and reported that Bb parasitized 100% of eggs of *Meloidogyne hapla* and delayed egg hatching by 36% and juvenile mortality by 18.1% in *Meloidogyne hapla*. Liu et al. (2008) conducted an *in vitro* assay to investigate the effect of Bb culture filtrate against eggs and juveniles of *M. hapla*. The reported that cultural filtrate of *B. bassiana* inhibited *M. hapla* juveniles by 99% compared to the chemical pesticide Aldicarb (89%). Zhao et al. (2013) evaluated nine isolates (nef2621, Snef2607, Snef2615, Snef2636, Snef2637, Snef2568, Snef2598, Snef2626 and Snef2601) of Bb applied as culture filtrate on four targeted nematodes including: *Meloidogyne incognita* (second-stage juveniles - J2), *Heterodera glycines* (J2), *Aphelenchoides besseyi* and *Caenorhabditis* spp. They reported differential Bb toxicity to each nematode. The Bb isolates caused high mortality of *M. incognita* (J2), *H. glycines* J2, and *Caenorhabditis* spp., but were less effective against *Aphelenchoides besseyi*. Although other studies have reported nematicidal activity of Bb, this was not supported by results of the present study. The difference in results could be related to methodology or the Bb isolates used.

We also investigated whether endophytic Bb has any antagonistic effect on root-knot nematode in a tomato crop with greenhouse studies. Based on results, root galling and egg number of RKN per root were not reduced with endophytic Bb on tomato plants, compared to endophyte-free control plants.

Data on various growth and development plant measures indicated that there were few differences between Bb-treated and endophyte-free plants. At 60 DAT, root weight was an exception. Roots treated with Bb and inoculated with RKN were consistently larger than controls (no Bb or RKN) or roots with Bb, and were larger than (Trial 1) or the same as (Trial 2) RKN only.

Our data on root galling and egg counts per root were more direct measures of RKN success on plants (or lack of inhibition by Bb). Interestingly, more root galling and higher egg counts/root were recorded for the RKN + Bb treatment than for RKN alone, and this difference was significant in one trial. Our results agree with Mwaura et al. (2017), who reported that the combination of Bb with two nematodes (*Ditylenchus destructor* and *D. dipsaci*) reduced potato tuber weight and yield, and caused higher nematode population density. Mwaura et al. (2017) evaluated Bb by soil inoculation together with the potato tuber rot nematode (*D. destructor*) and the stem and bulb nematode (*Ditylenchus dipsaci*) in two greenhouse experiments.

Our results raise several questions, such as, 'Does Bb have triggering factors that increase or aggravate RKN root galling and egg production in 'Rutgers' tomato? Does 'Rutgers' have any triggering genes in response to Bb infection that might increase nematode infection? Are our results specific for the cultivar studied? Additional research is needed to answer these questions.

In the second *in vitro* assay, using eggs extracted from greenhouse nematode treatments (RKN and RKN + Bb), with no additional Bb, there was a trend towards increased egg hatch with Bb + RKN, compared to RKN alone. This was supported with a significant difference at one time measurement point. In one trial, there were also significantly higher percentages of

immobile nematodes with RKN alone, compared to Bb + RKN at several time points. Conversely, there were significantly higher percentages of mobile nematodes with RKN + Bb than RKN alone. This suggest further that Bb 11-98 has little inhibitory effect on root-knot nematode, and may have a stimulatory effect.

Our results are contrary to those of Liu et al. (2008), who conducted *in vitro* and *in vivo* studies on the effect of Bb on northern root-knot nematode. They demonstrated that there was a nematicidal effect against northern root-knot nematode when Bb was applied as a "culture filtrate" soil drench in tomato plants. Bb reduced egg hatch increased nematode mortality *in vitro,* and reduced nematode populations in soil. Treatment with Bb also reduced gall formation and production of egg masses. Similarly, Kepenekci et al. (2017) found that Bb was effective on *Meloidogyne* and demonstrated that when Bb (isolates F-56 and F-63) was applied as conidial suspensions (10^6, 10^7 and 10^8CFU/ml), four times at different plant growth stages (15 days before planting, planting, 15, and 30 days after planting) on *Meloidogyne incognita* and *M. javanica* on tomatoes under greenhouse conditions, Bb had high nematicidal activity and reduced root galling, as well as increased crop yield. The highest concentration of Bb (log 8/ml) had the greatest inhibitory effect.

Entomopathogenic fungi are known as an effective method for plant parasitic nematode management (Butt et al., 2001). Some fungi show promising biocontrol potential against root-knot nematode (Jatala et al., 1979; Stirling, 1991; Tigrano-Milani et al., 1995). Almost 30 genera and 80 species including *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Purpureocillium lilacinum*, *Verticillium chlamydosporium* and *Arthrobotrys* spp., have been reported to show biocontrol potential against plant parasitic nematodes (Viaene and Abawi, 2000; Sun et al.,

2006 and Bakr et al., 2014). Paparu (2009) demonstrated that the fungus *Fusarium oxysporum* colonized banana plant and reduced burrowing nematode (*Radopholus similis*) numbers in roots after 12 weeks of endophyte inoculation to 8-week-old plants in a screenhouse study.

Bb isolates can produce several secondary metabolites, including beauvericin, beauverolides, bassianolides, oosporein, cyclosporine A, oxalic acid, and bassianolone. Antibacterial, antifungal, and cytotoxic activity have been reported for these compounds (Grove and Pople, 1980; Genthner et al., 1994). However, more investigation is needed on nematicidal properties of the bioactive compounds that Bb isolates may produce.

In conclusion, Bb 11-98 was not effective in reducing root-knot nematode activity, including egg hatch and root galling, in 'Rutgers' tomato. The reason for the lack of effectiveness of Bb 11-98 is not clear. The endophyte was applied as a seed treatment and soil drench. In addition, Bb appeared to increase root galling and egg count. Although isolates of Bb have been reported as effective biocontrol agents against *Meloidogyne* spp. and other plant parasitic nematodes, it was not the case in our research.

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Appendix 4

All tables and figures for Chapter 4 are in Appendix 4.

Table 4. Composition of treatments for Beauveria bassiana (Bb) conidia/RKN egg ratios.

Treatment #*	Bb conidia (#)	Bb suspension water (ml)	Sterile water (ml)	RKN eggs (#)	RKN suspension water from root extractions (ml)	Total water (ml)	Bb conidia/RKN egg ratio
1 (control)	0	0.00	0.26	60	0.04	0.3	0.00
2	25	0.02	0.24	60	0.04	0.3	0.42
3	76	0.06	0.20	60	0.04	0.3	1.27
4	126	0.10	0.16	60	0.04	0.3	2.10
5	176	0.14	0.12	60	0.04	0.3	2.93
6	227	0.18	0.08	60	0.04	0.3	3.78

*There were four replicates of each treatment.

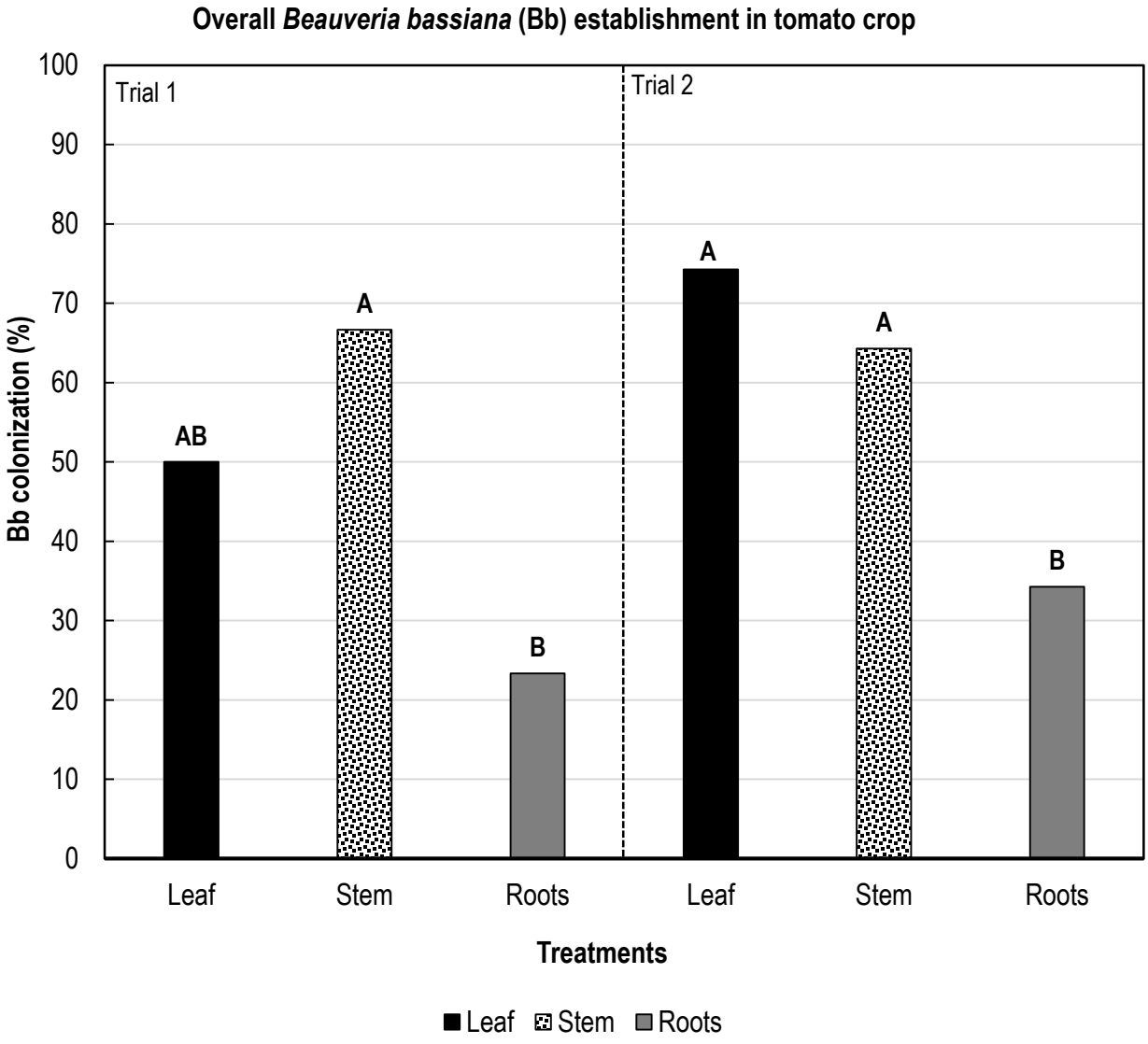


Figure 4.1. Across both cultivars, percent colonization by endophytic B. bassiana (Bb) of leaf, stem, and root samples of 'Mountain Spring' and 'Rutgers' tomato seedlings grown from Bb-treated seed.

Trials were analyzed separately. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.05$.

