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EFFECT OF ENDOPHYTE-INFECTED TALL FESCUE ON THE JAPANESE BEETLE AND OTHER SELECTED SOIL INSECTS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Jason B. Oliver

May 1990

I would like to dedicate this thesis to my parents, Dr. Jack Wallace and Martha Lou Oliver. Your support over the years has made it possible for me to complete my education and this thesis.

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ABSTRACT

Field and laboratory studies were conducted to establish if soil insects are adversely effected by Acremonium coenophialum-infected (E+) or non-infected plants (E-). Studies with Japanese beetle (JB) (Popillia japonica) indicate that toxic factors are present in roots of E+ plants. Survival of first instar JB was significantly lower in the presence of E+ roots or peat soil which had been exposed to E+ roots than in the presence of E- roots or soil which had been exposed to E- roots. Results indicate that toxins in soil have a limited activity period and might be loline alkaloids (pyrrolizidine), which are volatile and known to occur in roots of E+ plants or other unknown toxins. JB eggs appeared to be unaffected by E+ plants. Field studies with natural populations of JB or artificial infestations of eggs into field plants proved difficult and did not produce conclusive results. Potted plants infested with JB eggs and placed into the field had significantly more third instar larvae surviving in 80% E- plantings than 80% or 100% E+ plantings after a 3-month period. Preliminary evidence indicates that E+ plants may be resistant to JB larvae due to deterrence.

Other insects studied for effects of E+ plants were Cyclocephala sp., Conoderus sp., and sminthurid springtails. Field populations of Cyclocephala did not indicate

significant differences in abundance between E+ or E-, but field collected larvae placed in potted fescue plants for 1 month demonstrated significant reductions in E+ plants. Conoderus larvae were not significantly more abundant in E+ or E- field plants. Pitfall collections of Collembola indicate relative abundance of Sminthuridae were reduced in E+ field plots. Bourletiella (Deuterosminthurus) lippsoni Snider and Sminthurides (Sphaeridia) serratus Folsom and Mills were the two predominant species and both occurred in greater abundance in E- fescue plots than E+ plots.

Findings of this study indicate that soil insects are affected by the presence of A. coenophialum in fescue plants and that these effects occur in the root regions of the plant.

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CHAPTER I

LITERATURE REVIEW

TALL FESCUE

Tall fescue, Festuca arundinacea Schreb., is a cool-season perennial grass in the tribe Festuceae and family Gramineae. Tall fescue is believed to have originated in Europe (13, 30) and is now widely grown in the U.S. on approximately 12-14 million hectares (80). Tall fescue is best adapted to the transition zone between the northern and southern United States (14) and is the predominant cool-season grass grown in the southeast (14). Fescue is grown on more than 1,420,000 ha in Tennessee (58) for forage, pasture, turf, and soil conservation (13, 30, 92). Fescue is used widely because of its persistence and adaptive ability under environmental stresses such as drought (48, 89), high moisture conditions (13, 30, 31), poor soils (13, 30), grazing damage (86, 87, 92), pest damage (11, 25, 28, 45, 49, 56), injury (38), and because of high forage yields and quality (13, 30, 92).

Tall fescue is a highly heterozygous-polyploid, cross-pollinated plant relying on wind for pollination (30). Plant growth begins when weekly temperatures are above 4.4°C

(30) and seed heads are produced only in the spring. Perennial growth of the grass occurs by tillering (13).

Fescue pastures for many years have been associated with toxic syndromes in livestock (92). Livestock problems typically resemble "ergot poisoning" caused by ingestion of sclerotia of Claviceps purpurea (Fr.:Fr.) Tul., and Claviceps often was considered as a possible cause of livestock poisoning (31, 92). Livestock poisonings frequently are seasonal and characterized by reduced growth and difficulty in heat regulation. In addition, a more serious condition involving the sloughing of the extremities ("fescue foot") (86, 92) also occurs. Cunningham (31) suggested that "fescue foot" was not due to Claviceps, since mature sclerotia were present on the plants only from January to March, while symptoms typical of ingested sclerotia occurred year round. Symptoms also developed in cattle grazing pastures not infected with Claviceps. Cunningham suggested that the fescue plant itself was the source of fescue toxicity. Bacon et al. (7) suggested involvement of a mycotoxin since the condition occurs sporadically and seasonally.

Two species in the genus Balansia, isolated from fescue pastures, were found to produce ergot alkaloids similar to those in Claviceps (7). Balansia sp., which typically infect plants systemically, are closely related to C. purpurea.

In 1977, Bacon et al. (9) suggested that the systemic-endophyte, Epichloe typhina (Pers.) Tul., in tall fescue was associated with possible cattle toxicity. Endophytes had been reported in fescue previously (76) and were mentioned as a possible source of livestock toxicity in other grasses and weeds (7). The findings of Bacon and coworkers were the first report of a systemic endophyte in tall fescue possibly causing livestock toxicity. Additional information now exists that presence of this endophyte is correlated with livestock toxicity (6, 86, 87).

ENDOPHYTES

Endophyte literally means an organism that lives systemically within a plant. Endophytic fungi (Ascomycetes) in the family Clavicipitaceae and tribe Balansiae infect grasses (Graminae), sedges (Cyperaceae), and rushes (Juncaceae) (27). The majority of grass endophytes cause symptomatic infections such as host sterilization ("choke disease") in their hosts (6, 23, 80). Signs usually appear in the form of external stromata, with subsequent inhibition of flowering through production of fungal fruiting bodies such as ascospores and conidia (6, 25, 54, 80).

Certain endophytes produce no external signs of infection, and reproduce by growing into their hosts' seed without interfering with host reproduction (6, 80). These

endophytes are sometimes called non-choke-inducing (NCI) endophytes (48, 75). Tall fescue, as well as perennial ryegrass and some fine fescues, have NCI endophytes (6, 38, 75, 80).

FESCUE ENDOPHYTE

Grass/Fungal Relationship

Taxonomy

Fescue endophyte is a clavicipitaceous fungus (6). Bacon et al. (9) originally classified the fungus in tall fescue as a biotype of E. typhina, the causal organism of "choke disease" in most grasses. E. typhina is a common endophyte (or pathogen) that infects a wide range of grasses (54, 91) as well as fescue (76). Bacon et al. (9) suggested that the endophyte of fescue evolved from a pathogenic ancestor to a highly compatible, completely systemic fungi that no longer requires reproductive propagules. In 1982, Morgan-Jones and Gams (63) reclassified the fescue endophyte as Acremonium coenophialum Morgan-Jones and Gams based on conidial morphology. Origin of the fescue endophyte is difficult to establish on the basis that the endophyte only produces the asexual state of conidia, which makes classification difficult (6). Other NCI endophytes in fine

fescue species and perennial ryegrass may be related to E. typhina or Acremonium (57, 75).

Endophyte Life Cycle

Acremonium coenophialum develops systemically between plant cells of the tall fescue plant. Infected plants (E+) produce no visible symptoms and can be distinguished from non-infected plants (E-) only by microscopic analysis (9, 47, 61), immunoassay (70), fungal tissue culture (5, 29), or bioassay (56). In the mature plant, the fungus occurs principally in the leaf sheaths, seeds, crowns, and stems, with lesser amounts in the leaf blades (6, 48, 82) and is generally believed not to enter the roots (39, 47). However, Siegel et al. (82) reported low amounts of endophyte in the roots. The fungus remains in the seed for a short period of time after seed germination. The seedling is invaded within 2 weeks (6). The fungus is disseminated only in infected seed.

Mutualism

The grass-endophyte relationship is believed to be mutualistic (80). Unlike pathogens, the endophyte does not appear to alter host cell morphology (4, 6, 47). The fungus is completely dependant upon the plant for survival and

propagation. If A. coenophialum was formerly a pathogen as suggested by Bacon (9), then pathogenic qualities may have been lost by natural selection for a less virulent biotype. Some studies (18, 21) suggest that the former pathogenic nature of the endophyte can be induced by subjecting both the plant and endophyte to stressful situations such as nutrient deficiencies. Prevalence of infected plants suggests differential fitness of infected and uninfected plants; mutualism is thought to evolve in situations where both organisms contribute to each other's total fitness and survival (18).

Siegel et al. (80) listed four benefits the fungus derives from the relationship with the fescue plant: nutrition, long term protection, improved dissemination, and survival. Because A. coenophialum develops endophytically, it is assumed all of its nutrition and growth factors are derived from its host. The prevalence of fungal hyphae in the leaf sheath area of the plant may be related to carbohydrate procurement from vascular transport of photosynthesis products. A. coenophialum also receives protection from environmental stress by growing intercellularly within host tissues (6).

Dissemination of the fungus is enhanced through its seed-borne nature (6). Seed-borne reproduction assures the majority of F1 progeny of E+ plants are infected (38) and is highly efficient compared to reproduction dependent upon

spores with limited viability encountering suitable hosts in the environment (6). Seed-borne reproduction allows the endophyte to remain viable in the seed for 1 year or more, and to resume growth upon germination of the host (6, 38, 48).

Plant benefits derived from the fungal association are expressed as improved plant stress responses (38, 48) that result from enhanced plant growth or protection from herbivores. The value of the mutualistic relationship may be enhanced under environmental stress, so that E+ plants are selectively favored over E- plants (4). Endophyte-infection level does increase in pastures over time, implying a selective advantage for infected plants (2, 6, 12, 28). Improved plant growth from endophyte infection includes increased plant tillering and forage production (4, 24, 89), recovery from injury (4, 38), resistance to weed invasion (38), improved persistence and density (4, 38, 89), increased seed production (6), and pest resistance (25, 89). Improved plant growth may lead to better pasture stand with higher forage quality and less maintenance (6, 38, 89). In addition, fescue endophytes enhance plant performance by providing protection against herbivores either indirectly, through increased plant tolerance as a result of better plant growth (80), or directly, by the production (or inducement in the plant) of toxins that are antibiotic or antixenotic to herbivores (26, 94).

Alkaloids

A. coenophialum-infected plants possess a variety of alkaloids not found in E- plants. The production of alkaloids has been the focus of considerable research due to their probable role in livestock toxicosis and pest resistance. Most members of the Balansiae group have been found to produce alkaloids similar to those produced by C. purpurea (6, 60).

One possible benefit of alkaloid production is plant defense. Several studies have demonstrated that endophyte-associated alkaloids have detrimental effects on herbivores (14, 26, 87, 94), implying alkaloids found in fescue may serve as a plant defense mechanism. Production of alkaloids is costly in terms of metabolic pathways involved (i.e., use of photosynthesis products and nitrogen (44, 74)). Presence of alkaloids must therefore provide a selective advantage to the plant that produces them (74). Graminous plants typically do not produce toxins (44) and therefore, alkaloid production associated with endophyte may be advantageous to the plant for protection. Alkaloids frequently accumulate in plant regions with greatest growth, where herbivore damage could be potentially greatest (44, 45, 74). Fluctuations in alkaloid concentration with season and growth stages may correspond to times that the plants are

most susceptible to herbivore damage (74), such as during stressful summer periods.

Several alkaloid groups are associated only with the presence of A. coenophialum and their production may result from the endophyte or the plant in response to the fungus. Three major groups of alkaloids have been reported in tall fescue: ergot alkaloids (60, 67, 68, 93), loline alkaloids (pyrrolizidine) (16, 73), and diazaphenanthrene alkaloids (14, 15). Other less prevalent types include β-carbolines (94), sympathetic amines (32), and sterols (33).

Ergot alkaloids are produced by all members of the family Clavicipitaceae (6, 8, 27, 67, 68) including C. purpurea, the ergot fungus. Ergot alkaloids possess an ergoline nucleus and fall into one of three groups: ergopeptides, clavines, and lysergic acid amides (60, 93). Yates and Powell (93), Yates et al. (94), and Bacon et al. (6) provide lists of some of the known ergot alkaloids in tall fescue and other Balansiae. Ergovaline is the principal ergot peptide occurring in E+ tall fescue (60, 93). Concentration of ergot alkaloids varies seasonally (80, 87) and with the level of nitrogen fertilization (60). Porter et al. (67) isolated ergot alkaloids from endophyte cultures, suggesting that the fungus and not the plant produces ergot alkaloids. Ergot alkaloids produced by A. coenophialum are translocated throughout the plant sheath and leaves (6, 60).

