To the Graduate Council:

I am submitting herewith a thesis written by Kelly Silas Parman entitled "Evaluation of *Lygus lineolaris* (Hemiptera: Miridae) as a Vector of Bacterial Soft Rot in Broccoli and Arthropods Associated with Broccoli in Tennessee." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Gary L. Lentz, Major Professor

We have read this thesis
and recommend its acceptance:

Ernest C Bernard

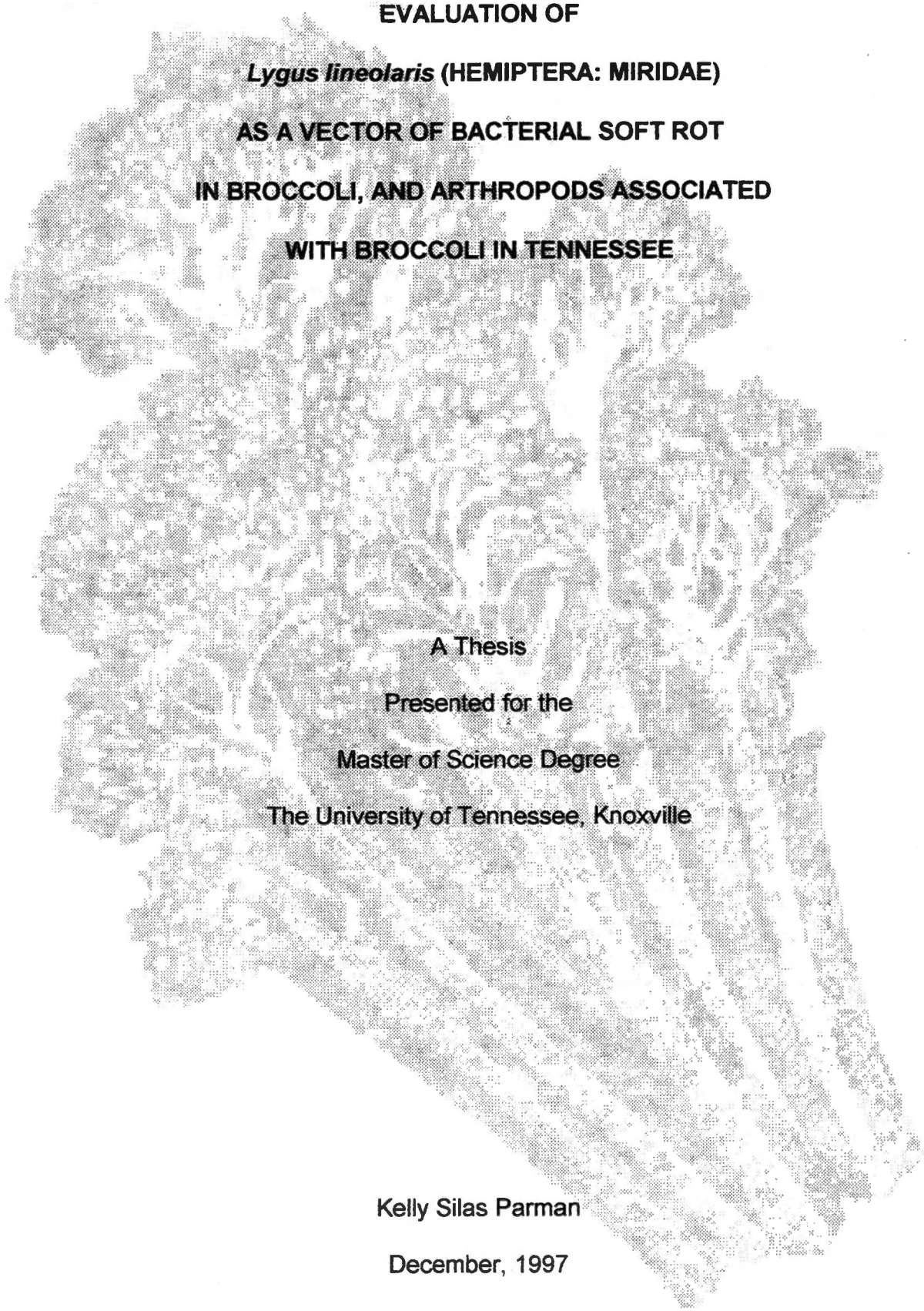
Craig H. Canaday

Charles A. Mullins

Accepted for the Council:

Associate Vice Chancellor
and Dean of the Graduate School

EVALUATION OF *Lygus lineolaris* (HEMIPTERA: MIRIDAE) AS A VECTOR OF BACTERIAL SOFT ROT IN BROCCOLI, AND ARTHROPODS ASSOCIATED WITH BROCCOLI IN TENNESSEE



A Thesis

Presented for the

Master of Science Degree

The University of Tennessee, Knoxville

Kelly Silas Parman

December, 1997

DEDICATION

This thesis is dedicated

to my husband

Bradley J. Parman

He pulled me out of the stars,

and put my feet on the ground.