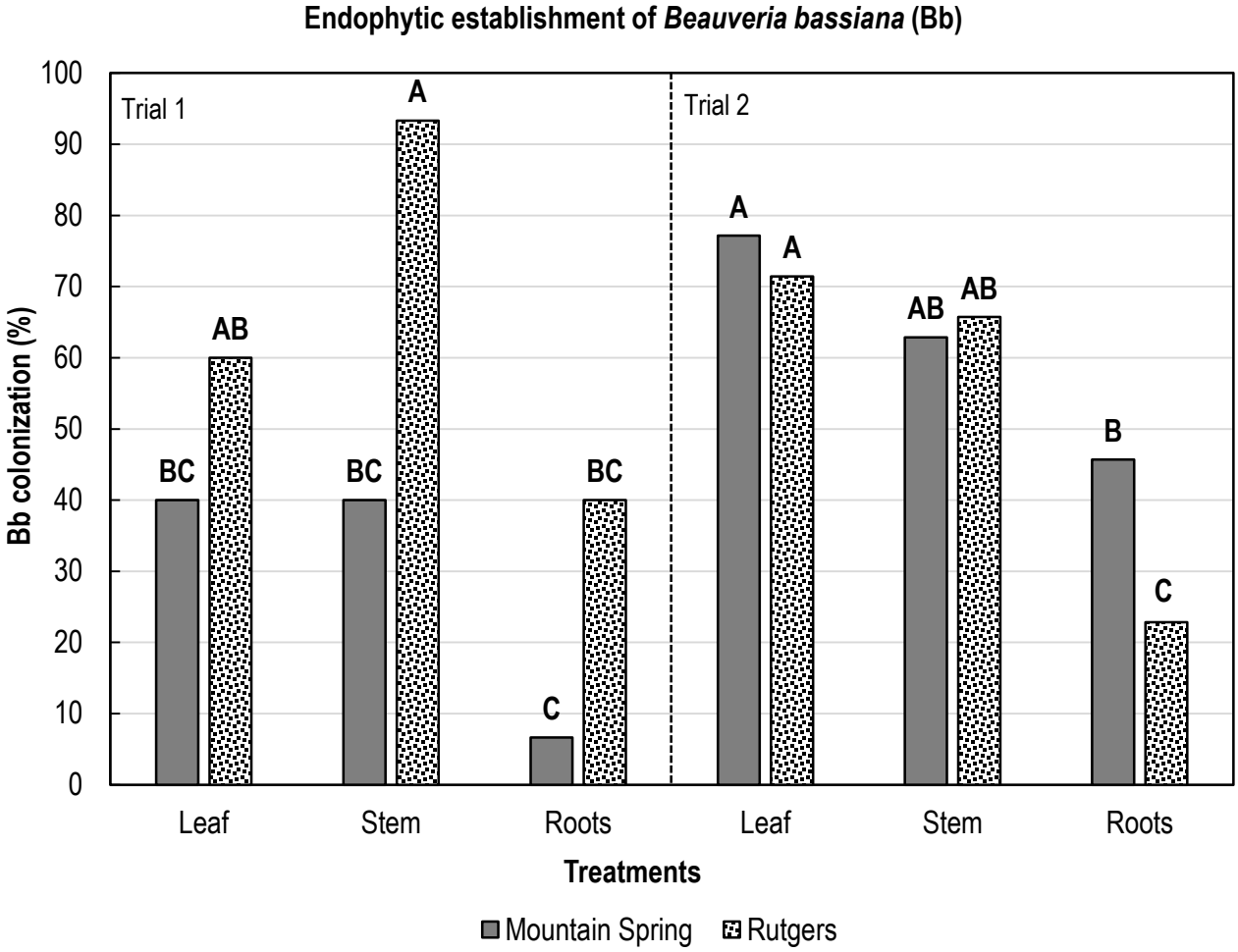


Figure 4.2. Percent colonization by endophytic B. bassiana (Bb) of leaves, stems, and roots of 'Mountain Spring' and 'Rutgers tomato seedlings grown from Bb treated seed.

Trials were analyzed separately. Bars with the same letter are not different according to an F-protected LSD at $P \leq 0.05$.

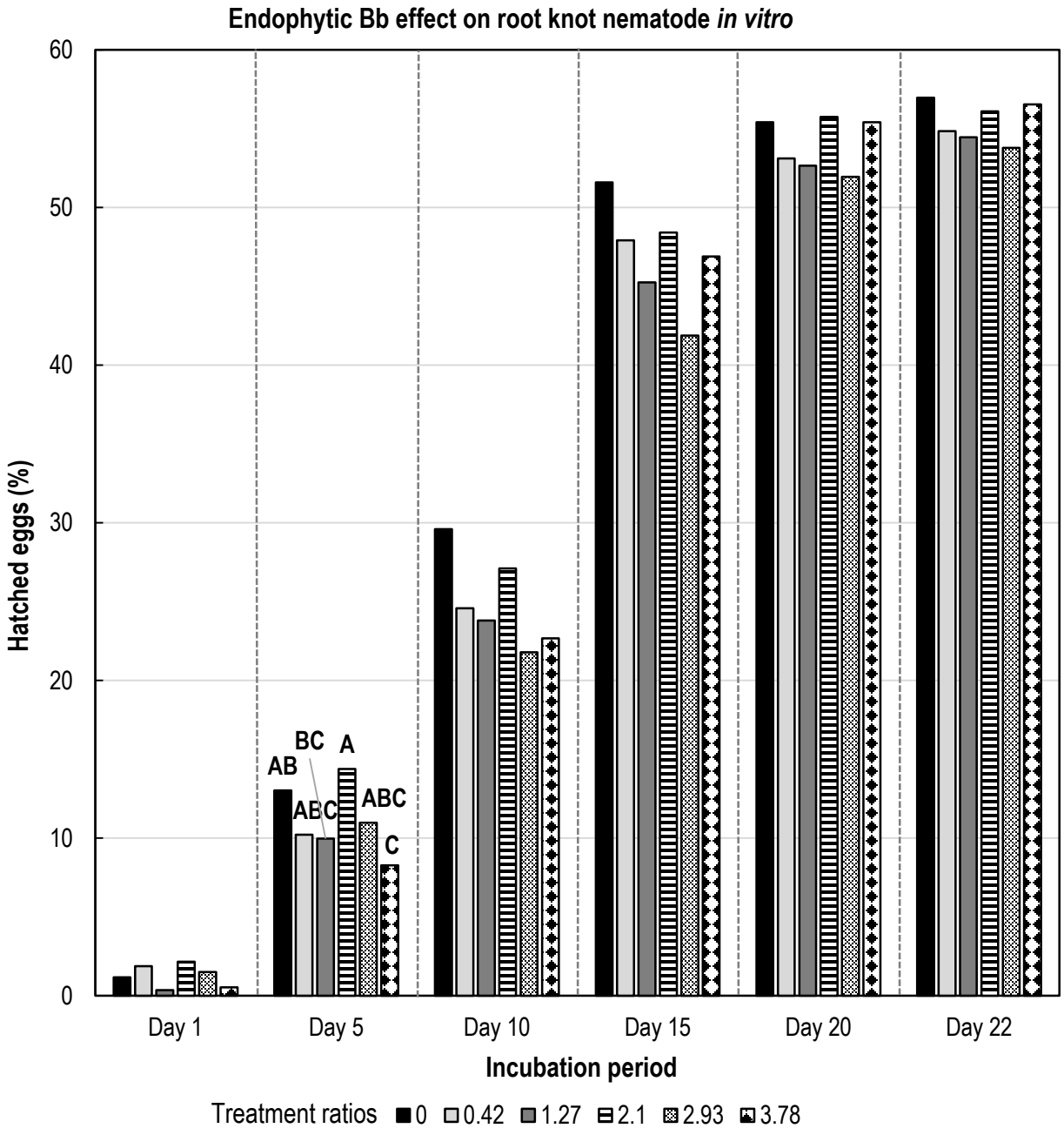


Figure 4.3. Endophytic B. bassiana (Bb) effect on percent hatched root-knot nematode eggs in an in vitro assay over 22 days.

Treatment ratios = [(number of fungal spores/number of nematode eggs)]/volume (ml) of water. Analyses were performed separately for each day. Bars with the same letter or no letter are not different according to an F-protected LSD at $P \leq 0.10$.

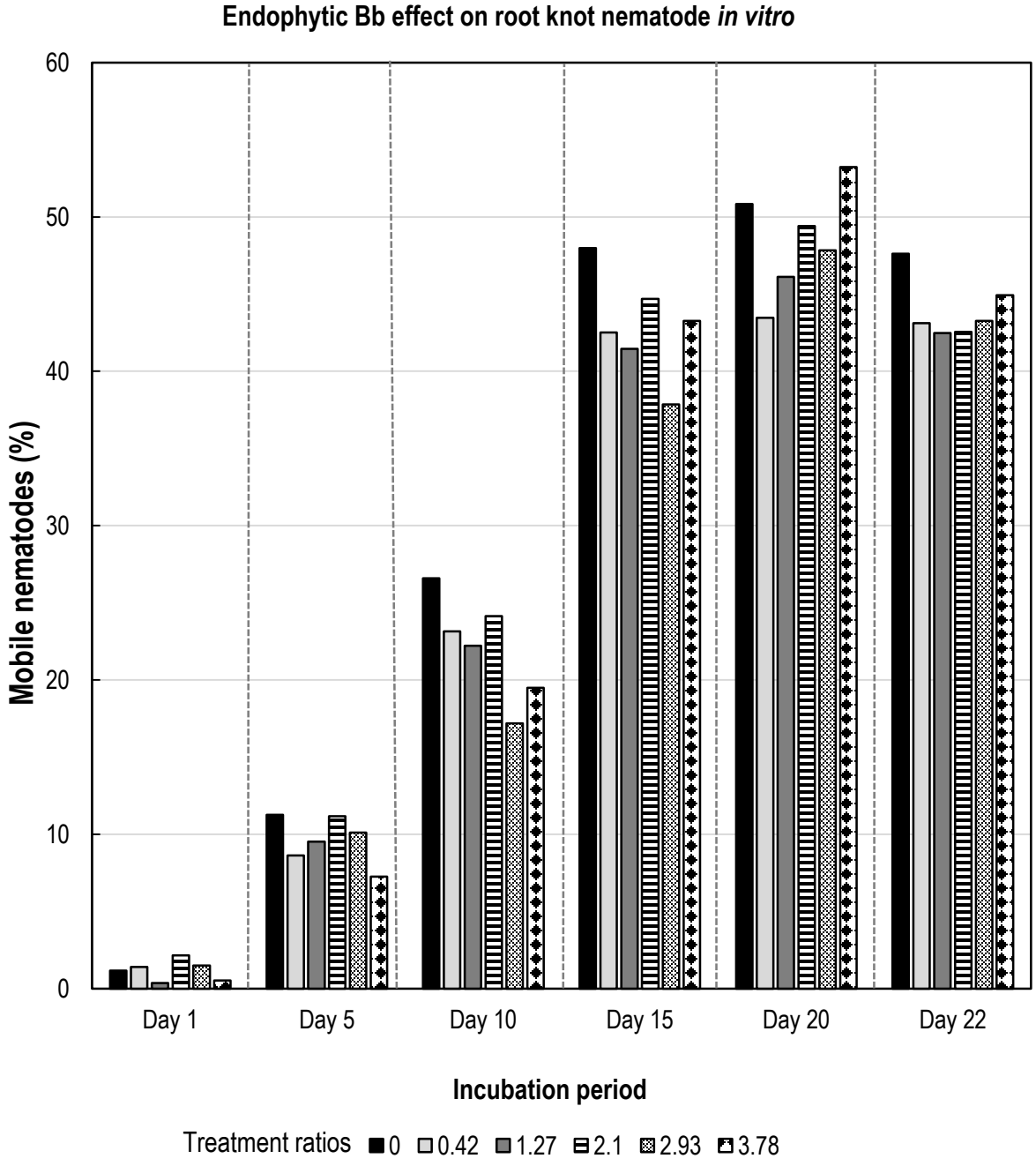


Figure 4.4. Endophytic B. bassiana (Bb) effect on percent mobile root-knot nematodes in an in vitro assay over 22 days.