Lolines are present in E+ fescue in high concentrations. Evidence suggests lolines are specific to A. coenophialum-infected plants. They are not found in grasses infected with other members of the tribe Balansiae (80, 81). Bush et al. (16) found concentrations of 3.02 mg/g of N-acetyl and N-formyl loline in E+ plants compared to 0.036 mg/g in E- plants. N-formyl loline has been found in the roots of A. coenophialum-infected fescue (variety 'Mustang') at concentrations of 93 μg and not in the roots of E- plants (personal communication, Daniel A. Potter, University of Kentucky, Lexington, Ky 40546). Jones et al. (50) suggests the origin of loline alkaloids may be an interaction between the fungus and plant since neither organism was found to produce the alkaloids separately in tissue cultures. Others (80, 81) report loline production by artificially infecting perennial ryegrass with A. coenophialum. Production of lolines may involve a complex interaction between host and endophyte.

Loline concentrations are high in tall fescue, but seasonal fluctuations do occur; highest levels are found in late summer (14). Lolines have been found in the highest concentrations in new fescue growth (45). The water-soluble nature of lolines (personal communication, Richard G. Powell, Bioactive Constituents Group) might facilitate their movement in the plant's vascular system.

Perloline (a diazaphenanthrene) occurs in very high concentrations (3.0 mg/g dry matter) in tall fescue and fluctuates seasonally, with highest concentrations present in the summer (14). Perloline presence however, does not appear to be related to A. coenophialum presence since plants high in lolines (i.e., endophyte-infected) may have low perloline content (16). Due to difficulty of separating of alkaloid fractions (94), it is possible that undiscovered compounds exist in E+ fescue.

Effects on Livestock

Tall fescue infected with A. coenophialum has a significant impact on animal agriculture. Cattle grazing E+ pastures are frequently afflicted with a condition referred to as "fescue toxicosis", a term which describes adverse effects on animals caused by fescue endophyte. Stuedemann and Hoveland (86) and Thompson and Porter (87) provide reviews on the effects of tall fescue on livestock and Yates (92) provides a historical review. In general, fescue toxicosis in cattle can be classified into three categories: reduction in growth, reduction in milk production (agalactia), and impaired reproduction (86). Common symptoms in cattle include an inability to tolerate summer temperatures, reduced grazing, roughened hair coat, nervousness, fat necrosis, and the more extreme condition of

gangrenous fescue foot (61, 86, 87, 92). Adverse effects have been observed in other livestock such as horses (41) and sheep (86) grazing fescue as well as vertebrate laboratory animals fed E+ seed (64). Ergot alkaloids are considered prime candidates as the cause of fescue toxicosis because of their similarity to the toxic alkaloids of C. purpurea (68), their potent physiological nature (6), and the fact that fescue foot resembles ergotism (60). Solomon et al. (84) found ergot alkaloids to have a vasoconstrictive action on cattle veins. However, other alkaloids (e.g., lolines, perloline, and halostachine) present in tall fescue, are also toxic to livestock (14, 15, 16, 32, 73). Toxic symptoms appear to be distinct from the symptoms shown by cattle grazing ryegrass infected with the related A. loliae infection. Ryegrass staggers in sheep (36) is generally believed to be caused by lolitrem alkaloids (40).

Many tall fescue pastures in the southeast have a high incidence of endophyte infection (58, 79); therefore the potential for livestock loss is large. Yates and Powell (93) state that 95% or more of the tall fescue grown in the United States is infected with A. coenophialum. Annual monetary losses in the cattle industry have been estimated at $800 million (Stuedemann as cited in Ball (10)). In Tennessee, a potential annual loss of $21 million has been reported by McLaren (62).

Since tall fescue is one of the best adapted grasses to the southeastern transition zone, other cool-season or warm-season grass selections may be limited for forage use (14). Also, the advantages endophyte infection provides to fescue may make it impractical to use E- fescue on a wide scale. Prestidge et al. (69) and Bacon (5) both suggest that it might be possible to select endophyte isolates for factors resistant to pests and nontoxic to livestock. Clay (25) suggests that with improved inoculation methods, it may be possible to genetically alter endophytes to provide desired traits.

Effects on Insects

Insects can contribute significantly to damage in tall fescue pastures (19). Soon after the implication that A. coenophialum presence was associated with toxicity in livestock (9), resistance of E+ plants to various insects was discovered. Pest resistance in the fescue plant by endophyte-infection could provide considerable selective advantage over E- plants (6, 25). Endophytes are advantageous for insect "biological control", because they reduce chemical pesticide use, are self-maintaining through seed-transmission, have minimal effects on the environment, and are very selective for grass pests while remaining effective against a broad spectrum of pest types (25, 48).

Insects adversely affected by infection of tall fescue with A. coenophialum include the argentine stem weevil (11), flour beetle (20), fall armyworm (28, 45), oat-bird cherry aphid (37, 49, 56), greenbug (49), corn-flea beetle (52), a sharpshooter leafhopper (52), and various other leafhoppers. Methanol extracts of E+ fescue affect the oat-bird cherry aphid (49), argentine stem weevil (69), and the large milkweed bug (49, 94). Adverse effects include increased mortality (20, 28, 45, 49, 94), reduced incidence in field samples (52), reductions in growth (28, 45) and development time (28), and feeding deterrence (11, 37, 45, 49, 56, 69).

These studies show that there is considerable variation in the degree of toxicity of E+ fescue to certain groups of insects. For example, fall armyworm typically showed reduced growth and development time, but mortality was relatively low (28, 45); whereas, flour beetle populations were heavily reduced (20). Oat-bird cherry aphid and greenbug were completely deterred from feeding (49). Some insects such as the tobacco hornworm and tobacco budworm (49), the rose grain aphid (56), the English grain aphid (49), and several leafhoppers (52) apparently are not affected by E+ fescue. Variability in insect response to E+ fescue is influenced by a number of factors. These factors include plant growth stage and nutrition, endophyte toxin production, and species specific variation among insects. Species specific factors include biochemical machinery for

toxin breakdown (3, 44), type of feeding such as chewing or piercing-sucking (45, 48), location of feeding (45), insect age (45), and general condition of the insect.

Although not all insects are affected by E+ plants, the endophyte may still provide a broad means of plant protection. Endophyte-infected plants provide resistance against insects in one of three ways: tolerance, antibiosis (toxic), or antixenous (deterrent) (25). Endophyte-infected plants recover from insect damage better than E- plants (2, 39). Because E+ plants produce more biomass, leaf area, and forage (24, 89, 90), it is possible they can tolerate significantly heavier feeding damage than E- plants as well as recover faster. Resistance of E+ plants to insects can also occur as antibiosis or antixenosis. Plant feeding studies showing higher mortality in insects frequently did not distinguish between antibiosis or deterrence (28, 52). Studies which have established a mode of resistance show deterrence (i.e., higher mortality from reduced feeding) (11, 45, 49, 56, 69) to be the principal form of resistance. Insect groups, such as the flour beetle and fall armyworm (20, 45), which are less strongly deterred by endophyte (i.e., continue to feed normally or at some slightly reduced level) ultimately show some level of antibiosis. Resistance qualities in E+ plants are present at a very early age (e.g., 12-day-old seedlings) even before the endophyte has entered the growing plant from the seed (56, 85). The exact

physiological action endophytes have against insects are not known and have been examined only in a few studies with perennial ryegrass endophyte (1, 3).

Several studies have attempted to establish a link between the numerous alkaloids in E+ plants and the detrimental affects observed in plant feeding studies (26, 45, 49, 81, 94). Most of the major alkaloid classes in fescue have been examined, including ergot peptides (26, 81, 94) and lolines (45, 49, 81), diazaphenanthrenes (94), and β-carbolines (94). As expected, because of the toxic properties of alkaloids in general (44) most fescue alkaloids examined have produced some inhibitory effects on insects; however, some groups were less toxic than others.

Ergot-alkaloids, although potentially very important in causing livestock toxicity, do not appear to be greatly involved with insect resistance (26, 81, 94). Prestidge et al. (69) working with fungal cultures of A. coenophialum, established that the fungus can produce insecticidal compounds active against the Argentine stem weevil. Their findings might suggest some ergot alkaloid activity against insects since the endophyte is known to produce these alkaloids in culture (67).

Loline alkaloids have been suggested by several researchers as a source of insect resistance. Yates et al. (94) found the loline-lysergic acid fraction isolated from fescue seed to be the most toxic to the large milkweed bug,

while pure N-formyl loline had the lowest lethal dose value for the milkweed bug. Johnson et al. (49) reported that the oat-bird cherry aphid and large milkweed bug were deterred by a methanol extract of fescue seed containing higher concentrations of N-formyl loline and N-acetyl loline than non-deterrent ethyl acetate and hexane extracts. However, they did not consider the fact that ergot alkaloids are also removed by methanol (93). Siegel et al. (81) also reported loline alkaloids to be toxic to the oat-bird cherry aphid and greenbug aphid. Loline alkaloids are abundantly present in tall fescue (14, 16) and could provide a very important source of insect resistance. Since many alkaloid studies have been done with the fall armyworm, a generalist feeder of grasses, it is possible that this insect species is not very responsive to the alkaloids present in grasses. Hardy et al. (45) suggested there was a lack of responsiveness by the fall armyworm to loline alkaloids due to its preference for new growth vegetation containing high concentrations of loline-type alkaloids.

Other alkaloids tested by Yates et al. (94), such as halostachine (sympathetic amine), nonharmane (β-carboline), and ergonovine (lysergic amide), were toxic to the large milkweed bug. They also found evidence of synergistic relationships between alkaloids by demonstrating the effects of perloline and ergocryptine were enhanced when combined. Thus, alkaloids are a source of resistance against insects

both as deterrents and toxicants, but questions about alkaloid interactions, their origin (plant or fungus), and differential susceptibility of insect taxa are still unanswered.

Effects on Soil Nematodes

The presence of A. coenophialum has been associated with the reduction of plant-parasitic soil nematodes. The endoparasitic nematode, Pratylenchus scribneri Steiner has reduced numbers on E+ fescue (51, 89, 90). Egg numbers and masses of the endoparasitic nematode Meloidogyne marylandi Jepson and Golden were also suppressed on E+ fescue (51). Other ectoparasitic nematodes the spiral nematode, Helicotylenchus dihystera (Cobb) (65), the stubby root nematode, Paratrichodorus minor (Colbran) (65), and Tylenchorhynchus acutus Allen (90) have fewer numbers on E+ in comparison with E- fescue.

Detrimental effects on soil nematodes would appear to suggest that toxic factors are present in fescue roots. Hinton and Bacon (47) state that A. coenophialum does not occur in fescue roots, while Siegel et al. (82) reports having detected very low amounts of endophyte in fescue roots. Factors potentially toxic to soil nematodes may enter the fescue roots by translocation through the vascular tissues (phloem) or by other means, since alkaloids have

been found in areas of the plant where the endophyte is not known to occur (6, 45, 60, personal communication, Daniel A. Potter).

Fescue Endophyte and Soil Insects

Effects of A. coenophialum on soil insects have not been studied. Presence of "true" soil insects such as Japanese beetle larvae and other white grub species was unaffected in studies with endophyte-infected perennial ryegrass (2, 39), and researchers attributed the lack of symptoms to the fact that A. loliae is not known to occur in ryegrass plant roots. Since A. loliae-infected ryegrass has been found to contain alkaloids different than those of A. coenophialum-infected tall fescue (80), it is possible that A. loliae infected ryegrass might not affect soil insects if these different compounds are not present in the roots. However, since E+ fescue adversely affects soil nematodes, it is probable that toxins exist in the roots, and that these toxins might affect soil insects.

Root-feeding insects are a very important group of pests in turf and forage grasses (19, 35, 55), and their damage may be comparable to foliage feeding insects (19). Current research provides evidence that alkaloids associated with the endophyte are toxic to insects (26, 45, 49, 81, 94) and are translocated throughout the plant (6, 45, 56, 60,

85) and that soil nematodes demonstrate reduced survival in the presence of E+ fescue (65, 89, 90). Therefore, the major research objective of this thesis was to determine if soil insects are negatively affected by the presence of A. coenophialum in tall fescue variety 'Kentucky 31'. To examine endophyte influences on soil insects, two research areas were examined: 1. The effects of endophyte presence on larval and egg stages of the Japanese beetle (JB) (Popillia japonica Newman) (Scarabaeidae: Coleoptera) in both laboratory and field. 2. the effects of endophyte presence on other soil insect populations occurring in the field.