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ABSTRACT

The production of broccoli in the United States increased 22% from 1987 to 1992. However, broccoli production in Tennessee decreased. The number of farms producing broccoli decreased 30%, and harvested acreage decreased 89%. Part of this decline is due to bacterial soft rot, a common and potentially threatening disease in broccoli production. This disease is caused by soil-borne bacteria, primarily *Pseudomonas* spp. and secondarily *Erwinia carotovora,* after prolonged periods of rain and high humidity.

Brassica spp. are among the wide range of host plants of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). Host plants include alfalfa, corn, cotton, legumes, mustard, strawberry, and several weed species. This research explored the relationship of the tarnished plant bug and bacterial soft rot in broccoli, and evaluated the insect fauna in broccoli fields at the West Tennessee Experiment Station (WTES), Jackson, TN, and the Plateau Experiment Station (PES), Crossville, TN. Broccoli was evaluated in late spring (WTES), summer, and early fall (PES).

Two plantings of broccoli, differing by tillage and irrigation practices, were grown in each season. One crop, the soft rot nursery (SRN), was conventionally tilled and watered with overhead irrigation to induce bacterial soft rot. In the test field, broccoli seedlings were planted into untilled soil and watered with drip irrigation. Two soft rot-susceptible cultivars, Green Comet and Premium Crop,

were used for treatments, and plants of a resistant cultivar, Arcadia, served as border rows. A planting of mustard served as a tarnished plant bug reservoir.

Test treatments consisted of tarnished plant bugs placed on diseased heads in the SRN, confined to the broccoli head with organza cages, and allowed to feed for two days, then removed and placed on healthy broccoli heads in the test plot. Two control treatments were included. One control consisted of insects placed on disease-free heads in the test plot (insect control treatments), and the other consisted of disease-free heads caged without insects, also in the test plot (plant control treatments). Five and ten days after caging in the test field, disease severity was rated on a scale of 0 to 5, where 0=no symptoms, 1=1%, 2=10%, 3=30%, 4=60%, and 5=100% of head affected with necrotic or water-soaked appearing areas.

In spring, soft rot was observed in 65% of the test treatment broccoli heads. Both control groups were heavily diseased (81% for insect control treatments, 77% for plant control treatments). In the summer crop, 24% of test treatments, 35% of insect controls, and 33% of the plant control treatments developed soft rot symptoms. In the fall planting, 40% of test treatment broccoli heads, 50% of the insect controls, and 41% of the plant controls displayed disease symptoms. Disease incidence did not differ among treatments and controls in each season; thus, the presence of the tarnished plant bug did not increase the incidence of bacterial soft rot in broccoli.

Faunal surveys were performed in spring and early fall months, with a total of insect families collected. Insect numbers in the spring increased as sampling

dates progressed, but declined in the late summer months. Pest insects collected included members of Chrysomelidae (leaf beetles), Miridae (tarnished plant bug), Pentatomidae (harlequin and stink bugs), Pieridae (imported cabbage worm) and Plutellidae (diamondback moth). Beneficial and predatory families included Anthocoridae (minute pirate bugs), Lygaeidae (big-eyed bugs), and Pteromalidae (pteromalid wasps). Differences were found in the number of families and of insects in each field. More insect families occurred in spring and late summer in test plots than in the SRN. Among locations, the spring season had a higher number of families than the late summer season. Further investigations of leaf damaging insects that may vector pathogens or increase disease incidence in broccoli is warranted.

TABLE OF CONTENTS

CHAPTER **PAGE**

CHAPTER PAGE

LIST OF FIGURES

FIGURE **PAGE**

LIST OF TABLES

TABLE **PAGE**

CHAPTER 1

REVIEW OF THE LITERATURE

Broccoli, *Brassica oleracea italica* (Brassicaceae), is a popular vegetable grown in America for over 200 years. Its relatives include cabbage, cauliflower, and Brussels sprouts. Two types of American-grown broccoli are Italian, or sprouting broccoli, and heading or cauliflower-like broccoli. Broccoli was first commercially produced in New York in the 1920s. In 1923, the D'Arrigo Brothers Company made plantings of the Italian type in California. By 1925, the commercial broccoli market was well established (Mann 1996).