Treatment ratios = [(number of fungal spores/number of nematode eggs)]/volume (ml) of water. Data for each day was analyzed separately. Bars with the same letter or no letter are not different according to an F-protected LSD at $P \leq 0.05$.

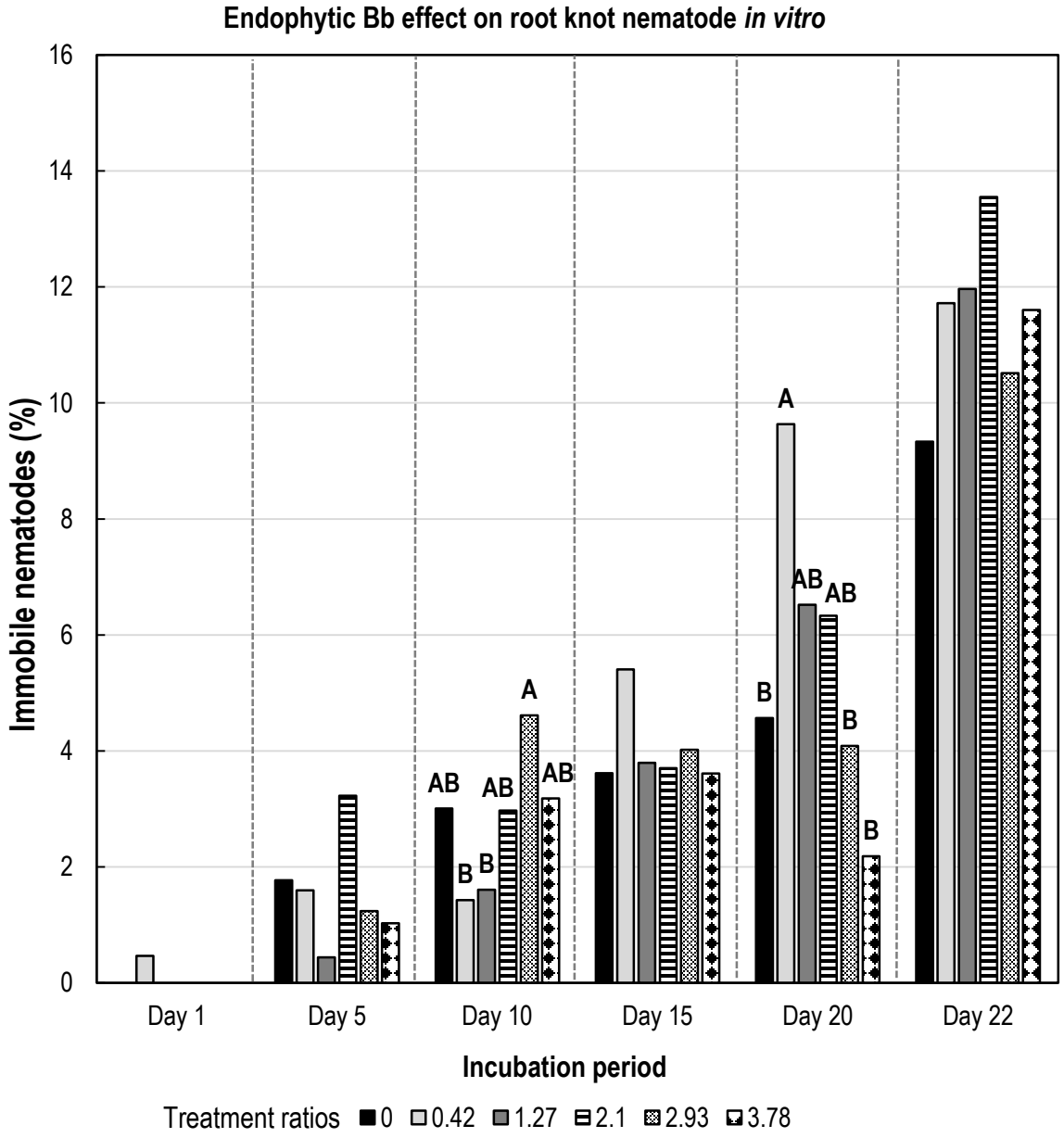


Figure 4.5. Endophytic B. bassiana (Bb) effect on percent immobile root-knot nematodes in an in vitro assay over 22 days.

Treatment ratios = [(number of fungal spores/number of nematode eggs)]/volume (ml) of water. Data for each day was analyzed separately. Bars with the same letter or no letter are not different according to an F-protected LSD at $P \leq 0.05$.

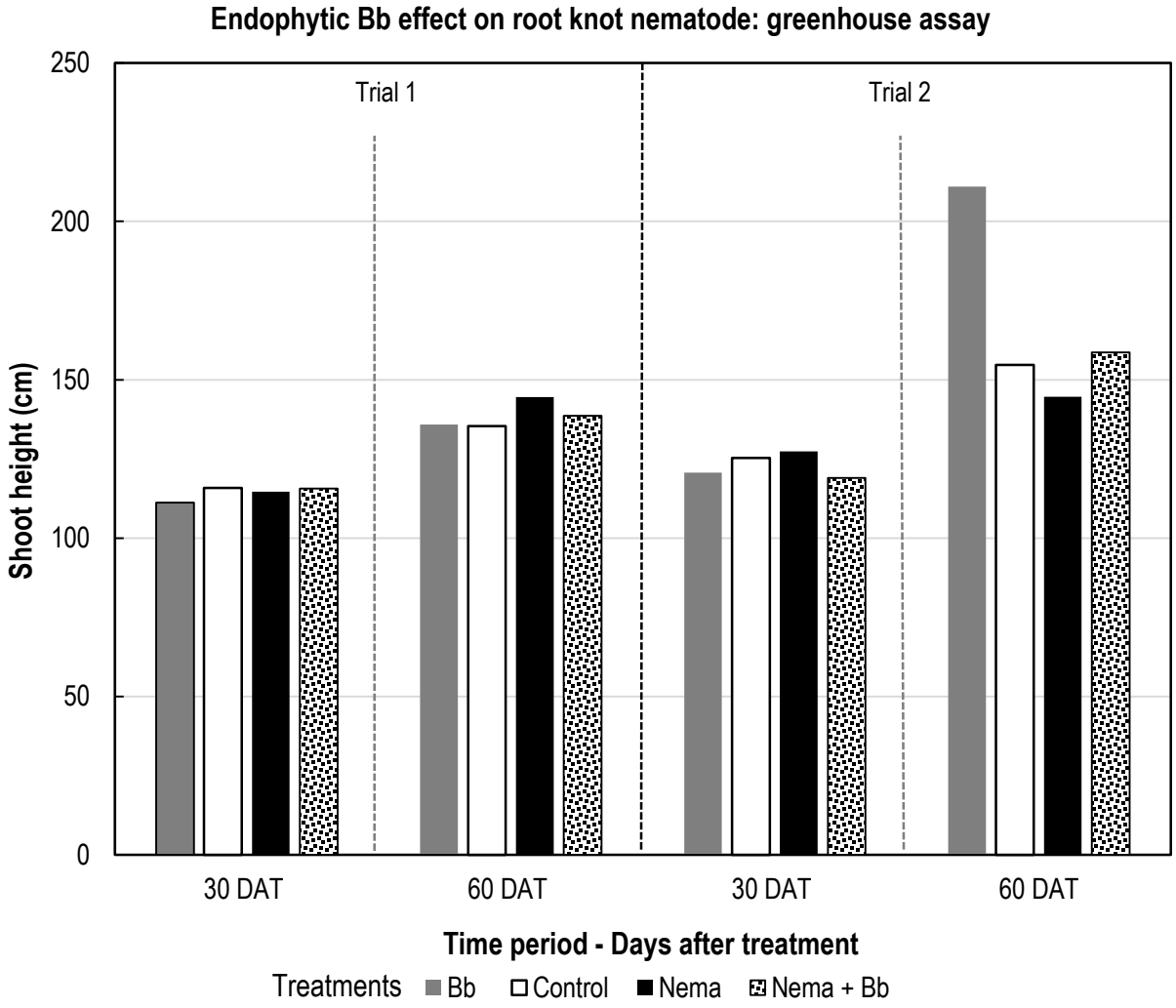


Figure 4.6. Effect of endophytic B. bassiana (Bb) and root-knot nematode on shoot height (cm) of tomato evaluated in a greenhouse assay.

Treatments were Bb = *Beauveria bassiana*, Nema = Root-knot nematode, Nema + Bb, and Control. Plants were evaluated at 30 days and 60 days after treatment (DAT), for two trials. Separate analyses were conducted for each incubation period and trial. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.05$.