CHAPTER II

MATERIALS AND METHODS

GENERAL

Insect Culture

JB was the principal soil insect used in this study, because the insect spends the majority of its life cycle in the soil (35), the larval stage is a pest of grasses (35), and the adult stage is seasonally abundant and can be cultured in the laboratory (35, 42). In order to perform laboratory and field experiments with JB, it was necessary to collect eggs from field-captured adults. Adult beetles were maintained in cages in a growth room (28 ± 1°C/Gro-Lite® 16:8 (L:D) photoperiod) and fed Pennsylvania smartweed (Polygonum pensylvanicum L.). Eggs were collected by supplying adults with pans containing oviposition medium (Appendix A). Eggs were subsequently collected by sifting the medium through a 12-mesh (1.70 mm) sieve and collecting eggs that passed through the sieve with a fine, moistened artist brush (42). Eggs not immediately used in experiments were stored in 120-ml plastic specimen cups containing oviposition medium. Hatchability of JB eggs used in various experiments was established by placing eggs on moistened

filter paper and counting hatch (personal communication, Michael Klein, USDA ARS Japanese Beetle Lab OARDC, Wooster, OH 44691).

Field collections of JB and other soft-bodied coleopterous larvae were boiled, preserved in 70% ethyl alcohol, and identified (19, 66, 71, 72). Instar status of field collected JB larvae was determined by head capsule measurement (59).

Plants, Endophyte Testing, and Statistics

Plants or seeds used in this thesis were variety 'Kentucky 31'. Fescue plants transplanted into field plots, providing tiller sources for experiments, or serving as plants for infestation with soil insects were grown in Promix® potting soil for 8-12 months in advance of their use. Prior to their use all plants were tested for endophyte by sampling tillers three separate times and testing with Protein-A sandwich, enzyme-linked immunosorbent assay (PAS-ELISA) (70).

All experiments consisting of three or more treatment groups were analyzed with ANOVA, and mean separation by Duncan's Multiple Range Test (77). Experiments comparing

two treatment groups were analyzed with ANOVA and mean separation by paired T-test (77).

JAPANESE BEETLE

Laboratory Studies

Egg Stage

Experiment I (1989). JB eggs were exposed to the roots of 13-day-old tall fescue seedlings. Glass bottles (120 ml) with lids, and bottoms lined with filter paper were steam-sterilized in an autoclave. Sterile tap water (2 ml) and four E+ or E- seeds (scarified in 60% sulfuric acid for 35 min and sterilized with 2.12% sodium hypochlorite solution for 20 min and allowed to imbibe water overnight) were placed into each bottle. Control treatments received 2 ml of water and no seeds. All treatments were replicated 16 times and placed on a lab bench in a randomized complete block design under a Gro-Lite® with a 16:8 (L:D) photoperiod at 28 ± 1°C. Thirteen days after plant germination, plants had grown several centimeters in length and roots were developed on the filter paper. Fifteen 1-day-old JB eggs were placed in direct contact with the fescue roots. Daily

egg hatch was monitored for 20 days, after which it was assumed no additional hatching would occur. Average daily hatch and period of egg stadia were examined.

Experiment II (1989). JB eggs were exposed to the roots of 7-8-month-old tall fescue tillers. Potted E+ and E- fescue plants were removed from their pots, tillers separated, and their roots washed in tap water to remove soil. Twenty-one tillers of both E+ and E- plants were set aside and had their leaves and roots trimmed. E+ or E- tillers were placed on a moistened paper towel (cut 25 cm^2) with the roots on the towel and the foliage extending past the edge of the towel. E+ and E- plant treatments were replicated seven times.

Twenty 1-day-old JB eggs per plant treatment were placed against the roots and the towels were folded to surround the roots and eggs. A control treatment consisted of moistened paper towels with eggs and no plants. Completed towels were placed into plastic bags as treatment groups, sealed, and placed on a lab bench under a Gro-Lite® with a 16:8 (L:D) photoperiod at 28 ± 1°C. Larval head capsules (body portions of the larvae had deteriorated in the moist towels) were counted on day 13 and 14 to determine egg hatch.

First Instar Survival

Experiment III (1988). JB eggs and larvae were exposed to the roots of E+ and E- tall fescue seedlings. Eggs were collected for 7 days and placed daily into separate round plastic containers (21 cm diameter x 8 cm height) with 1 liter of oviposition medium (Appendix A). A cardboard divider wrapped in aluminum foil was used to divide the container into sections of equal size, and E+ and E- seed (7 g of each seed type) were sown in separate sides. Eggs collected for a given day were equally divided between the two sides and covered with oviposition medium for a final total of 576 eggs/side. Containers were maintained on a lab bench under a Gro-Lite® with a 16:8 (L:D) photoperiod at 28 ± 1°C. To provide an initial food source for newly hatched JB larvae, white clover (1 g per side) and red clover (0.5 g per side) were sown into each container 13 days after eggs were introduced for the respective pan. Filter paper was placed over the seed and moistened daily until fescue and clover seed germinated. Contents of each container was sifted with a 20-mesh sieve on day 22 from original egg introduction to evaluate larval survival.

Experiment IV (1989). Survival of first instar JB larvae in oviposition medium (Appendix A) exposed to the root zone and leachate from E+ and E- plants was examined. Promix® (0.5 dry liters) was moistened until saturated, molded to 15 x 21 cm x 1.3 cm depth, and placed on top of a 20-mesh nylon insect screen (20 x 26 cm) similar to other reports (42, 43). Six Promix® pads were prepared. Two pads received 1000 broadcast E+ seeds, two received 1000 E- seeds, and two received no seeds (control). Paper towels (two layers) were moistened and placed over the seeds to facilitate germination. Oviposition medium (1.5 dry liters) was mixed and moistened to 25% moisture by weight and placed into plastic pans (15.5 x 21.5 by 11 cm height). Grass pads were then placed over the oviposition medium, with the insect screen separating the Promix® pad from the oviposition medium. Fescue roots growing through the screen penetrated into the oviposition medium. The grass pads were watered as needed when the Promix® began to dry. Grass pads were removed from the pans 34 days later and the oviposition medium formerly beneath the pads sifted through a 12-mesh sieve, air dried for 1 day, and stored at 28 ± 1°C.

E+, E-, and control laboratory-prepared soils were moistened to 20% moisture and JB eggs were introduced. Soils were used at two different storage ages: 8 days or 25

days from original plant removal from the soil. Twenty 1-day-old JB eggs were placed into each treatment soil in 120-ml specimen cups (60 ml of soil medium per cup). Specimen cups were placed on a lab bench in a randomized complete block design under a Gro-Lite® with a 16:8 (L:D) photoperiod at 28 ± 1°C.

Thirteen days after introduction of eggs, larval survival was examined by sifting (10-mesh sieve) the contents of specimen cups containing 8-day-old soil at the time of egg introduction (five replicates), or 25-day-old soil (four replicates). In addition, one group of specimen cups (three replicates) containing eggs in soil stored for 25 days before egg introduction was sifted on day 17 to evaluate the effects of longer development in the cups. Survival and group weight measurements for first instars were examined.

Experiment V (1989). Survival of first instar JB larvae in soil collected from the root zone of E+ or E- tall fescue plants was examined. Plants were transplanted in the field at the UT Plant Science Farm 8 months prior to soil collection. In late July, a 25 cm^2 x 15 cm deep root/soil mass was removed at each E+ and E- transplant location. Soil not exposed to plants was also collected 1.5 m from the

E+ and E- plants in a fallow area. Soils were air dried for 1 day, sifted through a 12-mesh sieve, and stored at 28 ± 1°C.

An equal portion of dry peat (not exposed to plants) was hand mixed into each of the E+, E-, and control field soils, then 120-ml plastic specimen cups were half-filled with one of the soil mixes. Twenty 1-day-old JB eggs were added to each cup. Soil storage age at the time of egg introduction was 8 days. Each treatment was replicated 10 times. Specimen cups were placed on a lab bench in a randomized complete block design under a Gro-Lite® with 16:8 (L:D) photoperiod at 28 ± 1°C. Thirteen days after egg introduction, contents of specimen cups were sifted and larval survival and group weight measurements were examined.

Field Studies

Field Collected Larvae

Experiment VI (1988). In early July, USDA Survey Traps, modified to allow beetle escape, were placed between E+ and E- tall fescue grass plots in field sections A and B (Appendix B). The traps were baited with cotton balls soaked in a food and floral attractant pheromone (3:7

eugenol and phenylethyl proprionate) placed into 16-ml specimen vials under the trap's roof. All baits were placed 0.9 m from the ground on metal rods. Baits were removed from the plots in early September. In mid-September, a 25 cm^2 x 15 cm deep sample, including a plant and surrounding soil, was removed from each corner of the grass plots in section A and examined for JB larvae as well as other soil insects. Plants were returned to their original holes and watered. Insects were collected from section B by the same method.

Field Introductions

Experiment VII (1988). Third-instar JB were field-collected at the UT Plant Science Farm and introduced in late November into eight E+ and eight E- tall fescue plants in section C (Appendix B). Each plant received four larvae placed individually into holes 12.7 cm deep by 3.2 cm diameter made with a core sampler. Holes were made 2.5 cm from the plant, equally spaced around the plant. Soil removed by the core sampler was placed back over the larvae. In mid-May, 1989, each previously infested plant was sampled by removing a 25 cm^2 x 15 cm deep sample, including the

plant and surrounding soil, and examined for JB larvae. Plants were returned to their original holes and watered.

Experiment VIII (1989). JB adults and eggs were introduced into separate E+ and E- plants in section C (Appendix B). In mid-July, 40 mating pairs of adult JB were collected and introduced into field cages (wooden-frame 0.9 x 2.1 m square x 0.9 m tall with aluminum-insect screen). Each cage was placed over one E+ and one E- plant and secured into a trench with soil. Smartweed clippings were placed in 120-ml plastic specimen cups containing water by stretching Parafilm® over the cup top and inserting plant stems through a slit in the Parafilm®. Cups with smartweed were then buried between the E+ and E- plants. Approximately 1 month later, cages were removed.

In late July, 4-5-day-old JB eggs were collected from caged laboratory beetles for placement into section C field plots. Eggs were temporarily placed into 16-ml specimen vials (10 eggs/vial) with oviposition medium (Appendix A). After 1-2 weeks, two sod cores (3.2 cm diameter x 10 cm depth) were removed from E+ or E- plants and one vial of 10 eggs was shaken into each hole (20 egg treatment), or two vials of 10 eggs into each hole (40 egg treatment). The sod core was cut into halves, and the top half was returned to

the hole in the plant. Control plants did not receive any treatment and served as checks for natural JB field populations. In mid-September, a 25 cm^2 x 15 cm deep sample, including a plant and surrounding soil, was removed from each plant location and examined for JB larvae and other soil insects.

Potted Plants Infested with Eggs

Experiment IX (1989). In mid-March, individual E+ and E- tall fescue tillers were transplanted into clay pots (five tillers per pot) with sterile Promix®. Each pot had a 20-mesh aluminum-insect screen positioned in the bottom to prevent subsequent larval escape. Treatments infested with JB eggs were as follows: one E+ tiller surrounded by four E- tillers (80% E-), one E- tiller surrounded by four E+ tillers (80% E+), five E+ tillers (100% E+), or five E- tillers (100% E-). In addition, controls consisting of five E+ tillers (100% E+) or five E- tillers (100% E-), were left uninfested to serve as checks of natural introduction of eggs into the field experiment. Treatments were replicated 16 times. Plants were fertilized at planting, watered twice daily, and trimmed as needed during their growth period in the greenhouse.

In late July, the root zone in each pot was infested with 15, 4-5-day-old JB eggs by placing single eggs into holes 7 mm diameter by 3 cm depth. Holes were covered with oviposition medium after egg infestation, and plants were returned to the clay pots. Pots were placed into a fallow field area in a randomized complete block design. Pots were partially buried so that their rims remained approximately 2.5 cm above the soil surface. Plants were watered once daily during the summer and the area was kept weed-free. In late October, pots were removed from the field, the plants were completely dissected, and larvae recovered were counted and individually weighed.

OTHER SOIL INSECTS

Laboratory Studies

Potted Scarab Larvae

Experiment X (1989). Annual white grubs (AWG) (*Cyclocephala* sp.) from a bluegrass lawn infestation in Knox County, Tennessee, were collected in mid-September and stored for 24 h in moistened peat. Active AWG larvae were then collected from the peat and placed into the root zone

of potted E+ and E- plants, five larvae per pot. Pots were maintained in the greenhouse. Each treatment was replicated four times. Plants were watered twice daily. Forty-one days after infestation, plants were examined for surviving AWG.

Alkaloid Studies and Collembola

Experiment XI (1989). Effects of alkaloids, N-acetyl loline (NAL) and ergotamine tartrate (EG) on Folsomia candida (Willem) (Isotomidae) were examined. A colony of F. candida was established and maintained as described by Snider (83).