Broccoli is best grown in cool climates and seasons. Transplants can be produced in 5-7 weeks, and market-ready heads are produced 7-9 weeks after transplanting. Three to four tons of broccoli can be harvested from one acre (Mann 1992). Most commercial broccoli production is in California, where over 90% of the nation's broccoli is grown. Smaller-scale broccoli production is found in Arizona, Texas, Florida, the Carolinas, Washington, Wisconsin, Colorado, Oregon, and Maine (Mann 1992).

The production of broccoli in the United States increased 22% from 1987 to 1992. However, broccoli production in Tennessee, decreased. The number of farms producing broccoli decreased 30%, and harvested acreage decreased 89%

(United States Department of Commerce 1992). This decline is primarily due to production-related expenses and diseases.

Expenses in broccoli production include cool storage and transportation, wax-coated boxes for packaging, and labor. Although broccoli can be direct-seeded, most plants are greenhouse-seeded and seedlings are transplanted. Head maturation is frequently unevenly distributed; thus, several cuttings must be performed. In the field, several applications of fertilizers, insecticides, and disease-controlling measures may be necessary. Nitrogen fertilization is required, and additions of boron, molybdenum, and calcium often are necessary. Broccoli heads must be placed in a cold environment immediately after cutting and kept cool throughout storage and shipping

Pathogens of broccoli include fungi, viruses, nematodes, and bacteria. Fungal diseases include rotting of roots, leaves, and stems, often caused by *Rhizoctonia solani.* Other plant-pathogenic fungi of broccoli include *Cercospora brassicicola*, *Alternaria* spp., and *Fusarium* spp. Virus diseases include mosaic and yellows. Cyst lesion and root-knot nematodes may also damage broccoli plants. Bacterial diseases are common and include soft rot or head rot, caused by *Pseudomonas* spp. and *Erwinia carotovora* ssp. *carotovora,* leaf spot caused by *Pseudomonas syringae* pv. *maculicola*, black rot caused by *Xanthomonas campestris* pv. *campestris*, and Xanthomonas leaf spot caused by *X. campestris* pv. *armoraciae* (Williams 1994).

Bacterial soft rot is exacerbated by prolonged periods of wet conditions (Canaday 1991), and develops more rapidly when temperatures are warm (21 to 29C) (Coffey et al 1988). Soft rot-causing organisms of broccoli are found naturally as saprophytes in the soil. Bacteria are carried to the phyllosphere in splashed water droplets caused by rain or overhead sprinkler irrigation. Symptoms begin as small, water-soaked lesions, which develop into dark, necrotic areas. Crop losses due to bacterial soft rot may range from 30% to complete crop destruction (Hildebrand 1989). Increased disease incidence and severity with excessive nitrogen application in a susceptible cultivar (Premium Crop) has been reported (Canaday 1992). A resistant cultivar (Shogun), was not affected by nitrogen applications (Canaday 1992). Several bacterial soft rot-resistant cultivars including Arcadia, Shogun, and Green Defender are available to producers.

Pseudomonas spp. are responsible for most cases of soft rot. These bacteria degrade cell wall components and invade plant tissue. Fluorescent pseudomonads including *P. marginalis, P. fluorescens,* and *P. viridiflava* commonly are cultured from soft rot-affected broccoli. *Erwinia carotovora* ssp. *carotovora* and *E. carotovora* ssp. *atroseptica* also have been isolated from diseased tissues and may invade broccoli tissue after wounds are created (Hildebrand 1989).

Pseudomonas spp. capable of decaying unwounded broccoli tissues must possess surfactant activity and thus be able to alter the waxy surface of florets

(Hildebrand 1989). The waxy membrane suffers ionic leakage, altering permeability and allowing pathogen invasion. *P. fluorescens* biovar II, *P. fluorescens* biovar IV, and *P. marginalis* pv. *marginalis* have been identified as surfactant-positive organisms (Hildebrand 1989).