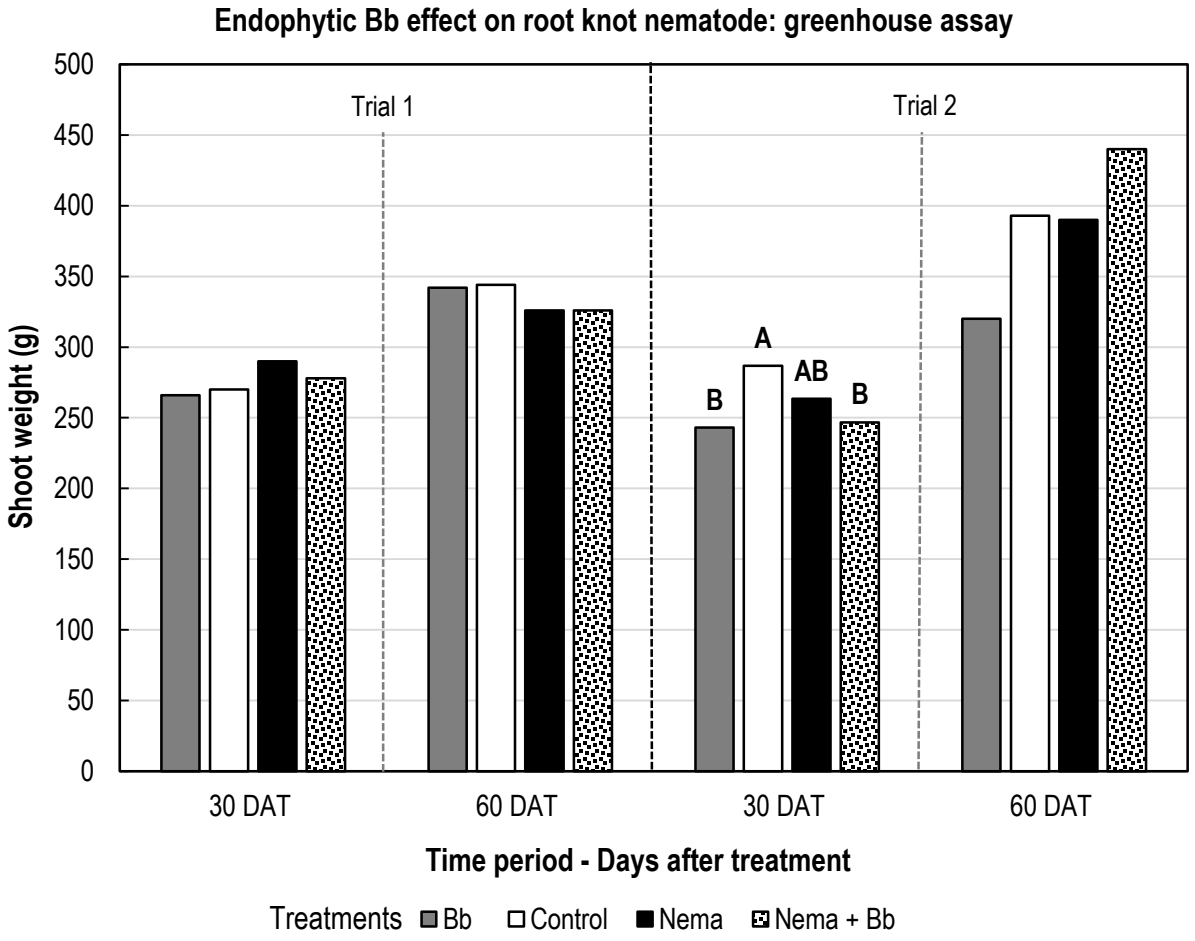


Figure 4.7. Effect of endophytic B. bassiana (Bb) and root-knot nematode on shoot weight (g) of tomato evaluated in a greenhouse assay.

Treatments were Bb = *Beauveria bassiana*, Nema = Root-knot nematode, Nema + Bb, and Control. Plants were evaluated at 30 days and 60 days after treatment (DAT), for two trials. Separate analyses were conducted for each incubation period and trial. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.10$.

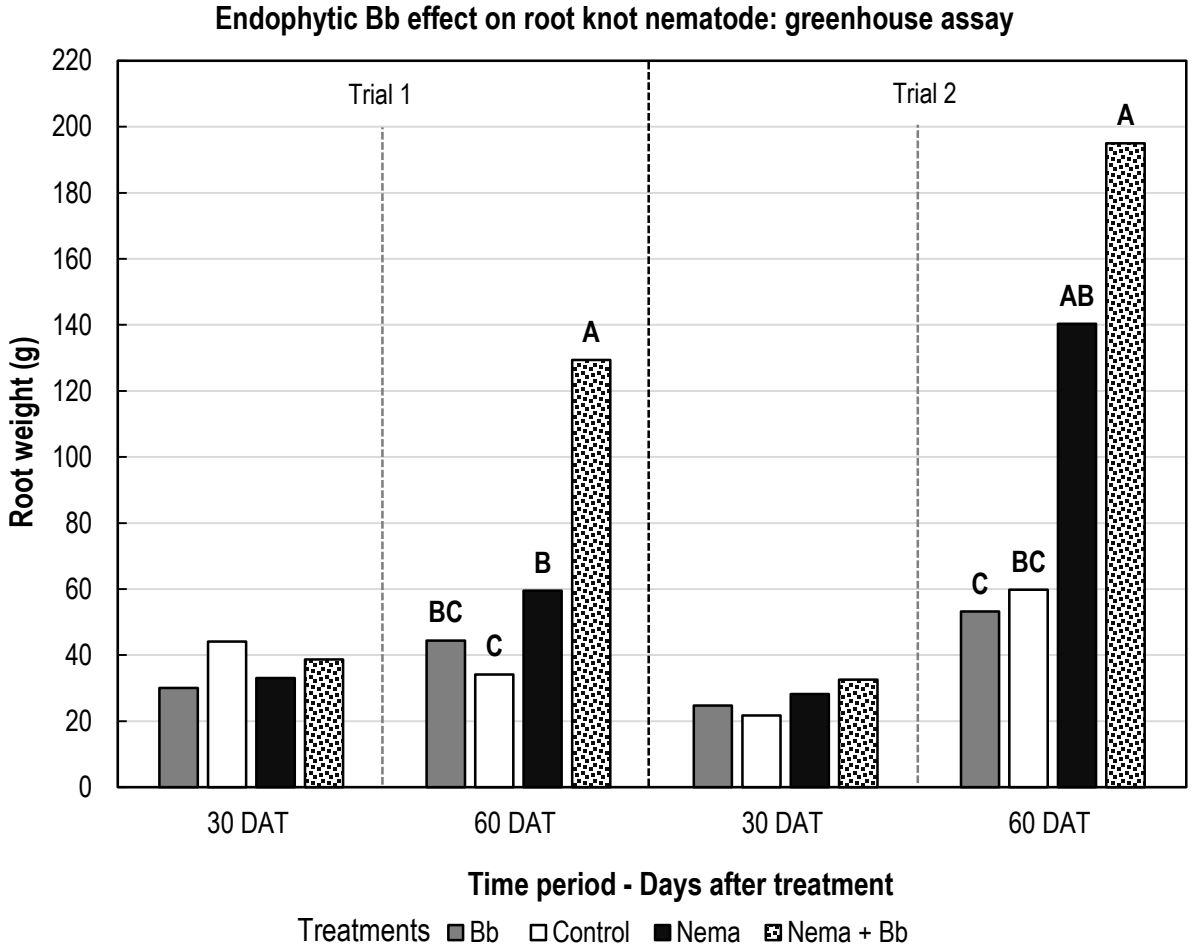


Figure 4.8. Effect of endophytic B. bassiana (Bb) and root-knot nematode on root weight (g) of tomato evaluated in a greenhouse assay.

Treatments were Bb = *Beauveria bassiana*, Nema = Root-knot nematode, Nema + Bb, and Control. Plants were evaluated at 30 days and 60 days after treatment (DAT), for two trials. Separate analyses were conducted for each incubation period and trial. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.05$.

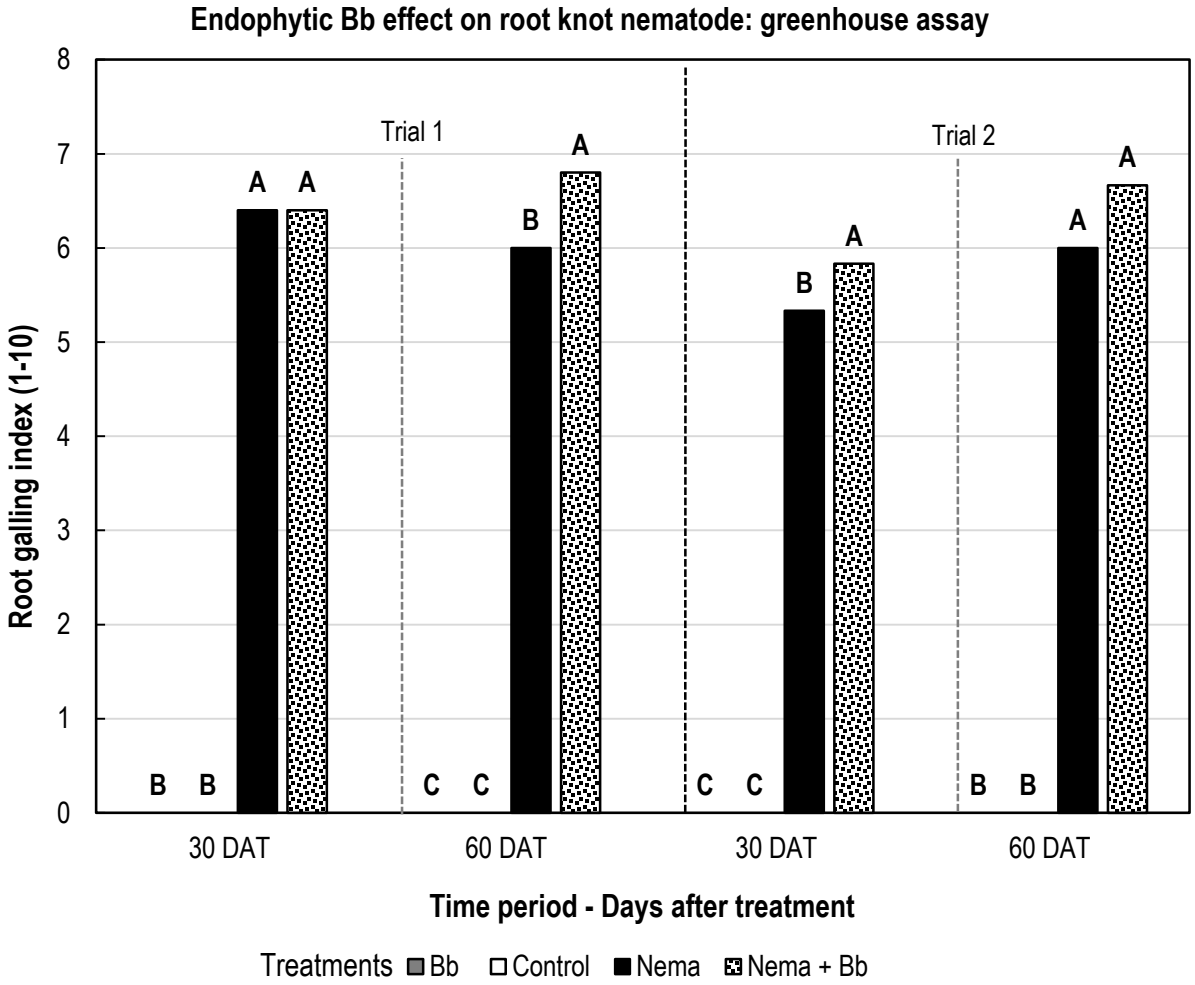


Figure 4.9. Effect of endophytic B. bassiana (Bb) and root-knot nematode on root galling index of tomato evaluated in a greenhouse assay.