Mortality and fecundity of F. candida were determined after feeding on brewer's yeast containing either NAL or EG. Dilutions of 1.0 mM EG, 1.0 mM tartaric acid (TA), and distilled water were made and 1 ml of each added to 0.472 g of brewer's yeast to produce a thick slurry. Yeast/alkaloid mixtures were frozen for 3 days, lyophilized, and frozen until use in experiments. NAL was prepared in dilutions of 2.0, 0.002, and 0.000002 mM NAL, and a distilled water control.

Ten individuals of F. candida of similar age were introduced into plastic rearing chambers (5 cm diameter x 4

cm height) with plaster-charcoal substrate. The EG, TA, and distilled water yeast extracts were placed onto the substrate. EG treatments were replicated twice, and the experiment was terminated after 5 days. Food was not replaced during the 5-day period and was sufficient for the experiment duration. Eggs were counted and removed daily and F. candida mortality was determined.

Ten F. candida of similar age were tested with the various NAL yeast mixtures. Experimental conditions were the same as previously stated for EG treatments, except that food was changed daily by placing ca. 15 mg of yeast on a wax paper disk. NAL treatments were replicated three times, and the experiment was terminated after 11 days. Egg number and F. candida mortality were determined.

Field Studies

Pitfall Trapping

Experiment XII (1988 and 1989). One pitfall trap was placed into each E+ and E- tall fescue grass plot in section A (Appendix B). Traps consisted of two plastic cups (one nested inside the other) placed flush with the soil surface, and a board placed over the cup to exclude rainfall.

Pitfall traps were filled with ethylene glycol. Traps were operated in alternate weeks from mid-July to mid-September in 1988, and mid-April to mid-September in 1989. At collection time, the inner cup was removed and the contents washed into a collection jar with water. The contents of the collection jars were rinsed onto a 140-mesh (0.0041 mm) sieve and back washed into 120-ml plastic specimen cups with 95% ethyl alcohol. Specimens were sorted to family level, and some groups were identified to species (22).

CHAPTER III

RESULTS AND DISCUSSION

GENERAL

Insect Culture

Methods utilized for caging adult beetles, collecting eggs, and storing eggs were sufficient for this research. In order to obtain large numbers of eggs (ca. 1000-2000) every other day, several thousand adult beetles were required.

Plants, Endophyte Testing, and Statistics

All plants and tillers utilized in experiments tested infected or non-infected unless otherwise indicated. Seed lots had endophyte incidence levels of 0% (E-) and 100% (E+) unless otherwise indicated.

JAPANESE BEETLE

Laboratory Studies

Egg Stage

Experiment I (1989). JB egg hatch after exposure to fescue seedlings was not significantly different between E+ and E- treatments, but was significantly different between E+ and control treatments (Table 1). The average period of egg stadia was also significantly longer in control than E+ treatment (Table 1).

If E+ plants are produce toxins that enter the rhizosphere, then eggs are not affected. Eggs were touching fescue roots throughout the experiment and were adhering to the roots through water tension. Seedling age may be the reason eggs were not differentially affected by E+ plants. However, both Latch et al. (56) and Stewart (85) have demonstrated that E+ foliage possesses toxins active against insects at a very early (i.e., before the endophyte has invaded seedling tissues). E+ toxins might enter seedling fescue roots by translocation. Other reasons for unapparent toxicity to JB eggs might include either a lack of toxins in E+ roots or a lack of toxic leachates from the roots, detoxification or binding of toxins possibly by the filter paper, selective exclusion of toxins by the egg membrane, or

Table 1. Number of Popillia japonica eggs which hatched and time required to complete hatch in contact with roots of Acremonium coenophialum-infected (E+) or non-infected (E-) 13-d-old fescue seedlings or control (no plants).

Treatment[a]	$\bar{X}$ ± SEM	
	No. Eggs Hatched	Time Required to Hatch (Days)
E+	14.3 ± 0.2a[b]	15.4 ± 0.3a
E-	13.9 ± 0.4ab	15.8 ± 0.3ab
Control	13.0 ± 0.4b	16.4 ± 0.4b

[a]Replicated 16 times with 15 eggs per treatment.
[b]Means within a column followed by the same letter are not significantly different ($p > 0.05$; Duncan's Multiple Range Test [SAS Institute 1987]).

a lack of susceptibility to E+ toxins by this stage of the insect. Since JB eggs have a water imbibing stage (35), any water soluble substances (e.g., lolines) may be excluded by the egg membrane.

Controls had less total egg hatch and required more time to hatch than E+ treatment. Although overall egg hatch in control and E- treatments were not significantly different, control treatment on a daily basis frequently had significantly lower hatch than E- treatment. Excess moisture might be the cause of differences between the control treatment and E+ and E- treatments, because control containers did not contain growing plants which could remove water from the filter paper.

PAS-ELISA test results indicated that four E+ replicates were not infected. Because of the small tiller size and difficulty in removing plant extract from the seedlings, it is possible that E+ replicates which tested non-infected were actually infected. The seed lot used for this experiment had tested ca. 100% infected when plants were previously tested. In addition, seed was stored under refrigeration to maintain endophyte viability (48), increasing the likelihood that all the replicates in this experiment were infected.

Experiment II (1989). JB egg hatch was not significantly different between E+, E-, or control treatments (Table 2). E+ plants did not reduce the hatchability of JB eggs. Reasons for the lack of affects on eggs may be similar to Experiment I. The paper towel folding process to enclose eggs and roots may have resulted in broken contact between eggs and roots.

First Instar Survival

Experiment III (1988). Larval survival was significantly higher in E- than E+ treatments (Table 3). The presumed E- fescue seed lot, tested after completion of this experiment, actually proved to be 50% infected and probably reduced larval survival in the E- treatment. The E+ fescue seed lot tested 100% infected. Differences in larval survival probably might have been greater in 0%/100% infection tests. E+ seeds and seedlings contain alkaloids such as lolines (49, 50, 94) which may be present in foliage at a very early age (56) and can have adverse effects on foliage-feeding insects (49, 56, 94). Soil nematodes are affected by E+ plants (51, 65, 89, 90). Since lolines occur in fescue roots (personal communication, Daniel A. Potter) they may be the source of resistance against JB larvae.

Table 2. Number of Popillia japonica eggs which hatched in contact with roots of Acremonium coenophialum-infected (E+) or non-infected (E-) 7-8-mo-old tall fescue tillers or control (no tillers).

Treatment[a]	Egg Hatch ($\bar{X}$ ± SEM)
E+	17.3 ± 0.8a[b]
E-	15.1 ± 0.7a
Control	17.0 ± 0.7a

[a]Replicated 7 times with 20 eggs per treatment.
[b]Means within a column followed by the same letter are not significantly different ($p > 0.05$; Duncan's Multiple Range Test [SAS Institute 1987]).

Table 3. Survival of 1st instar Popillia japonica from 1-d-old eggs placed into peat soil with roots of Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue seedlings.

Treatment[a]	$\bar{X}$ ± SEM	
	No. Surviving Larvae	Percent Survival
E+	0.3 ± 0.2a[b]	0.5a
E-	9.0 ± 3.0b	10.9b

[a]Replicated 7 times with egg number variable between replicates. Seedlings established by seed broadcast at time of egg introduction.

[b]Means within a column followed by the same letter are not significantly different ($p > 0.05$; paired T-test [SAS Institute 1987]).

In addition, lolines which are very abundant in foliar regions of the fescue plant (16, personal communication, Daniel A. Potter) may be leaching into the soil from the surface.

Experiment IV (1989). Larval survival was significantly higher in E- and control soils than E+ soils when they had been stored for 8 days after exposure to plants (Table 4); however, average larval weights and number of unhatched eggs were not significantly different (Table 4). Larval survival, average weight, and number of unhatched eggs were not significantly different in soils stored for 25 days after exposure to plants (Table 5 and 6).

Higher survival of first instar larvae in E- and control treatment soils stored for 8 days suggests that E+ plants are producing toxins which have leached into the soil. Since living plants were not present in the soil, toxins may have entered the soil from the roots of plants previously present. JB larvae appear to feed on the peat in the soil (indicated by dark contents of the gut) as reported by Schwartz et al. (78), and toxins from E+ plants may have been present in the peat. Another potential source for toxins in the soil could have been leachate from fescue seed or plant foliage on the soil surface.

Treatment soils stored for longer periods from initial exposure to plants (25 days at 28 ± 1°C) apparently lost

Table 4. Survival and weight of 1st instar Popillia japonica 13 d after introduction of 1-d-old eggs into soil which had been collected from the root-zone of Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue tillers and stored for 8 d after collection at 28 ± 1°C before use, or control soil (not exposed to plants).

Treatments[a]	$\bar{X}$ ± SEM		
	First Instar		No. Unhatched Eggs
	No. Surviving Larvae	Larval Wt (mg)[b]	
E+	10.0 ± 2.3a[c]	3.1 ± 0.1a	0.6 ± 0.4a
E-	18.6 ± 0.4b	3.0 ± 0.0a	0.0 ± 0.0a
Control	16.0 ± 0.7b	3.0 ± 0.1a	0.0 ± 0.0a

[a]Replicated 5 times with 20 eggs per treatment.
[b]Weights calculated from mean group weight of larvae surviving for each replicate.
[c]Means within a column followed by the same letter are not significantly different ($p > 0.05$; Duncan's Multiple Range Test [SAS Institute 1987]).

Table 5. Survival and weight of 1st instar Popillia japonica 13 d after introduction of 1-d-old eggs into soil which had been collected from the root-zone of Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue tillers and stored for 25 d after collection at 28 ± 1°C before use, or control soil (not exposed to plants).

Treatments[a]	$\bar{X}$ ± SEM		
	First Instar		No. Unhatched Eggs
	No. Surviving Larvae	Larval Wt (mg)[b]	
E+	15.3 ± 1.3a[c]	3.4 ± 0.1a	1.5 ± 0.9a
E-	14.0 ± 1.6a	3.5 ± 0.1a	2.8 ± 0.8a
Control	15.8 ± 1.3a	3.4 ± 0.1a	1.3 ± 0.6a

[a]Replicated 4 times with 20 eggs per treatment.
[b]Weights calculated from mean group weight of larvae surviving for each replicate.
[c]Means within a column followed by the same letter are not significantly different ($p > 0.05$; Duncan's Multiple Range Test [SAS Institute 1987]).

Table 6. Survival and weight of 1st instar Popillia japonica 17 d after introduction of 1-d-old eggs into soil which had been collected from the root-zone of Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue tillers and stored for 25 d after collection at 28 ± 1°C before use, or control soil (not exposed to plants).

Treatments[a]	$\bar{X}$ ± SEM		
	First Instar		No. Unhatched Eggs
	No. Surviving Larvae	Larval Wt (mg)[b]	
E+	6.7 ± 0.9a[c]	4.6 ± 0.6a	2.0 ± 1.0a
E-	6.0 ± 0.6a	4.0 ± 0.5a	0.3 ± 0.3a
Control	5.3 ± 1.5a	3.2 ± 0.6a	1.0 ± 0.6a

[a]Replicated 3 times with 20 eggs per treatment.
[b]Weights calculated from mean group weight of larvae surviving for each replicate.
[c]Means within a column followed by the same letter are not significantly different ($p > 0.05$; Duncan's Multiple Range Test [SAS Institute 1987]).

their toxicity (Table 5 and 6). In addition, prolonged larval exposure to the conditions in the specimen cups apparently increased larval mortality in all treatments (Table 6), suggesting the rearing technique is not adequate for prolonged first instar storage.

Loline alkaloids are known to affect insects (49, 81, 94) and do occur in fescue roots (personal communication, Daniel A. Potter). Loline alkaloids are also very water soluble (personal communication, Richard G. Powell), which might facilitate their leaching into the surrounding soil from the fescue plant. The volatile chemistry (73) of loline-type alkaloids might also account for the decrease in larval mortality in soils stored for 25 days before their use in experiments.

Average larval weights were not significantly different between treatments stored for 8 or 25 days, suggesting that toxicological interactions occurring between E+ soils and JB may be moderate. No differences in number of unhatched eggs were observed, indicating that antibiotic effects on the egg stage of JB may be minimal. The lack of effects on eggs is supported by other experiments (Experiments I and II) and thus, mortality probably occurred in the first instar larvae. Other researchers have demonstrated that early instar insects are more sensitive to the effects of endophyte-infected plants (45).

Experiment V (1989). Larval survival was not significantly different between E+, E-, or control soils (Table 7). Average larval weights and number of unhatched eggs were also not significantly different (Table 7).