Pectolytic activity is a second capability of soft-rotting bacteria. Upon inoculation, organisms capable of degrading cell wall pectin induce necrotic areas beyond the inoculum site (Liao et al. 1993). Bacterial pathogens of broccoli producing pectinases include *Erwinia carotovora* ssp. *carotovora*, *E. carotovora* ssp. *atroseptica*, *P. marginalis* pv. *marginalis*, and *P. fluorescens* biovar II (Hildebrand 1989). Organisms that produce one of the two virulence components require other bacteria for disease formation. For example, *Pseudomonas viridiflava* does not have the ability to produce the surfactant required for infection of unwounded tissue, but does have pectolytic enzymes needed for disease spread beyond the inoculation point. When *P. viridiflava* is combined with *P. fluorescens* biovar V, a surfactant-producing organism lacking pectolytic activity, disease occurs (Hildebrand 1989).

Pseudomonas spp. implicated in bacterial soft rot produce a fluorescent yellow-green pigment upon exposure to ultraviolet light (254 nm) when cultured on King's B medium. The fluorescent pigment is produced by siderophores released by the bacterial cell in response to a low-iron environment. Siderophores are iron-chelating molecules that absorb and transfer iron from

outside of the bacterial cell membrane to the inside of the cell. This capability of soft-rotting *Pseudomonas* spp. is a survival and virulence mechanism.

Wounds from wind damage, adverse weather conditions, cuttings, and insect feeding also are entry portals for bacteria. Insects that feed on broccoli create wounds and injury through direct chewing of the plant parts or piercing of the plant surface. Lepidopteran larvae and flea beetles primarily feed by chewing on leaves. Aphids, thrips, and plant bugs pierce the plant surface and feed on plant juices. The tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois), is a piercing-sucking hemipteran. The insect pierces the broccoli floret and injects polygalacturonase, a digestive enzyme (Leigh 1976). The digested plant juices are then aspirated through the insect's proboscis. The piercing-sucking feeding of tissues and liquids by the TPB creates internal and external wounds, and causes enough damage to make this insect a pest in many crops (Tingey and Pillemer 1977).

The tarnished plant bug is widely distributed throughout the United States, and has more than 300 host plant species, including alfalfa, broccoli, corn, cotton, mustard, soybean, strawberries, and many weeds (Young 1986). Of the 385 host plants listed by Young (1986), approximately one-third were agriculturally important plants. In Arkansas, Louisiana, and Mississippi, the preferred field crop of TPB is cotton, *Gossypium hirsutum* (Snodgrass et al 1984). TPB feeding often causes unappealing fruit shapes. Terms of malformation such as "cat facing" in apples and peaches (Strong 1970), "buttoning" in strawberries (Day 1991), and

"stingers" in sugarbeets (Landis 1997) describe plant parts injured by TPB feeding. The tarnished plant bug preferentially feeds on youngest, most tender areas of the plant, including developing reproductive organs, leaf primordial and apical meristematic tissues (Strong 1970). The insect also seeks tender tissues for oviposition (Ridgway and Gyrisco 1960).

The tarnished plant bug overwinters as an adult in weed and crop debris. Females oviposit in mid to late spring into tender plant tissues. Under usual conditions, first-generation eggs hatch within 3 weeks after oviposition. Nymphs develop into adults through 5 instars over a period of 3 weeks, for a total first generation time of 6 weeks. In the summer generation, postembryonic development occurs in approximately 4 weeks. A third generation may develop in early fall (Ridgway and Gyrisco 1960). In the southern U.S., TPB may be active throughout the year (Snodgrass et al 1984).

Insects are important vectors of disease-causing pathogens. Plant-pathogenic bacteria transmitted by insects include *Erwinia tracheiphila*, a wilt-causing bacterium vectored by chrysomelid beetles to cucurbits, and *E. stewarti*, which causes Stewart's wilt of corn and is vectored by corn flea beetles. Cicadellidae and Cercopidae (Homoptera) may vector *Xylella fastdiosa*, a phloem-infecting bacteria. Several other pathogens including viruses, fungi, spiroplasmas, mycoplasma-like organisms, and phytoplasmas have been associated with insect vectors. The tarnished plant bug, however, have not been implicated.

Surveys of beneficial and pest insects in crops provide information useful for management. Sampling practices may include sweep-net sampling, vacuum aspiration, and drop-cloth methods (Southwood 1978). Sweep net sampling is an inexpensive and accurate method of sampling, provided that proper timing and adequate samplings are performed. Sampling with vacuum aspiration removes arthropods from the plant and traps them in a fabric bag. This method of sampling can be used for plants with dense canopies, or in crops in which the sweep method could damage plants. Schotzko and O'Keefe (1986) compared efficiencies of the sweep net and vacuum aspiration methods, for collecting *Lygus hesperus* in soybeans. Sweep-net sampling provided reliable estimates of *L. hesperus* adults, but was not as effective for collecting nymphs. Vacuum aspiration sampling gave better estimates of both adult and nymph numbers. Schotzko and O'Keefe (1986) suggested that vacuum aspiration may have sucked insects from outside of the sampling area, and recommended that sampling be performed on spaced plants, free of weeds, and for a minimal amount of time. Drop-cloth sampling, another relative sampling method, typically is used in row crops. It consists of shaking plants over a cloth laid between the rows and collecting insects deposited on it.