Treatments were Bb = *Beauveria bassiana*, Nema = Root-knot nematode, Nema + Bb, and Control. Plants were evaluated at 30 days and 60 days after treatment (DAT), for two trials. Separate analyses were conducted for each incubation period and trial. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.05$.

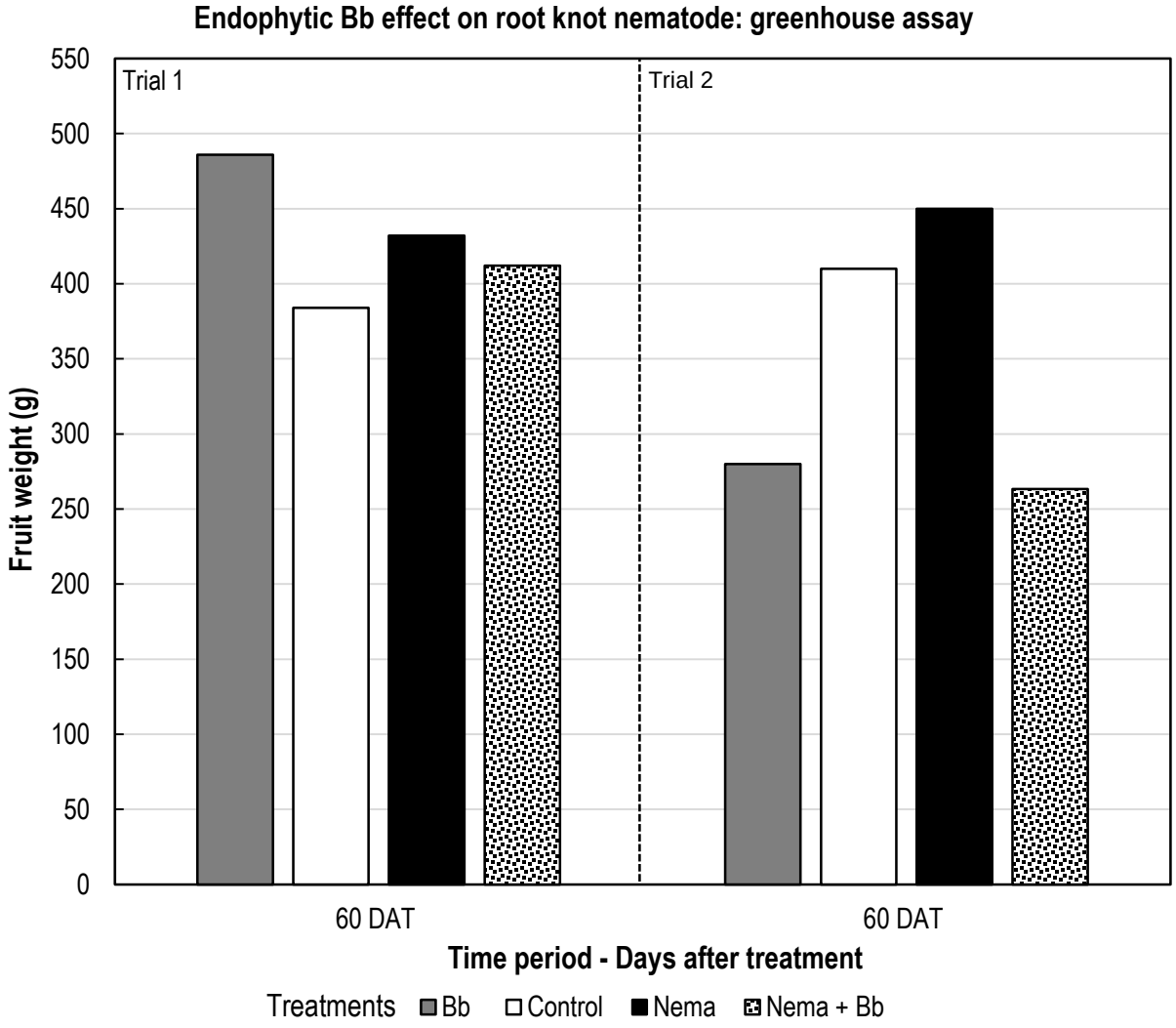


Figure 4.10. Effect of endophytic B. bassiana (Bb) and root-knot nematode on fruit weight (g) of tomatoes evaluated in a greenhouse assay.

Treatments were Bb = *Beauveria bassiana*, Nema = Root-knot nematode, Nema + Bb, and Control. Plants were evaluated at 60 days after treatment (DAT), for two trials. Separate analyses were conducted for each incubation period and trial. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.05$.

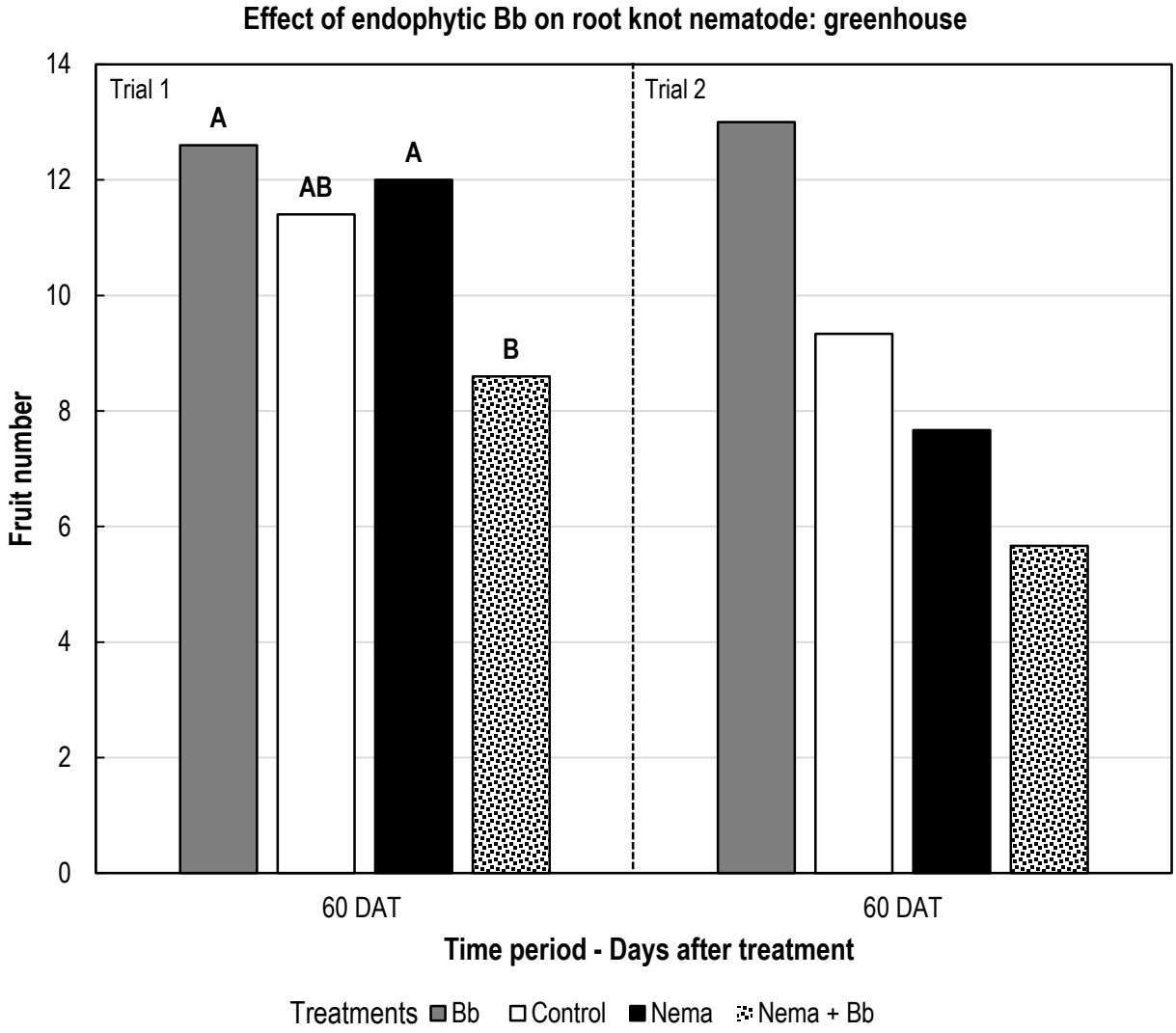


Figure 4.11. Effect of endophytic B. bassiana (Bb) and root-knot nematode on fruit number of tomatoes in a greenhouse assay.

Treatments were Bb = *Beauveria bassiana*, Nema = Root-knot nematode, Nema + Bb, and Control. Plants were evaluated at 60 days after treatment (DAT), for two trials. Separate analyses were conducted for each incubation period and trial. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.10$.