Field soil utilized in this experiment had no measurable effects on JB. The E+ soil prepared for the experiment had been exposed to an E+ plant for ca. 8 months prior to its collection, providing adequate time for any toxins to accumulate in the soil. The soil was stored for 8 days at 28 ± 1°C prior to use. The storage time was not longer than previously reported for the toxic soil in Experiment IV. Because the soil collected from the field was a clay soil low in organic matter and plant material was removed by sifting, peat not previously exposed to fescue plants was added to the soil for food. If the field soil was toxic to JB larvae when ingested, the addition of "clean" peat would reduce intake of soil and account for the lack of adverse affects on JB larvae.

Table 7. Survival and weight of 1st instar Popillia japonica 13 d after introduction of 1-d-old eggs into soil which had been collected from the root-zone of Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue field plants and stored for 8 d after collection at 28 ± 1°C before use, or control soil (not exposed to plants).

Treatments[a]	$\bar{X}$ ± SEM		
	First Instar		No. Unhatched Eggs
	No. Surviving Larvae	Larval Wt (mg)[b]	
E+	17.4 ± 1.7a[c]	2.9 ± 0.1a	2.1 ± 1.8a
E-	17.8 ± 0.6a	2.8 ± 0.1a	1.1 ± 0.5a
Control	17.2 ± 0.8a	2.8 ± 0.0a	0.5 ± 0.2a

[a]Replicated 10 times with 20 eggs per treatment.
[b]Weights calculated from mean group weight of larvae surviving for each replicate.
[c]Means within a column followed by the same letter are not significantly different ($p > 0.05$; Duncan's Multiple Range Test [SAS Institute 1987]).

Field Studies

Field Collected Larvae

Experiment VI (1988). Third instar JB abundance was not significantly different between E+ or E- field plots in section A (Table 8). No JB larvae were found in section B (Table 8). Conoderus (Elateridae: Coleoptera) larval prevalence was not significantly different between E+ or E- plots in either section A or B (Table 9).

Recovery of JB larvae was low from both section A and B field plots and adverse effects associated with E+ plants were not established. Drought conditions occurring during this year may have contributed to low adult populations (i.e., reduced oviposition and larval recovery). Because JB adults prefer to oviposit in suitable sites near their host plants (35) and a food and floral attractant pheromone was used in this experiment, pheromone baiting of adults probably contributed to the presence of larvae in the plots. JB larval presence as a result of natural adult populations was insignificant in field experiments (VII, VIII, IX) not using pheromone baits, suggesting pheromone attraction may enhance oviposition into nearby plots. Wireworm populations did not indicate a greater suitability between either E+ or E-, indicating E+ may not be detrimental to them.

Table 8. Abundance of 3rd instar Popillia japonica in Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue field plots at the University of Tennessee Plant Science Farm surrounded by adult pheromone baits, summer of 1988[a].

Treatment	Larval No. ($\bar{X}$ ± SEM) Section A[b]	Section B
E+	1.0 ± 0.7a[c]	0.0 ± 0.0a
E-	3.3 ± 1.3a	0.0 ± 0.0a

[a]Pheromone baits were USDA Survey Traps modified to allow beetle escape and baited with 3:7 eugenol + phenylethyl proprionate.
[b]Sections A and B each consisted (4 replicates) of 9 plant plots in a 3 x 3 grid pattern with the 4 corner plants of each plot sampled for larvae.
[c]Means within a column followed by the same letter are not significantly different ($p > 0.05$; paired T-test [SAS Institute 1987]).

Table 9. Abundance of larval Conoderus in Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue field plots at the University of Tennessee Plant Science Farm, summer of 1988.

Treatment	Larval No. ($\bar{X}$ ± SEM) Section A[a]	Section B
E+	0.0 ± 0.0a[b]	0.8 ± 0.5a
E-	2.0 ± 0.9a	0.5 ± 0.5a

[a]Sections A and B each consisted of (4 replicates) of 9 plant plots in a 3 x 3 grid pattern with the 4 corner plants of each plot sampled for larvae.
[b]Means within a column followed by the same letter are not significantly different ($p > 0.05$; paired T-test [SAS Institute 1987]).

Field Introductions

Experiment VII (1988). No JB larvae were found in any of the 16 plants previously infested. Introduction method (placement of larvae in soil beside the plants and not directly in the plant) could have contributed to the complete mortality.

Experiment VIII (1989). A total of three JB larvae were recovered from the entire experiment. The three larvae were recovered in the caged-adult treatment from E- plants. Reasons for low larval recovery from caged-adult or egg-infestation treatments are unknown. Egg hatch tests from the egg stock used in the experiment indicated 96 to 100% viability. It was assumed that egg hatch was similar in the field, and other factors such as infestation technique or field conditions were the cause of mortality. More larvae were expected in the caged-plant treatments than were found. Beetles were active and alive within these cages for at least 2 weeks. The soil around field plants was sufficiently soft (due to previous rainfall) for female oviposition, and the food supply remained succulent in the cage for at least a week. Previous experience with adult oviposition has demonstrated that after a few days in captivity, beetles adapt and begin to oviposit. Reasons for

poor adult oviposition or subsequent low larval survival in caged-plant treatments were undetermined.

Other soil insects found in the field plots included Conoderus and AWG. Mean numbers of these insects were not significantly different between E+ and E- plants (Table 10). Because of low populations of AWG, JB, and Conoderus, we have insufficient data to state antibiotic or deterrent compounds were present.

Potted Plants Infested With Eggs

Experiment IX (1989). Numbers of third instar JB were significantly higher in the 80% E- treatment than in 80% E+ or 100% E+ (Table 11). The 100% E- treatment did not differ significantly from the 80% E- or 80% E+ and 100% E+ treatments. Control treatments were not significantly infested with JB larvae implying that natural adult populations did not affect experimental results. Average larval weight and length were not significantly different among any of the four egg-infestation treatments (Table 11). Egg hatch tests from the egg stock used in the experiment indicated 96% egg viability. Egg hatch in potted plants was probably high.

Survival was higher in both 80% E- and 100% E- than in the E+ treatments. Survival may have been favored by absence of endophyte infection. The 80% E- and 80% E+

Table 10. Abundance of larval Conoderus and Cyclocephala in Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue field plots at the University of Tennessee Plant Science Farm, summer of 1988.

Treatment	Larval No. ($\bar{X}$ ± SEM)	
	Conoderus	Cyclocephala
E+	0.4 ± 0.1a[a]	0.1 ± 0.1a
E-	0.2 ± 0.1a	0.3 ± 0.1a

[a]Means within a column followed by the same letter are not significantly different ($p > 0.05$; paired T-test [SAS Institute 1987]).

Table 11. Survival of 3rd instar Popillia japonica 3 mo after eggs were placed into potted tall fescue plantings with selected levels of Acremonium coenophialum infection.

Treatment[a]	X ± SEM		
	Larval Survival	Larval Growth[b]	
		Wt (mg)	Length (cm)
80% E-	4.7 ± 0.7a[c]	180.5 ± 9.8a	2.0 ± 0.0a
100% E-	3.4 ± 0.7ab	176.3 ± 11.7a	1.9 ± 0.1a
100% E+	2.8 ± 0.7b	178.5 ± 7.5a	1.9 ± 0.0a
80% E+	1.9 ± 0.4b	177.5 ± 16.7a	1.9 ± 0.1a

[a]Replicated 16 times with 15 eggs per treatment.
[b]Larval weight and length calculated from measurements of individual larvae surviving in each treatment.
[c]Means within a column followed by the same letter are not significantly different ($p > 0.05$; Duncan's Multiple Range Test [SAS Institute 1987]).

treatments which were both opposite in tiller transplant arrangements, were also exactly opposite in terms of larval survival. If the level of toxins entering the soil is proportional to the number of infected tillers, then the 80% E- treatment would be expected to have higher survival than the 80% E+ treatment. However, contrary to the results, 100% E- might also be expected to have higher survival than 80% E+ as well. Ahmad et al. (3) found evidence that the number and mass of southern armyworms molting to fourth instar were initially higher on endophyte-infected perennial ryegrass and attributed this advantage to a slight allelochemical stimulation of growth by toxins. Clay and Cheplick (26) also report that low levels of ergot peptide alkaloids increased fall armyworm larval weights and leaf consumption over controls not receiving any alkaloids. In the present experiment, low alkaloid levels in the 80% E- treatment might have stimulated larval survival and growth. Larval weights and lengths were greater for the 80% E- treatment than any other treatment, although differences were not significant. The trend toward higher larval weights and increased body lengths could indicate allelochemical stimulation.

Larval migrational direction might also account for the difference between 80% E- and 80% E+ treatments. JB larvae are capable of considerable movement in sod (46). If toxins (e.g., loline alkaloids) present in fescue roots or leached

into the soil deter larval feeding, then larval migration would tend to be away from areas highest in toxin concentration. Larvae in 80% E- treatments, therefore, would migrate to the outer edges of the potted plants. The outer edges of the potted plants consisted mostly of root material and appeared to dry out faster than the interior regions in the pot which contained mostly Promix®. It is possible that differential moisture, as well as migration directions in the clay pots, contributed to enhanced survival in some pots (80% E-) and decreased survival in others (80% E+).

OTHER SOIL INSECTS

Laboratory Studies

Potted Scarab larvae

Experiment X (1989). Survival of AWG was significantly higher on E- plants than on E+ plants (Table 12), demonstrating that toxins may be present in E+ roots or leached into the soil from E+ plants. However, since larvae were collected from E+ plants 1 month after introduction, adverse effects related to E+ presence do not produce 100% mortality in this species.

Table 12. Survival of field collected Cyclocephala larvae placed into Acremonium coenophialum-infected (E+) or non-infected (E-) potted tall fescue plants.

Treatments[a]	Larval Survival ($\bar{X}$ ± SEM)
E+	2.3 ± 0.3a[b]
E-	3.8 ± 0.3b

[a]Replicated 4 times with 5 larvae per pot.
[b]Means within a column followed by the same letter are not significantly different ($p > 0.05$; paired T-test [SAS Institute 1987]).

Alkaloid Studies and Collembola

Experiment XI (1989). F. candida survival was 100% for all treatments in both EG and NAL experiments and thus not significantly different (Tables 13 and 14). Egg production was also not significantly different for EG, TA, and distilled water treatments (Table 13) or NAL treatments (Table 14).

F. candida is not strongly affected by EG or NAL. Survival was 100% even at high concentrations of 1.0 mM of EG or 2.0 mM of NAL. F. candida antibiosis or feeding deterrence was not distinguished by this study, but some feeding was assumed to have occurred in all treatments based on accumulations of excrement in the containers. F. candida might be detoxifying the alkaloids present in the yeast similar to the way it does some insecticides like DDT (17). Because F. candida closely associates with the euedaphon, it may be equipped with the mechanisms for detoxifying potentially harmful compounds that other arthropods examined in fescue alkaloid studies (26, 49, 81, 94) may not possess. Lack of adverse symptoms produced by the ergot peptide alkaloids has been reported for other arthropods (81, 94) and the lack of symptoms in F. candida by EG may support these studies. Loline alkaloids, however, have been shown to produce adverse effects in insects (49, 81, 94) and may also have effects on F. candida based on the slight

Table 13. Survival and fecundity of Folsomia candida after feeding on brewer's yeast with the ergot peptide, ergotamine.

Treatment[a]	$\bar{X}$ ± SEM	
	Survival	Egg Production[b]
EG	10.0 ± 0.0a[c]	50.8 ± 21.3a
TA	10.0 ± 0.0a	70.2 ± 5.9a
DW	10.0 ± 0.0a	47.2 ± 22.7a

[a]Replicated 2 times with 10 F. candida per treatment. EG = ergotamine tartrate (1.0 mM), TA = tartaric acid (1.0 mM), DW = distilled water.

[b]Eggs removed daily as counted.

[c]Means within a column followed by the same letter are not significantly different ($p > 0.05$; Duncan's Multiple Range Test [SAS Institute 1987]).

Table 14. Survival and fecundity of Folsomia candida after feeding on brewer's yeast with the loline alkaloid, N-acetyl loline.

Treatment[a]	$\bar{X}$ ± SEM	
	Survival	Egg Production[b]
NAL		
2.0	10.0 ± 0.0a[c]	32.6 ± 8.5a
0.002	10.0 ± 0.0a	39.5 ± 3.2a
0.000002	10.0 ± 0.0a	41.1 ± 1.3a
DW	10.0 ± 0.0a	42.6 ± 2.9a

[a]Replicated 3 times with 10 F. candida per treatment. NAL = N-acetyl loline: 2.0 mM, 0.002 mM, and 0.000002 mM, and DW = distilled water.