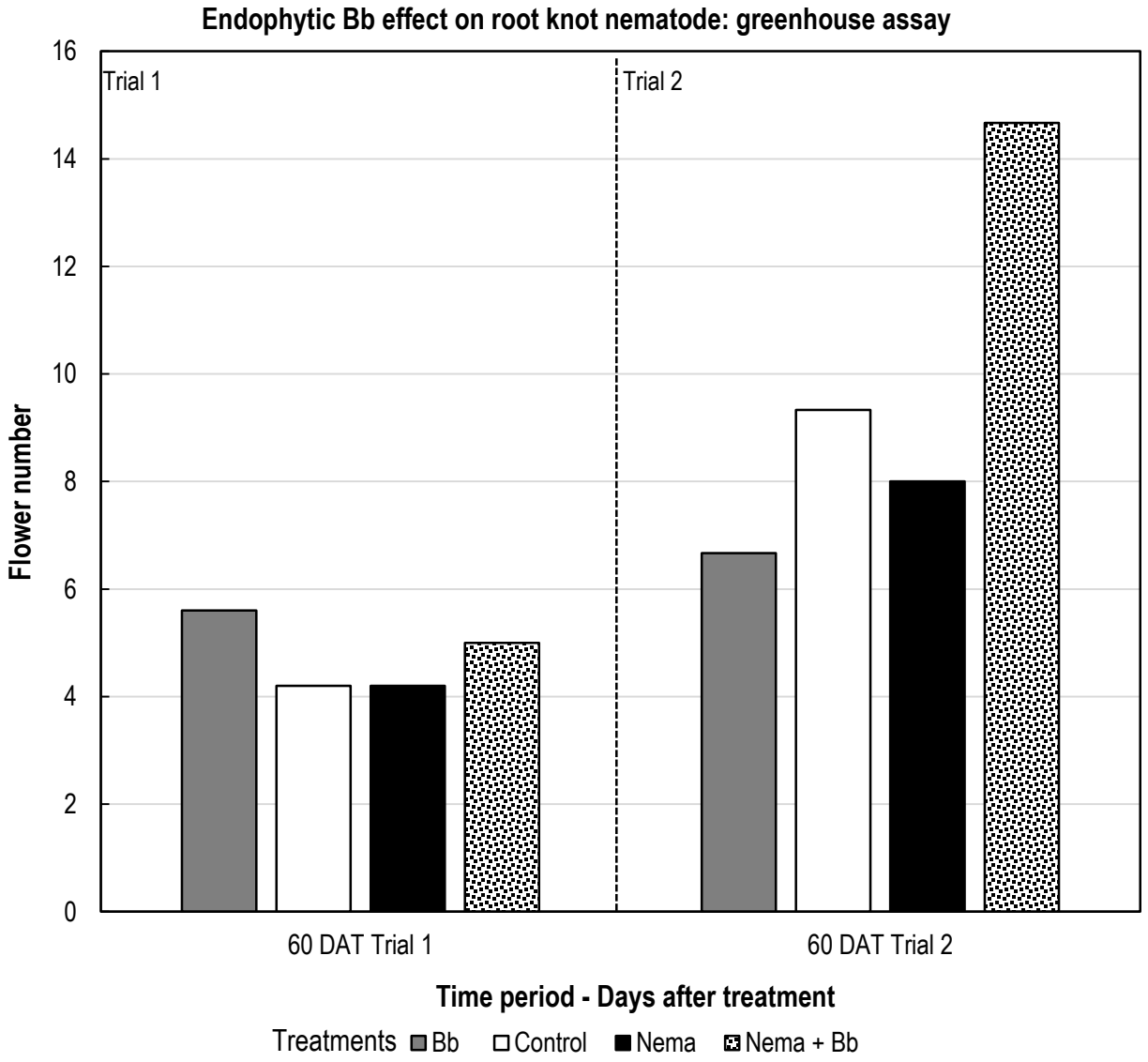


Figure 4.12. Effect of endophytic B. bassiana (Bb) and root-knot nematode on flower number of tomato plants in a greenhouse assay.

Treatments were Bb = *Beauveria bassiana*, Nema = Root-knot nematode, Nema + Bb, and Control. Plants were evaluated at 60 days after treatment (DAT), for two trials. Separate analyses were conducted for each incubation period and trial. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.05$.

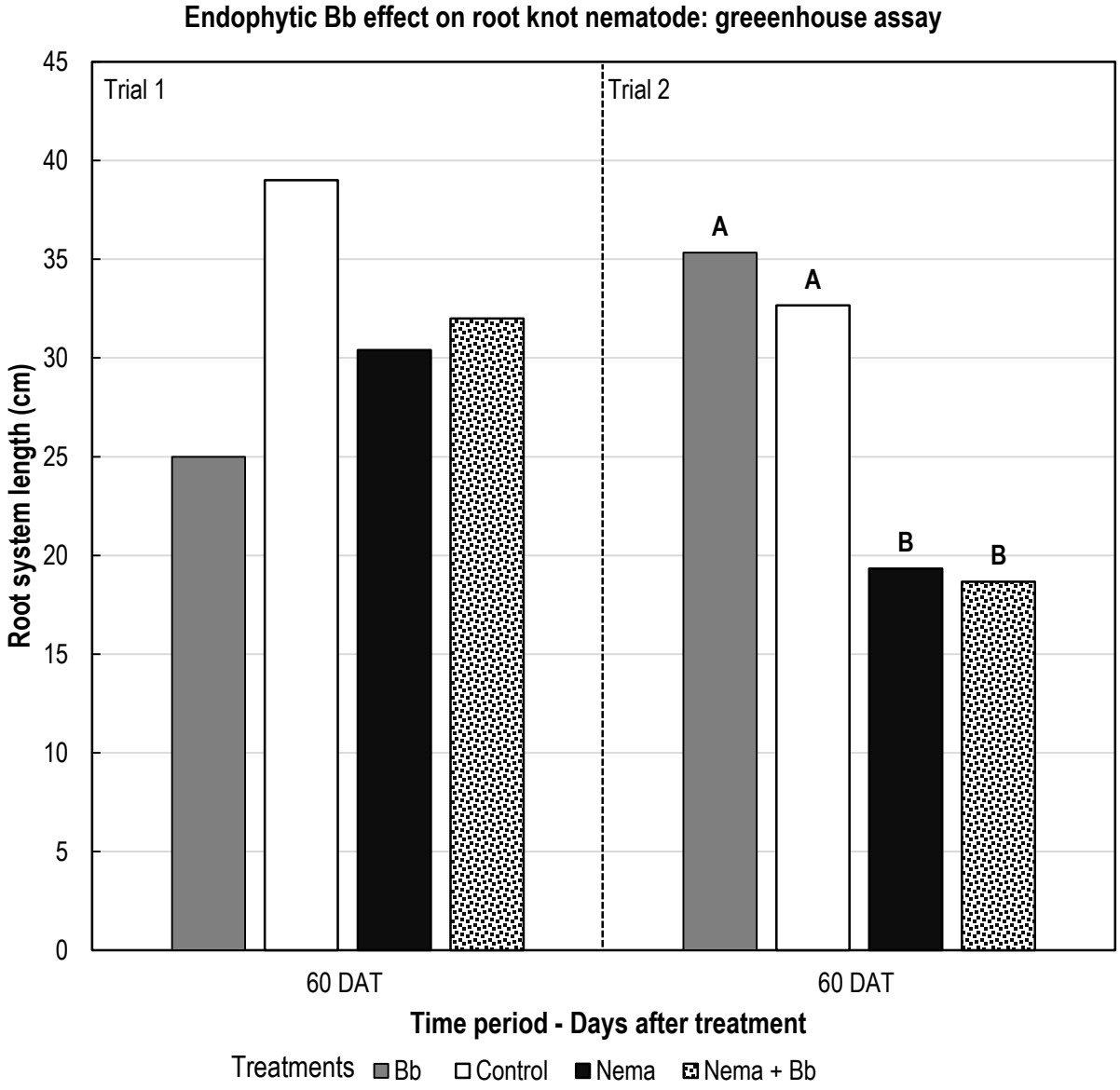


Figure 4.13. Effect of endophytic B. bassiana (Bb) and root-knot nematode on root system length (cm) of tomato in a greenhouse assay.

Treatments were Bb = *Beauveria bassiana*, Root-knot nematode, Nema + Bb, and Control. Plants were evaluated at 60 days after treatment (DAT), for two trials. Separate analyses were conducted for each incubation period and trial. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.05$.

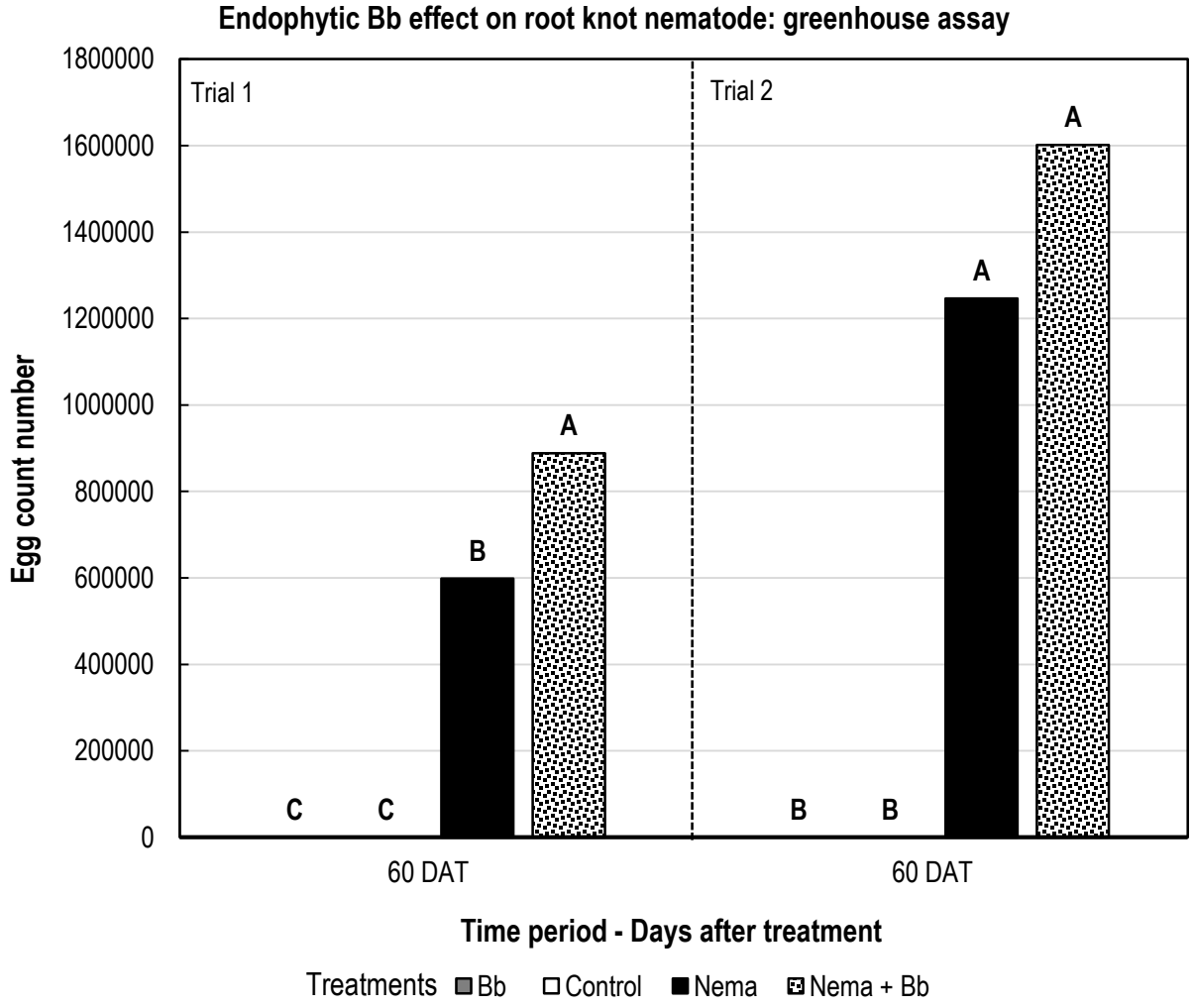


Figure 4.14. Effect of endophytic B. bassiana (Bb) on root-knot nematode egg count on roots of tomato in a greenhouse assay.

Treatments were Bb = *Beauveria bassiana*, Nema = Root-knot nematode, Nema + Bb, and Control. Plants were evaluated at 60 days after treatment (DAT), for two trials. Separate analyses were conducted for each incubation period and trial. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.05$.