[b]Eggs removed daily as counted.

[c]Means within a column followed by the same letter are not significantly different ($p > 0.05$; Duncan's Multiple Range Test [SAS Institute 1987]).

reduction in fecundity seen at higher concentrations of the alkaloid. Since not all insects are adversely affected by ergot or loline alkaloids (45, 81), F. candida may be another example of an arthropod not susceptible to the effects of these alkaloids.

Field Studies

Pitfall Trapping

Experiment XII (1988 and 1989). Collembola were the most abundantly represented order of arthropods present in the 1988 and 1989 pitfall samples and were used as indicators of the effects of endophyte-infection on euedaphic arthropods.

Collembola in the family Sminthuridae were consistently more prevalent in E- than E+ plots in both 1988 and 1989 (Fig. 1). Bourletiella (Deuterosminthurus) lippsoni Snider and Sminthurides (Sphaeridia) serratus Folsom and Mills were the two principal species present in 1988 samples. Bourletiella (Bourletiella) hortensis (Fitch) was also found, but it occurred infrequently. B. lippsoni was significantly more prevalent in weekly samples from mid-July to mid-September in 1988 (Table 15). S. serratus was common in late summer in 1988 and was significantly more abundant in E- than E+ plots (Table 15). In 1989, B. lippsoni and S.

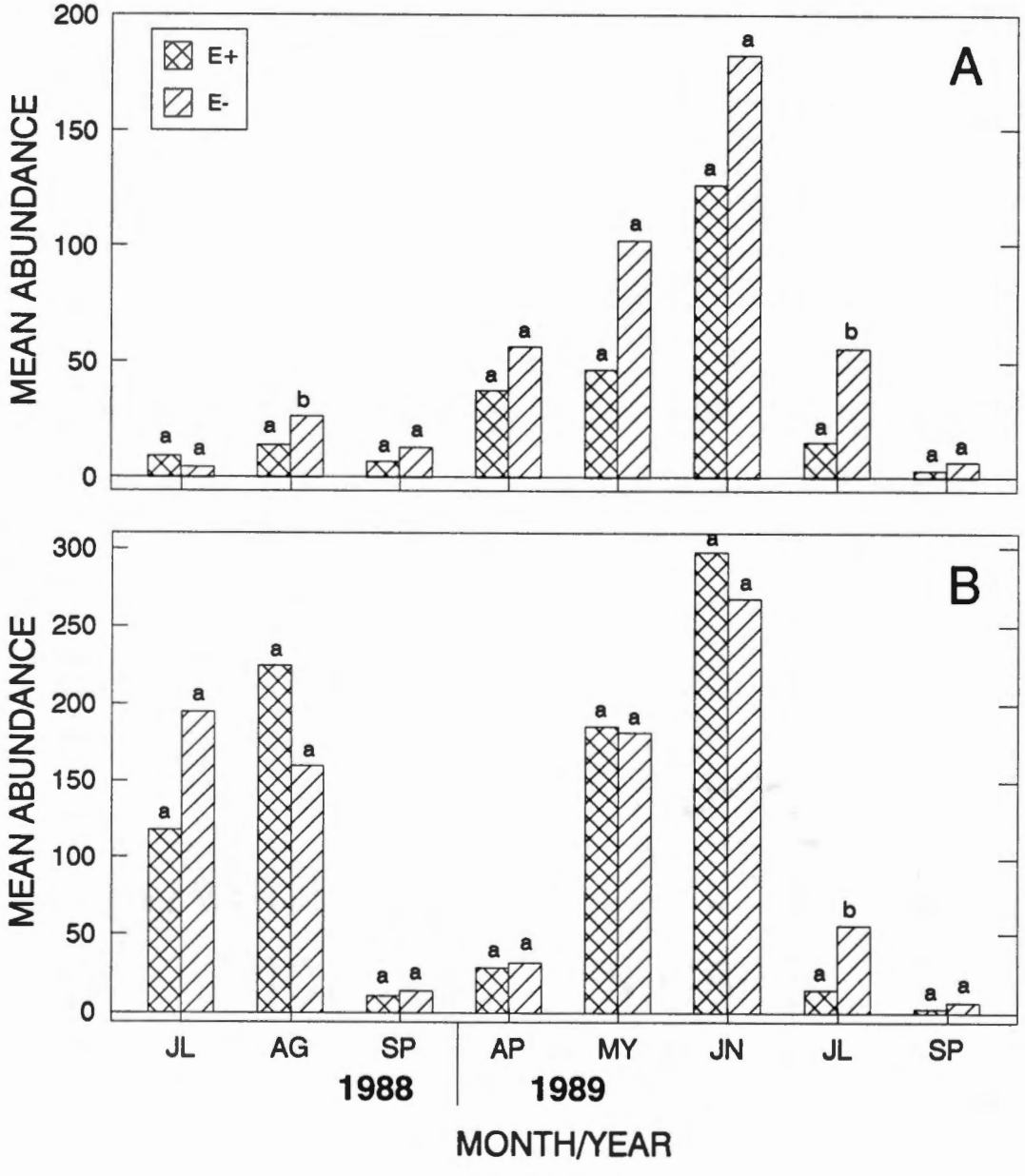

Fig. 1. Abundance of Sminthuridae (A) and Entomobryidae (B) (Collembola) in pitfall traps located in Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue plots at the University of Tennessee Plant Science Farm.

Table 15. Abundance of Bourletiella lippsoni and Sminthurides serratus in pitfall traps located in Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue plots at the University of Tennessee Plant Science Farm, summer 1988.

Treatment	$\bar{X}$ ± SEM	
	B. lippsoni	S. serratus
E+	7.0 ± 4.0a[a]	7.0 ± 0.0a
E-	20.5 ± 3.5b	28.5 ± 3.5b

[a] Means within a column followed by the same letter are not significantly different ($p > 0.05$; paired T-test [SAS Institute 1987]).

serratus were also present from July to September. Although specimens in 1989 collections were not all identified, samples suggest that B. lippsoni and S. serratus were the two major species of sminthurids.

Abundance of springtails in the Entomobryidae was variable and apparently unrelated to endophyte presence in 1988 and 1989 (Fig. 1). Entomobryids were not identified past family level; however, samples indicated that Pseudosinella violenta (Folsom) and Entomobrya sp. were present.

Abundance of springtails in the Isotomidae was apparently unrelated to endophyte presence in 1989 (Fig. 2). Few isotomids were collected in 1988, possibly because sampling period was initiated later in the summer. Isotomids were not identified past family level since populations were similar in size in E+ and E- plots in 1989 and appeared to fluctuate with season and not between E+ and E- treatments.

Sminthurid populations were affected by the presence of E+ fescue. The hemiedaphic life style (i.e., foliage-climbing) of some members in the sminthurid family (34, 53) might indicate a more intimate association with the fescue plant and explain the differences in abundance between E+ and E- plots. Some sminthurids (e.g., B. lippsoni) were collected from fescue foliage and observed to chew on leaves in the laboratory. Sminthurids may have adverse reactions

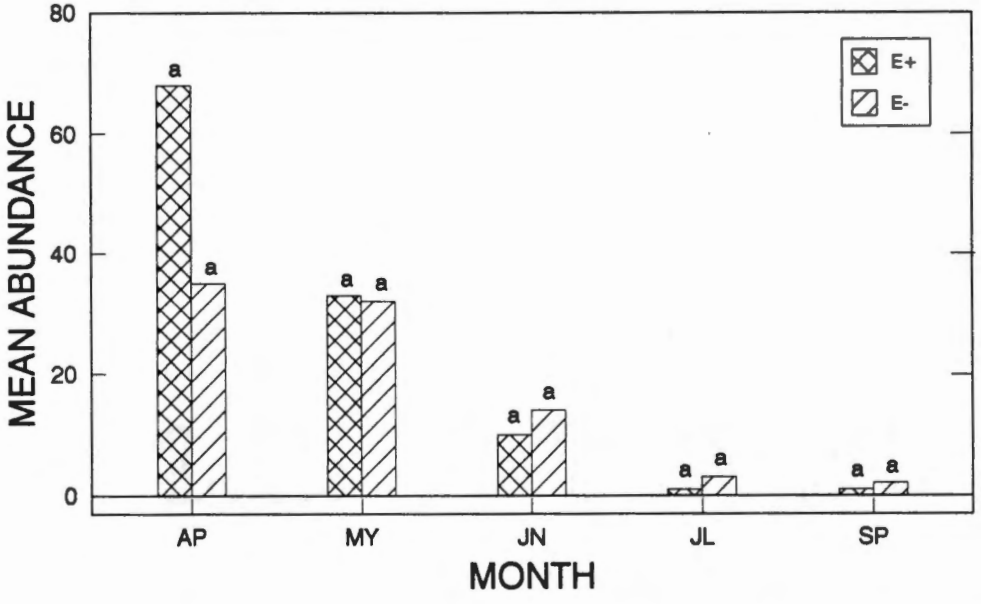

Fig. 2. Abundance of Isotomidae (Collembola) in pitfall traps located in Acremonium coenophialum-infected (E+) or non-infected (E-) tall fescue plots at the University of Tennessee Plant Science Farm, 1988.

to E+ plants in a manner similar to some foliage-feeding insects (28, 37, 45, 49, 52, 56). Most Isotomidae and Entomobryidae are adapted for euedaphic or epiedaphic life-styles (34). The lack of observed effects on isotomid and entomobryid populations in E+ plots might indicate that some soil arthropods are not affected by E+ plants or that these arthropods do not interact with the fescue plants as closely as sminthurids, which are in frequent contact with the foliage. Most Collembola are saprophagous and do not actually feed on living plant tissues (34). Sminthurid species examined in this study might routinely engage in plant feeding like other members of the family (88). Plant feeding might make sminthurids more susceptible to E+ toxins than other springtail families. Volatile compounds in E+ plants, such as the lolines, might also deter sminthurids that come into contact with the plant.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Presence of A. coenophialum presence in tall fescue reduced survival (or prevalence) and possibly deterred larval JB. Effects of E+ fescue on JB were not as severe as reported in some insect studies (20, 28, 49, 94). Factors responsible for reductions in soil nematode prevalence (51, 65, 89, 90) might also affect JB survival. Alkaloids, such as the lolines reported to be present in E+ fescue roots (personal communication, Daniel A. Potter) or ergot peptides, both of which have some known anti-insect activity (26, 45, 49, 81, 94), may be responsible for negative effects on JB in this study.

First instar JB larval populations were most significantly reduced by E+ roots. Effects on early instars may be similar to findings of Hardy et al. (45), in which neonate fall armyworm were much more sensitive to the allelochemical activity of E+ than older fourth instar larvae. Potter (personal communication) also indicated findings of more adverse effects of E+ plants on early instar JB. Experiments with first instar JB indicated tillers at a relatively immature state (ca. 5-30 days old) may still produce antibiotic (or deterrent) effects. The negative effects appear to be enhanced when the fescue plant

is present (Experiment III), but one experiment (Experiment IV) indicated that peat soils were still toxic to JB even after plant removal, suggesting E+ roots may leach or exude toxins. Over time, peat soils exposed to E+ fescue roots apparently lose their toxicity when kept at room temperature (28 ± 1°C) (Experiment IV). Toxic substances with limited activity may be leaching into the rhizosphere. Loline alkaloids are volatile (73) (i.e., limited activity period) and water soluble (personal communication, Richard G. Powell) (i.e., may leach from roots) and could be the source of toxicity. Lolines have been found in E+ fescue roots (variety 'Mustang') (personal communication, Daniel A. Potter). In addition, loline alkaloids which are very abundant in the foliar regions of the fescue plant could be leaching into the soil from the surface. Lolines deter feeding (49) and have antibiotic (81, 94) effects on insects, and may be the cause of toxic or deterrent properties of E+ fescue roots to JB larvae.

Feeding on E+ fescue does not produce 100% mortality in JB larval populations. Some JB larvae survived from egg to third instar apparently unaffected in E+ plants for as long as those on E- plants (Experiment IX), but numbers were reduced on E+ plants. Type of resistance, whether antibiosis or feeding deterrence, was not established. Resistance to JB through feeding deterrence is reasonable, because several insect foliage-feeding studies (11, 45, 49,

56, 69) suggest E+ plants are principally insect resistant due to deterrence and not antibiosis.