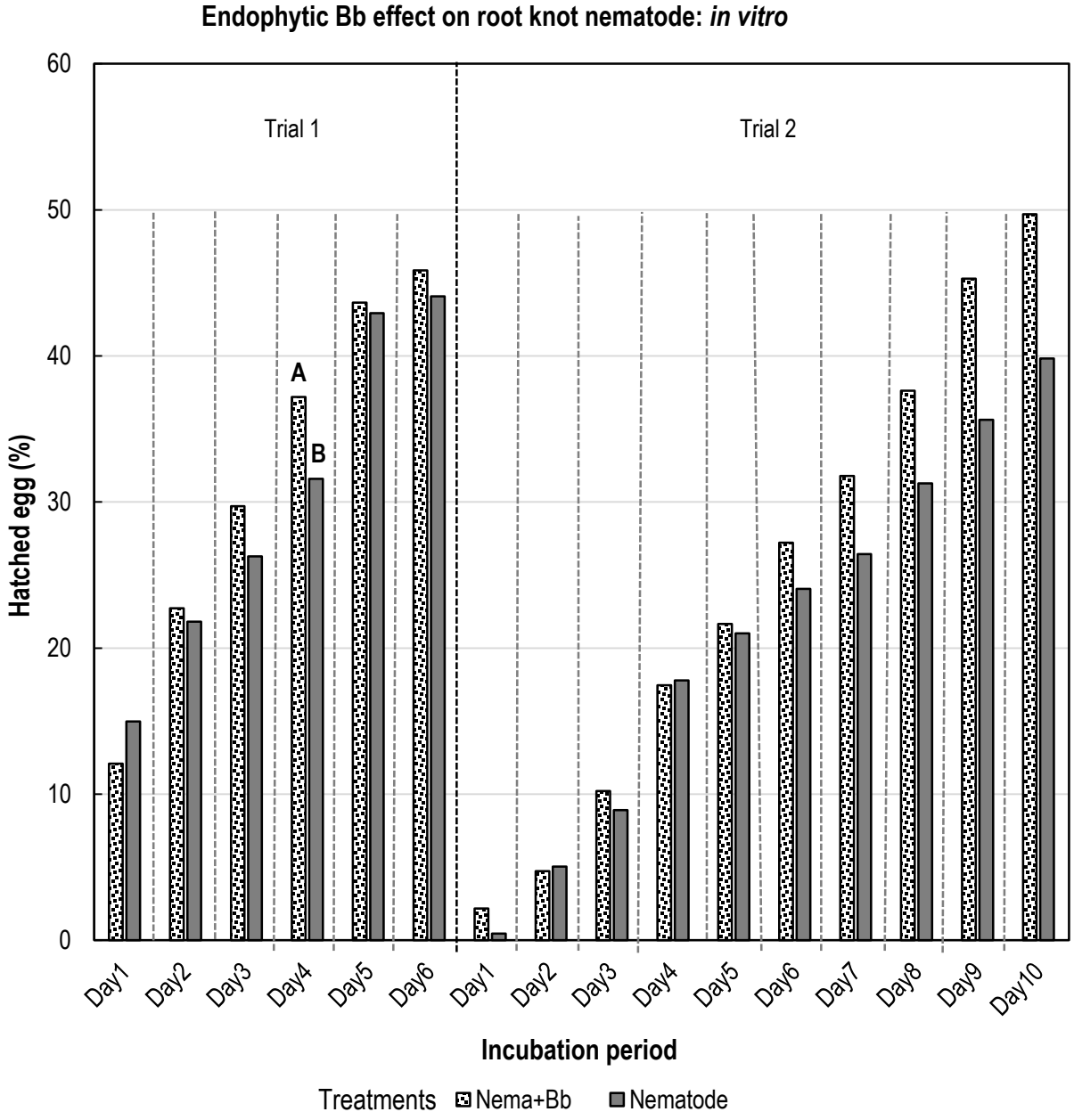


Figure 4.15. Effect of endophytic B. bassiana (Bb) on percent egg hatch of root-knot nematode extracted from tomato roots in a greenhouse assay.

Treatments were Nema + Bb = Root-knot nematode + *Beauveria bassiana,* Nema = Root-knot nematode. Separate analysis was performed for each day. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.05$.

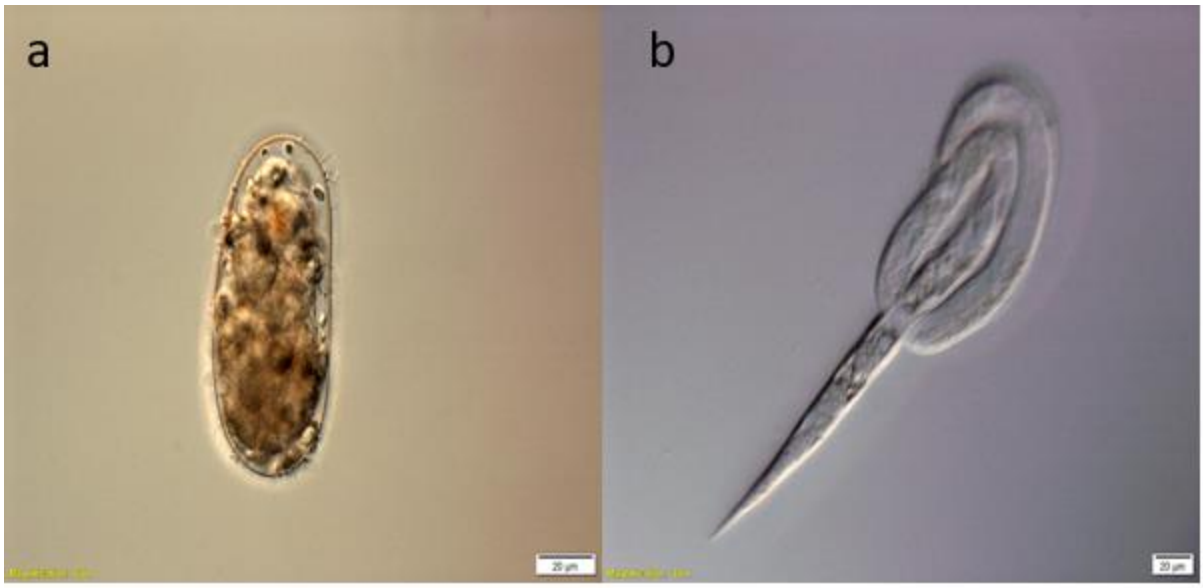


Figure 4.16. A) Root-knot nematode egg observed in endophytic B. bassiana (Bb) treatment; B) Root-knot nematode juvenile observed in no Beauveria treatment (control) in the in vitro assay.

(Photo credit: Dr. E. Bernard, University of Tennessee, Knoxville).

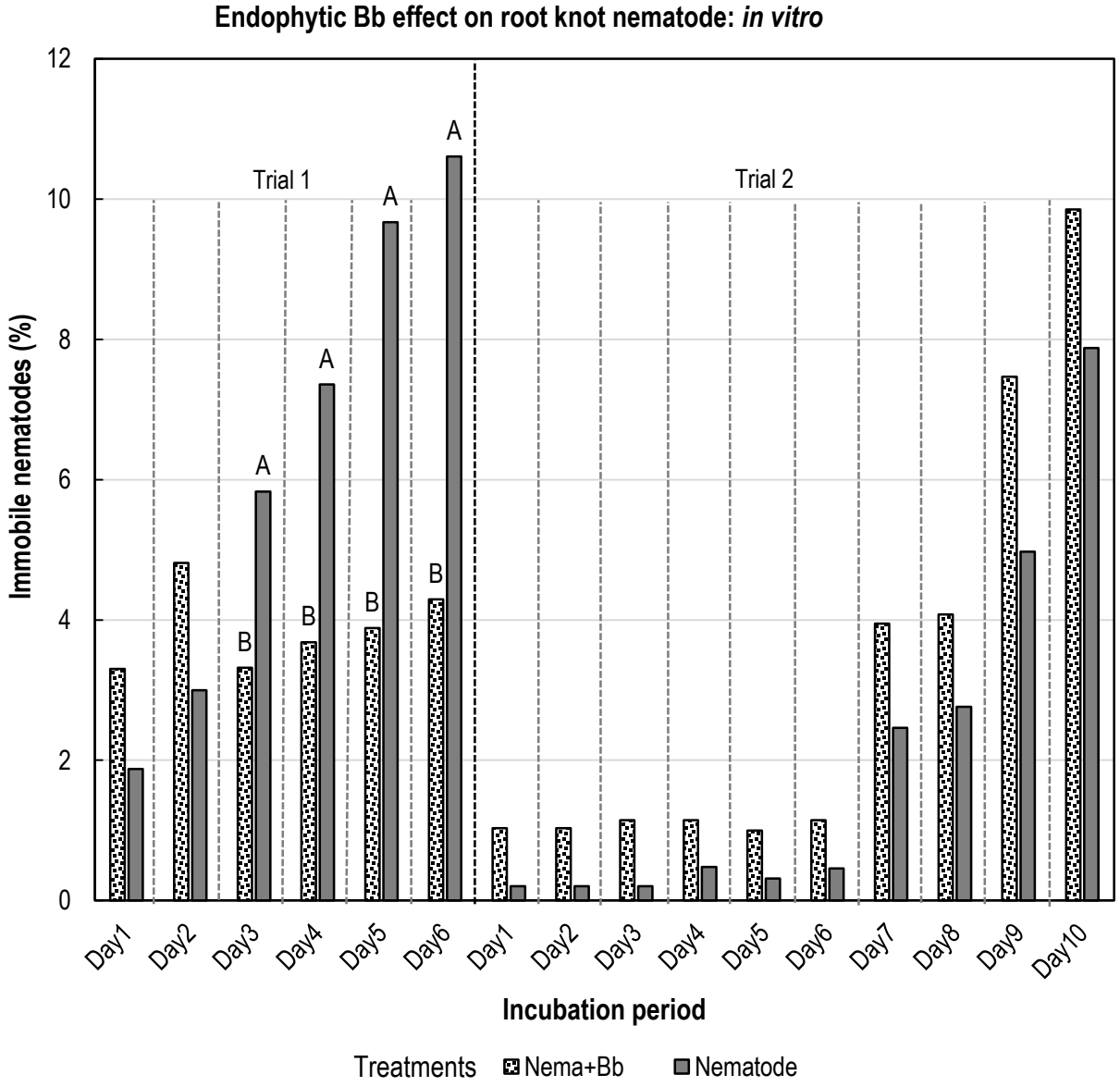


Figure 4.17. Effect of endophytic B. bassiana (Bb) on percent immobile root-knot nematode extracted from tomato roots in a greenhouse assay.

Treatments were Nema + Bb = Root-knot nematode + *Beauveria bassiana,* Nema = Root-knot nematode. Separate analysis was performed for each day. Bars with the same letter or no letter are not different according to an F-protected LSD at $P \leq 0.05$.

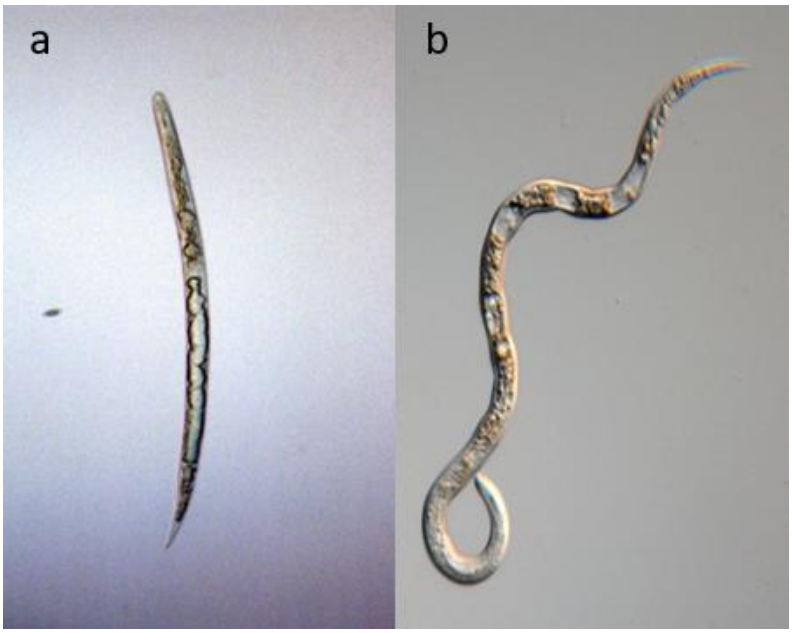


Figure 4.18. A) Immobile nematode, B) Mobile nematode observed in the in vitro assay of endophytic B. bassiana (Bb) conidia effect on root-knot nematode.

(Photo credit: Dr. E. Bernard, University of Tennessee, Knoxville).

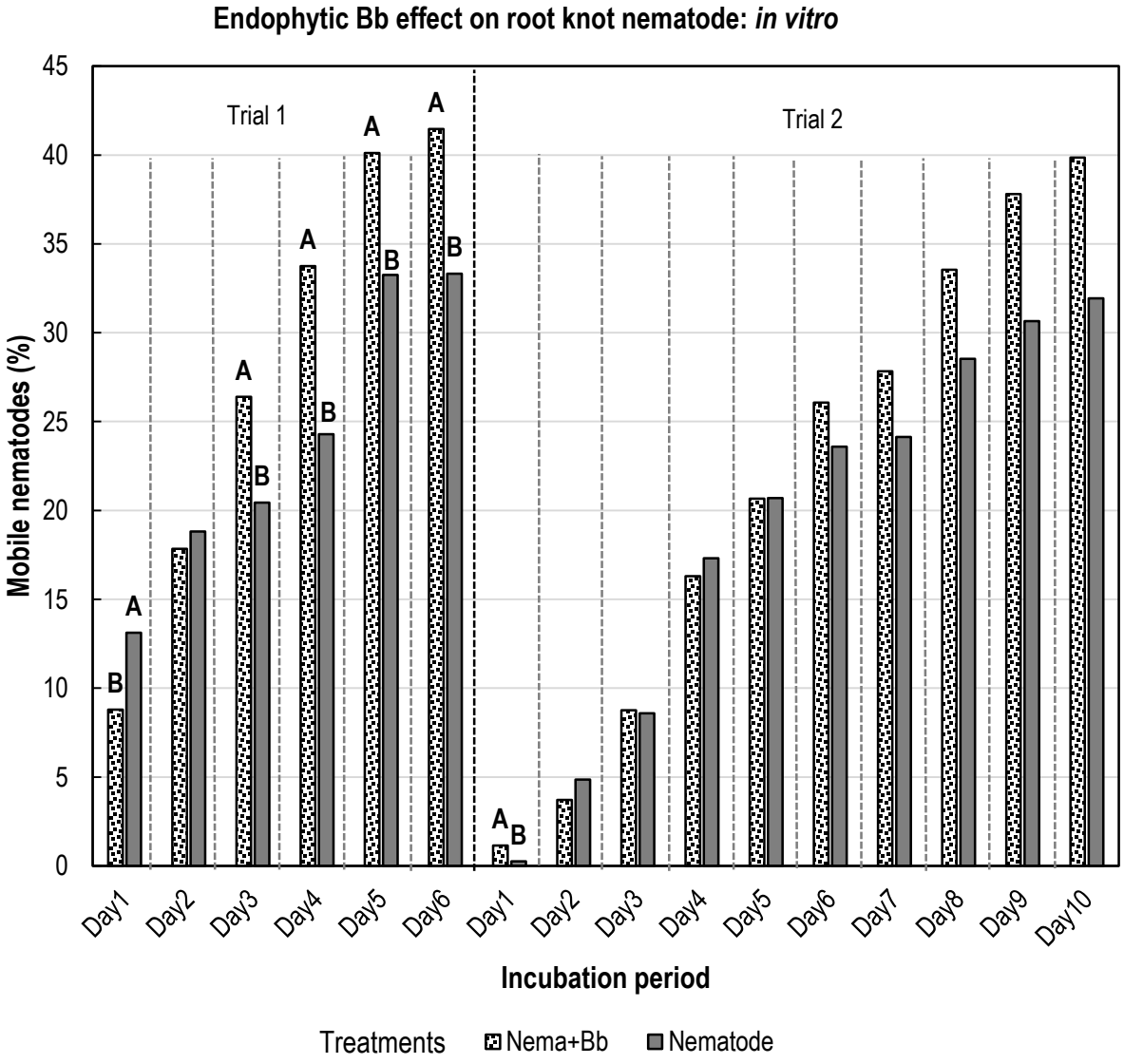


Figure 4.19. Effect of endophytic B. bassiana (Bb) on percent mobile root-knot nematode extracted from tomato roots in a greenhouse assay.

Treatments were Nema + Bb = Root-knot nematode + *Beauveria bassiana,* Nema = Root-knot nematode. Separate analysis was performed for each day. Bars with the same letters are not different according to an F-protected LSD at $P \leq 0.05$.

SUMMARY

There are several important issues that affect agricultural ecosystems, including the use of chemical pesticides, which can be very effective in reducing pest loads. However, there are also disadvantages, including higher cost for producers and environmental pollution. Alternative management strategies for chemical pesticides include biopesticides. Many biopesticides are beneficial endophytes. They live in mutual association with plant hosts, and protect plants from biotic and abiotic stresses, by direct and indirect mechanisms. In turn, plants provide shelter and nutrients to the endophyte.

Although biopesticides can be effective, they are often limited by inconsistent performance. The goal of this research project was to identify factors that influence the ability of the entomopathogenic fungal endophyte, *Beauveria bassiana*, to colonize plants, kill insects, and suppress activity of plant pathogens. A meta-analysis approach was used to evaluate many studies over multiple years. In addition, little is known about the ability of *B. bassiana* to control plant-parasitic nematodes. To answer that question, this project included experiments to assess the ability of *B. bassiana* to control southern root-knot nematode (Meloidogyne incognita) on tomato.

Three separate, quantitative meta-analyses were conducted on research articles published during the period of 2002 to 2019. Overall, the first objective was to assess the endophytic colonization efficacy of *B. bassiana* across different plant taxa. The second objective was to determine the effect of *B. bassiana* on mortality of many insect species, and the third objective was to determine efficacy of *B. bassiana* to suppress activity (growth, disease severity or disease incidence) of plant pathogens. The fourth objective on root-knot nematode control, was focused on laboratory and greenhouse experiments to determine the ability of *B. bassiana*

to suppress egg hatch and percentages of mobile and immobile nematodes. The meta-analyses conducted in this research are the first studies to our knowledge focused solely on endophytic *B. bassiana* and its unique relationship with plants, insects and plant pathogens.

The meta-analysis of endophytic *B. bassiana* and plant colonization was focused on comparing endophytic colonization by *B. bassiana* of plants treated with the fungus, versus control plants, which were not treated. Across all studies, with different plant taxa, endophytic colonization by *B. bassiana* was 29%. There were 33 factors identified that influenced the extent of colonization, including isolate of *B. bassiana*; concentration and form of fungal inoculum; application method and site of application on the plant; and plant taxa, growth habit, and type of root system. Various experimental factors were also influencers, including the method of confirming the presence of *B. bassiana* in plant tissue and length of incubation period before assessment.

The focus of the meta-analysis of endophytic *B. bassiana* and its efficacy against insect pests was on the difference in percent insect mortality between treatments with *B. bassiana* and controls that did not receive the fungus. Across all studies, endophytic *B. bassiana* caused 39% insect mortality across diverse insect species. Thirty-two influential factors were identified, including, fungal isolate; source of the isolate; form of inoculum and concentration of *B. bassiana*; application method; extent of plant colonization; and insect species, and its physical and feeding characteristics.

The meta-analysis of endophytic *B. bassiana* on suppression of plant pathogen activities was focused on the difference in suppression of plant pathogens associated with *B. bassiana* treatments versus non-treated controls. Plant pathogen activities were defined as pathogen

growth, disease severity, or disease incidence. Across all studies, endophytic *B. bassiana* reduced pathogen activity by 31%, and 34 influencing factors were identified. Factors that affected the extent pathogen suppression by B. bassiana included isolate of B. bassiana; geographic and substrate source of the isolate; form of B. bassiana inoculum and concentration; application method and extent of plant colonization; plant pathogen species and characteristics; and plant species.

For the three meta-analyses, the geographic location and whether the experiments were conducted in the field, protected culture, e.g. greenhouse, or the laboratory, impacted efficacy of the fungus. Significant variability across studies was evident in all three analyses supporting the important role of the species of insects, plants, and plant pathogens, as well as isolate of *B. bassiana* evaluated.

In nematode experiments, it was confirmed that *B. bassiana* could endophytically colonize 'Rutgers' tomato, which was selected for greenhouse studies due to is susceptibility to southern root-knot nematode. However, *B. bassiana* was not effective in reducing percentages of egg hatch, and mobile and immobile nematodes in *in vitro* or *in planta* studies. Interestingly, root galling and egg count/root system were enhanced in *B. bassiana* treated tomato plants. The presence of viable *B. bassiana* from roots was confirmed by collecting egg suspensions from root galls and plating them on Beauveria Selective Medium. Colonies of *B. bassiana* were verified on the medium, however, no parasitism of nematode eggs was observed.

In summary, the research findings of this study confirmed that endophytic *B. bassiana* endophytically colonizes plants, and effectively parasitizes insects and suppresses plant pathogens across a wide variety of experimental conditions and different plants, insects, and

plant pathogens. Although *B. bassiana* endophytically colonized 'Rutgers' tomato, it was not effective in reducing activities of the southern root-knot nematode. In fact, nematode egg hatch and root galling were enhanced in plants treated with *B. bassiana*.

Overall, these research findings act as a valuable tool to enhance our current understanding and knowledge on the endophytic and multi-trophic lifestyle of *B. bassiana*. The meta-analyses provide new information for development of improved, efficacious, ecofriendly, and sustainable plant disease management strategies, and provide insight for future research on plant protection with endophytic *B. bassiana*, and other fungal endophytes.

VITA

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