E+ fescue may not be entirely detrimental to JB larvae. JB larvae potted in plants (Experiment IX) consisting of 80% E- had higher survival, although not significant, than 100% E- treatments, and significantly higher survival than 80% E+ or 100% E+ plants. The presence of some E+ tillers in the 80% E- treatment could have enhanced larval survival, possibly through directing larval migration into more favorable regions of the plant. Stimulation of larval development by alkaloids is also possible, because both Ahmad et al. (3) and Clay and Cheplick (26) report enhancement in insect condition (i.e., molting time, weight increase) under certain conditions when insects were exposed to E+ plant tissue or low levels of fescue-related alkaloids, compared to insects not exposed to either. Toxins present in fescue at low concentrations (i.e., 80% E-) may provide an allelochemic stress that produces a positive response in JB, such as increased survival, weight gain, etc.

Field research with JB did not indicate dramatic differences in larval presence between E+ and E- plots. Pheromone-attracted adult beetles (Experiment VI) apparently did not oviposit heavily in field plots and subsequent larval recovery was low and not significant between E+ and E- field plots. Egg infestation and third instar transfers

(Experiments VII and VIII) to established field plants were not successful. Transfer of JB eggs into potted plants (Experiment IX) was successful in producing comparable larval populations.

The JB egg stage in these studies was resistant to adverse effects of E+ fescue. Eggs placed against the roots of 12-day-old seedlings or 7-8-month-old tillers did not show differential hatchability between E+ or E- fescue. In addition, studies in which eggs were placed into soil previously exposed to E+ or E- fescue roots, had equivalent numbers of unhatched eggs between E+ and E- treatments even when first instar survival was reduced (Experiment IV). If E+ fescue does produce toxic factors which enter the rhizosphere, then JB eggs are either not susceptible to the effects, are not receiving the toxins due to environmental conditions (e.g., absorption of toxins by the filter paper), or are selectively excluding the compounds at the egg chorion. JB eggs might be selectively excluding toxins since the eggs have a water imbibing stage and some of the known toxins (i.e., lolines) are water soluble.

Other soil insects examined for adverse effects of E+ fescue were a scarab (AWG) and an elaterid (Conoderus) species. AWG was not significantly affected by E+ plants in field plots. However, AWG larvae collected from a bluegrass lawn were significantly reduced when placed into potted E+ plants, indicating some antibiosis or feeding deterrence

does occur in this species. Some AWG, however, survived 37 days on E+ plants without observable adverse effects. Elaterid larvae were not significantly affected by either E+ or E- field plants in 1988 or 1989. Wireworms were more abundant in some E+ grass plots than in E- plots.

Collembola populations sampled by pitfall trapping were abundant in both E+ and E- plots. Populations of entomobryid and isotomid springtails were consistent between E+ and E- plots. Isotomid populations decreased late in the summer in both 1988 and 1989 and might have been regulated by factors other than endophyte presence. Sminthurids were usually significantly more abundant in E- fescue for most weeks sampled. Sminthurids collected in 1988 consisted principally of Bourletiella lippsoni and Sminthurides serratus. The sminthurids appear to interact more intimately with fescue than do entomobryids or isotomids. Sminthuridae were observed chewing on fescue tiller clippings in the laboratory.

Preliminary studies (Experiment XI) involving the feeding of fescue-related alkaloids, NAL and EG, in yeast to F. candida indicated that survival and fecundity were not significantly affected. NAL treatments did slightly reduce oviposition of F. candida at higher concentrations, suggesting alkaloids might be mildly affecting fecundity, but differences were not significant. Fescue alkaloids examined did not cause F. candida mortality.

The results of research presented in this thesis indicate soil insects are affected by presence of A. coenophialum in fescue plants, but not to the degree that some foliage-feeding insects are affected. These effects occur in plant regions previously not known to be toxic to insects. Loline alkaloids may be involved with the resistance to JB and other soil insects either through their production or translocation into the root regions of the fescue plant, or possibly by their leaching from plant foliage or seed at the soil surface. In addition to loline alkaloids, other undiscovered toxins might also be involved.

REFERENCES CITED

1. Ahmad, S., S. Govindarajan, C.R. Funk, and J.M. Johnson-Cicalese. 1985. Fatality of house crickets on perennial ryegrass infected with a fungal endophyte. Entomol. Exp. Appl. 39: 183-190.

2. Ahmad, S., J.M. Johnson-Cicalese, W.K. Dickson, and C.R. Funk. 1986. Endophyte-enhanced resistance in perennial ryegrass to the bluegrass billbug, Sphenophorus parvulus. Entomol. Exp. Appl. 41: 3-10.

3. Ahmad, S., S. Govindarajan, J.M. Johnson-Cicalese, and C.R. Funk. 1987. Association of a fungal endophyte in perennial ryegrass with antibiosis to larvae of the southern armyworm, Spodoptera eridania. Entomol. Exp. Appl. 43: 287-294.

4. Arachevaleta, M., C.W. Bacon, C.S. Hoveland, and D.E. Radcliffe. 1989. Effect of the tall fescue endophyte on plant response to environmental stress. Agron. J. 81: 83-90.

5. Bacon, C.W. 1988. Procedure for isolating the endophyte from tall fescue and screening isolates for ergot alkaloids. Appl. Environ. Microbiol. 54(11): 2615-2618.

6. Bacon, C.W., P.C. Lyons, J.K. Porter, and J.D. Robbins. 1986. Ergot toxicity from endophyte-infected grasses: a review. Agron. J. 78: 106-116.

7. Bacon, C.W., J.K. Porter, and J.D. Robbins. 1975. Toxicity and occurrence of Balansia on grasses from toxic fescue pastures. Appl. Microbiol. 29(4): 553-556.

8. Bacon, C.W., J.K. Porter, and J.D. Robbins. 1981. Ergot alkaloid biosynthesis by isolates of Balansia epichloe and B. henningsiana. Can. J. Bot. 59: 2534-2538.

9. Bacon, C.W., J.K. Porter, J.D. Robbins, and E.S. Luttrell. 1977. Epichloe typhina from toxic tall fescue grasses. Appl. Environ. Microbiol. 34: 576-581.

10. Ball, D.M. 1987. An overview of fescue toxicity research. Agric. Pract. 5: 31-36.

11. Barker, G.M., R.P. Pottinger, and P.J. Addison. 1983. Effect of tall fescue and ryegrass endophytes on Argentine stem weevil. Proc. 36th N. Z. Weed Pest Control Conf. 36: 216-219.

12. Barker, G.M., R.P. Pottinger, P.J. Addison, R.A. Prestidge. 1984. Effect of Lolium endophyte fungus infections on behaviour of adult Argentine stem weevil. N. Z. J. Agric. Res. 27: 271-277.

13. Buckner, R.C. and L.P. Bush (ed.). 1979. Tall fescue. Crop Science Society of America, Inc. Madison, WI. 351 pp.

14. Bush, L.P. and R.C. Buckner. 1973. Tall fescue toxicity, pp. 99-112. In Anti-quality components of forages. Crop Science Society of America, Inc. Madison, WI. 140 pp.

15. Bush, L.P., H.R. Burton, and J.A. Boling. 1976. Activity of tall fescue alkaloids and analysis in in vitro rumen fermentation. J. Agric. Food Chem. 24: 869-872.

16. Bush, L.P., P.L. Cornelius, R.C. Buckner, D.R. Varney, R.A. Chapman, P.B. Burrus, II, C.W. Kennedy, T.A. Jones, and M.J. Saunders. 1982. Association of N-acetyl loline and N-formyl loline with Epichloe typhina in tall fescue. Crop Sci. 22(5): 941-943.

17. Butcher, J.W., E. Kirknel, and M. Zabik. 1969. Conversion of DDT to DDE by Folsomia candida (Willem). Revue d'Ecologie et de Biologie du Sol. 6: 291-298.

18. Carroll, G.C. 1986. The biology of endophytism in plants with particular reference to woody perennials, pp. 205-222. In N.J. Fokkema and J. Van Den Heuvel. Microbiology of the phyllosphere. Cambridge University Press. Cambridge. 392 pp.

19. Carter, C.C., T.N. Hunt, D.L. Kline, T.E. Reagan, and W.P. Barney. 1980. Insect and related pests of field crops some important, common and potential pests in North Carolina. The North Carolina Agriculture Extension Service. N.C. 214 pp.

20. Cheplick, G.P. and K. Clay. 1988. Acquired chemical defenses in grasses: the role of fungal endophytes. Oikos 52: 309-318.

21. Cheplick, G.P., K. Clay, S. Marks. 1989. Interactions between infection by endophytic fungi and nutrient limitation in the grasses Lolium perenne and Festuca arundinacea. New Phytologist 111: 89-97.

22. Christiansen, K. and P. Bellinger. 1981. The collemobola of North America north of the Rio Grande, families Neelidae and Sminthuridae, part 4. Grinnell College, Grinnell. Iowa. 1322 pp.

23. Clay, K. 1986. Grass endophytes, pp. 188-204. In N.J. Fokkema and J. Van Den Heuvel. Microbiology of the phyllosphere. Cambridge University Press. Cambridge. 392 pp.

24. Clay, K. 1987. Effects of fungal endophytes on the seed and seedling biology of Lolium perenne and Festuca arundinacea. Oecologia (Berlin) 73: 358-362.

25. Clay, K. 1989. Clavicipitaceous endophytes of grasses: their potential as biocontrol agents. Mycol. Res. 92 (1): 1-12.

26. Clay, K., and G.P. Cheplick. 1989. Effect of ergot alkaloids from fungal endophyte-infected grasses on fall armyworm (Spodoptera frugiperda). J. Chem. Ecol. 15 (1): 169-182.

27. Clay, K., T.N. Hardy, and A.M. Hammond, Jr. 1985. Fungal endophytes of Cyperus and their effect on an insect herbivore. Amer. J. Bot. 72 (8): 1284-1289.

28. Clay, K., T.N. Hardy, and A.M. Hammond, Jr. 1985. Fungal endophytes of grasses and their effects on an insect herbivore. Oecologia (Berlin) 66: 1-5.

29. Conger, B.V. and J.K. McDaniel. 1983. Use of callus cultures to screen tall fescue seed samples for Acremonium coenophialum. Crop Sci. 23: 172-174.

30. Cowan, J.R. 1956. Tall fescue, pp. 283-320. In Advances in agronomy, Vol. VIII. Academic Press, Inc. Publishers. N.Y. 423 pp.

31. Cunningham, I.J. 1949. A note on the cause of tall fescue lameness in cattle. Austral. Vet. J. 25: 27-28.

32. Davis, C.B., B.J. Camp, J.C. Read. 1983. The vasoactive potential of halostachine, an alkaloid of tall fescue (Festuca arundinaceae, Schreb) in cattle. Vet. Hum. Toxicol. 25: 408-411.

33. Davis, N.D., R.J. Cole, J.W. Dorner, J.D. Weete, P.A. Backman, E.M. Clark, C.C. King, S.P. Schmidt, and U.L. Diener. 1986. Steroid metabolites of Acremonium coenophialum, an endophyte of tall fescue. J. Agric. Food Chem. 34(1): 105-108.

34. Eisenbeis, G. and W. Wichard. 1987. Atlas on the biology of soil arthropods. Springer-Verlag. New York. 437pp.

35. Flemming, W.E. 1972. Biology of the Japanese beetle. USDA Agric. Res. Serv. Tech. Bull. 1449. 129 pp.

36. Fletcher, L.R. and I.C. Harvey. 1981. An association of a Lolium endophyte with ryegrass staggers. N.Z. Vet. J. 29: 185-186.

37. Ford, V.L. and T.L. Kirkpatrick. 1989. Effects of Acremonium coenophialum in tall fescue on host disease and insect resistance and allelopathy to Pinus taeda seedlings, 29-34. In C.P. West (ed.). Proc. Ark. Fescue Toxicosis Conf. Ark. Exp. Stat. Univ. Ark. Dec. 1989 Special Report 140.

38. Funk, C.R., P.M. Halisky, S. Ahmad, and R.H. Hurley. 1985. How endophytes modify turfgrass performance and response to insect pests in turfgrass breeding and evaluation trials. In Proc. 5th Internat. Turf Res. Conf., F. Lemaire (ed.), Avignon, France: 137-145.

39. Funk, C.R., P.M. Halisky, M.C. Johnson, M.R. Siegel, A.V. Stewart, S. Ahmad, R.H. Hurley, and I.C. Harvey. 1983. An endophytic fungus and resistance to sod webworms: association in Lolium perenne L. Bio/Technol. 1: 189-191.

40. Gallagher, R.T., E.P. White, and P.H. Mortimer. 1981. Ryegrass staggers: isolation of potent neurotoxins lolitrem A and lolitrem B from staggers-producing pastures. N.Z. Vet. J. 29(10): 189-190.

41. Garrett, L.W., E.D. Heiman, W.H. Pfander, and L.L. Wilson. 1980. Reproductive problems of pregnant mares grazing fescue pastures. J. Anim. Sci. 51(Suppl. 1): 237.

42. Goonewardene, H.F. 1985. Popillia japonica, pp. 269-278. In P. Singh and R.F. Moore (ed.). Handbook of insect rearing, Vol. 1. Elsevier Science Publishers B.V. N.Y. 488 pp.

43. Goonewardene, H.F. and D.B. Zepp. 1969. Feeding larvae of the Japanese beetle in the laboratory. J. Econ. Entomol. 62(2): 514-515.

44. Harborne, J.B. 1988. Introduction to ecological biochemistry. Academic Press. N.Y. 356pp.

45. Hardy, T.N., K. Clay, and A.M. Hammond, Jr. 1986. Leaf age and related factors affecting endophyte-mediated resistance to fall armyworm (Lepidoptera: Noctuidae) in tall fescue. Environ. Entomol. 15: 1083-1089.

46. Hawley, I.M. 1935. Horizontal movement of larvae of the Japanese beetle in field plots. J. Econ. Entomol. 28: 656.

47. Hinton, D.M. and C.W. Bacon. 1985. The distribution and ultrastructure of the endophyte of toxic tall fescue. Can. J. Bot. 63: 35-42.

48. Hurley, R., and C.R. Funk. 1985. Endophytic fungi and biological control of insects in turfgrasses. Proc. 24th Virginia Turfgrass Conf. and Trade Show.

49. Johnson, M.C., D.L. Dahlman, M.R. Siegel, L.P. Bush, G.C.M. Latch, D.A. Potter, and D.R. Varney. 1985. Insect feeding deterrents in endophyte-infected tall fescue. Appl. Environ. Microbiol. 49 (3): 568-571.

50. Jones, T.A., R.C. Buckner, P.B. Burrus, II, and L.P. Bush. 1983. Accumulation of pyrrolizidine alkaloids in benomyl-treated tall fescue parents and their untreated progenies. Crop Sci. 23(6): 1135-1140.

51. Kimmons, C.A., K.D. Gwinn, and E.C. Bernard. 1989. Nematode reproduction on endophyte-infected and endophyte-free tall fescue. Plant Disease. (In Press).

52. Kirfman, G.W., R.L. Brandenburg, and G.B. Garner. 1986. Relationship between insect abundance and endophyte infestation level in tall fescue in Missouri. J. Kan. Entomol. Soc. 59 (3): 552-554.

53. Klaver, E. 1975. Some aspects of the reproductive biology of Bourletiella (Cassagnaudiella) pruinosa (Tullberg, 1871) (Collembola: Sminthuridae). Bull. Zool. Mus. Univ. Amsterdam. 4: 179-186.

54. Kohlmeyer, J., and E. Kohlmeyer. 1974. Distribution of Epichloe typhina (Ascomycetes) and its parasitic fly. Mycol. 66: 77-86.

55. Ladd, T.L., Jr., and C.R. Buriff. 1979. Japanese beetle: influence of larval feeding on bluegrass yields at two levels of soil moisture. J. Econ. Entomol. 72(3): 311-314.

56. Latch, G.C.M., M.J. Christensen, and D.L. Gaynor. 1985. Aphid detection of endophyte infection in tall fescue. N. Z. J. Agric. Res. 28: 129-132.

57. Latch, G.C.M., M.J. Christensen, and G.J. Samuels. 1984. Five endophytes of Lolium and Festuca in New Zealand. Mycotaxon. 20: 535-550.

58. Long, E.A. and J.W. Hilty. 1985. Occurrence of Acremonium coenophialum in tall fescue in Tennessee. Plant Disease. 69(6): 467-468.

59. Ludwig, D. and W.F. Abercrombie. 1940. The growth of the head capsule of Japanese beetle larva. Am. Entomol. Soc. Ann. 33: 385-390.

60. Lyons, P.C., R.D. Plattner, C.W. Bacon. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. Science. 232: 487-489.

61. Martin, T. and W.C. Edwards. 1986. Protecting grazing livestock from tall fescue toxicity. Vet. Med. 81(12): 1162-1168.

62. McLaren, J.B. 1987. Scientists collaborating to solve fescue fungus problem. Univ. Tenn. Agric. 3: 12-16.

63. Morgan-Jones, G. and W. Gams. 1982. Notes on hyphomycetes. XLI. Mycotaxon. 15: 311-318.

64. Neal, W.D. and S.P. Schmidt. 1985. Effect of feeding Kentucky 31 tall fescue seed infected with Acremonium coenophialum to laboratory rats. J. Anim. Sci. 61(3): 603-611.

65. Pedersen, J.F., R. Rodriguez-Kabana, and R.A. Shelby. 1988. Ryegrass cultivars and endophyte in tall fescue affect nematodes in grass and succeeding soybean. Agron. J. 80(5): 811-814.

66. Peterson, A. 1960. Larvae of insects an introduction to neartic species, Part II. Edwards Brothers, Inc. Columbus, Ohio. 416pp.

67. Porter, J.K., C.W. Bacon, and J.D. Robbins. 1979. Ergosine, ergosinine, and chanoclavine I from Epichloe typhina. J. Agric. Food Chem. 27(3): 595-598.

68. Porter, J.K., C.W. Bacon, R.D. Plattner, and R.F. Arrendale. 1987. Ergot peptide alkaloid spectra of Claviceps-infected tall fescue, wheat, and barley. J. Agric. Food Chem. 35(3): 359-361.

69. Prestidge, R.A., D.R. Lauren, S.G. Van Der Zijpp, and M.E. Di Menna. 1985. Isolation of feeding deterrents to Argentine stem weevil in cultures of endophytes of perennial ryegrass and tall fescue. N. Z. J. Agric. Res. 28: 87-92.

70. Reddick, B.B. and M.H. Collins. 1988. An improved method for detection of Acremonium coenophialum in tall fescue plants. Phytopathology. 78(4): 418-420.

71. Riley, T.J. and A.J. Keaster. A pictorial field key to wireworms attacking corn in the midwest. Photocopy.

72. Ritcher, P.O. 1944. Dynastinae of North America with descriptions of the larvae and keys to genera and species (Coleoptera: Scarabaeidae). Ky. Agr. Exp. Sta. Bull. 476.

73. Robbins, J.D., J.G. Sweeny, S.R. Wilkinson, and D. Burdick. 1972. Volatile alkaloids of Kentucky 31 tall fescue seed (Festuca arundinancea Schreb.). J. Agric. Food Chem. 20(5): 1040-1042.

74. Robinson, T. 1979. The evolutionary ecology of alkaloids, pp. 413-448. In G.A. Rosenthal and D.H. Janzen, ed. Herbivores: their interaction with secondary plant metabolites. Academic Press, Inc. N.Y. 718 pp.

75. Saha, D.C., J.M. Johnson-Cicalese, P.M. Halisky, and M.I. Van Heemstra. 1987. Occurrence and significance of endophytic fungi in the fine fescues. Plant Disease. 71: 1021-1024.

76. Sampson, K. 1933. The systemic infection of grasses by Epichloe typhina (Pers.) Tul. Trans. Br. Mycol. Soc. 18: 30-47.

77. SAS Institute. 1987. SAS user's guide: statistics, version 5 ed. SAS Institute, Cary, N.C.

78. Schwartz, P.H., Jr., J.P. Jurimas, and L.A. Hickey. 1970. Substrata for rearing the Japanese beetle in the laboratory. Am. Entomol. Soc. Ann. 63(4): 1083-1085.

79. Shelby, R.A. and L.W. Dalrymple. 1987. Incidence and distribution of the tall fescue endophyte in the United States. Plant Disease. 71(9): 783-786.

80. Siegel, M.R., G.C.M. Latch, and M.C. Johnson. 1987. Fungal endophytes of grasses. Ann. Rev. Phytopath. 25: 293-315.

81. Siegel, M.R., L.P. Bush, N. Fannin, G.C.M. Latch, D.D. Rowan, and B.A. Tapper. 1989. Distribution and insecticidal activity of alkaloids in fungal endophyte-infected grasses. Phytopathology. 79: 1144.

82. Siegel, M.R., M.C. Johnson, D.R. Varney, W.C. Nesmith, R.C. Buckner, L.P. Bush, P.B. Burrus, II, T.A. Jones, and J.A. Boling. 1984. A fungal endophyte in tall fescue: incidence and dissemination. Phytopathology. 74: 932-937.

83. Snider, R.M. and J.W. Butcher. 1973. The life history of Folsomia candida (Willem) (Collembola: Isotomidae) relative to temperature. Great Lakes Entomol. 6(4): 97-106.

84. Solomons, R.N., J.W. Oliver, R.D. Linnabary. 1989. Reactivity of dorsal pedal vein of cattle to selected alkaloids associated with Acremonium coenophialum-infected fescue grass. Am. J. Vet. Res. 50(2): 235-238.

85. Stewart, A.V. 1985. Perennial ryegrass seedling resistance to Argentine stem weevil. N. Z. J. Agric. Res. 28: 403-407.

86. Stuedemann, J.A. and C.S. Hoveland. 1988. Fescue endophyte: history and impact on animal agriculture. J. Prod. Agric. 1(1): 39-44.

87. Thompson, F.N. and J.K. Porter. 1989. Fescue toxicosis in cattle: could there be a public health problem here?. Symposium Public Health Significance of Natural Toxicants in Animal Feeds, Alexandria, VA. Feb. 6-7, 1989.

88. Wallace, M.M.H. 1974. Present and probable world distribution of Sminthurus viridis and prospects for its biological control. Pedobiologia. 14: 238-243.

89. West, C.P., E. Izekor, D.M. Oosterhuis, and R.T. Robbins. 1987. Endophytic fungus effects on tall fescue tolerance of drought and nematodes. Ark. Farm Res. 36(6): 3.

90. West, C.P., E. Izekor, D.M. Oosterhuis, and R.T. Robbins. 1988. The effect of Acremonium coenophialum on the growth and nematode infestation of tall fescue. Plant and Soil. 112: 3-6.

91. White, J.F., Jr. 1987. Widespread distribution of endophytes in the poaceae. Plant Disease. 71(4): 340-342.

92. Yates, S.G. 1962. Toxicity of tall fescue forage: a review. Econ. Bot. 16(4): 295-303.

93. Yates, S.G. and R.G. Powell. 1988. Analysis of ergopeptine alkaloids in endophyte-infected tall fescue. J. Agric. Food Chem. 36(2): 337-340.

94. Yates, S.G., J.C. Fenster, and R.J. Bartelt. 1989. Assay of tall fescue seed extracts, fractions, and alkaloids using the large milkweed bug. J. Agric. Food Chem. 37: 354-357.

APPENDICES

APPENDIX A

Oviposition, Egg Storage, and Rearing Medium

1988. Soil medium consisted of peat (P) (Fafard® Canadian Sphagnum Peat Moss), soil (SL) (Sequatchy loam), and sand (S) (2:2:1 - P:SL:S). The mixture was prepared by sifting the soils through a 20-mesh sieve, steam sterilizing the soil for 1 hour, oven drying to remove moisture, and storing dry. Oviposition medium used for placement in oviposition cages or egg storage was rehydrated to 15% moisture (15 ml water/100 g medium) with steam sterilized tap water at time of use.

1989. Preparation of soil medium was similar to that in 1988, except peat and soil were sifted through a 12-mesh sieve, the ratio of P:SL:S was 4:1:1, and the mixture was rehydrated to 20% moisture with non-sterile tap water.

APPENDIX B

Plot Treatments (1988)

During May, a field area at the University of Tennessee Plant Science Farm (UTPSF) (section A) was tilled and fumigated with methyl bromide. E+ and E- tall fescue plants (variety 'Kentucky 31' grown from seed for 8-12 months and sampled at least three times with PAS-ELISA for endophyte presence prior to use) were transplanted into the fumigated area. Plants were spaced uniformly in alternating E+ and E- plots that were 0.9 m^2, each containing nine plants in a 3 x 3 grid pattern. Plots were replicated four times.

In July, grass plants (section B) were transplanted into a fallow field area at the UTPSF with the same planting arrangement and plant history as section A. Section B had not been fumigated with methyl bromide.

In October, grass plants (section C) consisting of E+ and E- tall fescue (same plant history as section A) were transplanted into a fallow field area at the UTPSF. There were 28 plots each consisting of one E+ and E- plant. Spacing between plants was 0.9 m. Section C had not been fumigated with methyl bromide.

VITA

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