The objectives of this research were to: (1) determine if *Lygus lineolaris* is a vector of the organisms involved in bacterial soft rot, or increases the incidence of bacterial soft rot in broccoli fields of Tennessee, and (2) perform faunal surveys of broccoli fields in two locations and seasons of Tennessee.

CHAPTER II

EVALUATION OF THE TARNISHED PLANT BUG, *Lygus lineolaris* (PALISOT DE BEAUVOIS), AS A VECTOR OF BACTERIAL SOFT ROT IN BROCCOLI

i. MATERIALS AND METHODS

a. Broccoli Plant Production and Field Plot Design

Three crops of broccoli were grown in 1996 to determine if the tarnished plant bug (TPB) was a vector of bacterial soft rot. One was grown at the West Tennessee Experiment Station (WTES), Jackson, TN, and two at the Plateau Experiment Station (PES), Crossville, TN. Each crop contained two fields of broccoli. In one field, broccoli cultivars were planted in plowed soil and watered frequently via overhead irrigation until bacterial soft rot was observed. This plot was the soft rot nursery (SRN). In the other field, broccoli seedlings were transplanted into non-tilled soil (WTES) or straw-covered soil (PES) and watered with drip or trickle irrigation. This was the test plot (TEST).

Each crop consisted of three cultivars of broccoli: Premium Crop and Green Comet served as vector testing cultivars, and Arcadia, a cultivar highly resistant to bacterial soft rot, was used for border rows. All plants were seeded in 128-cell Todd planting trays (Speedling, Sun City, FL) in a greenhouse at WTES,

watered daily, and fertilized as needed. When they reached transplant size, they were moved to fields at WTES and PES. Premium Crop and Green Comet were planted in rows of random order. Arcadia plants were planted in two or three rows before the first row and after the last row of tested plants.

Broccoli seedlings were transplanted to appropriate fields on March 26 (WTES), May 10, and June 17 (PES). Rows were 0.9 m apart with plants spaced 30 cm. Plants were side-dressed with ammonium nitrate as needed. Upon formation of button-sized florets in the SRN, frequent overhead irrigation (5 times daily) was performed. Irrigation continued for 10 days after disease formed to ensure adequate disease development for test treatments.

A planting of mustard at WTES approximately 500 meters from the broccoli crops served as a TPB reservoir. Insects were collected with a sweep net and placed in chambers constructed of PVC piping and mesh screens. Insect-filled chambers were placed in a cooler for transportation to PES.

b. Test and Control Treatments

Insect vector test treatments consisted of five TPB placed on a diseased head of broccoli in the SRN. Insects were contained on the broccoli head with an organza sleeve cage and were allowed to feed on the diseased head for 2 days. The insects were then removed and placed on a healthy head of broccoli in the non-tilled, drip-irrigated TEST plot. In addition to the test treatments, 2 control

treatments (insect and plant) were performed. Insect controls were TPB placed caged on healthy broccoli heads in the TEST plot. Plant controls were healthy broccoli heads caged without insects, also in the TEST plot. Test and control treatments were performed in three environments, May 22-June 6 (WTES), July 3-9, and August 20-22, 1996 (PES). In May (WTES), 281 treatments were performed. At PES, 78 treatments were performed in July, and 103 treatments in August.

All treatments were rated twice, once after 5 days and again after 10 days of initial caging, for disease symptoms. Disease ratings followed the scheme by Canaday (1991), where ratings of 0-5 described disease severity (Figure 1). Cages were fastened to wires strung above the plants to prevent them from resting on the broccoli heads, and were watered through the fabric as needed to keep the heads moist and provide conditions conducive to disease formation.

Rainfall and temperatures were recorded at all sites. Weather data were compared to disease incidence and severity at each location to determine if environmental conditions were conducive to disease formation. Expected temperature and rainfall ranges were obtained from National Oceanic and Atmospheric Administration (NOAA) (Climatological Data Annual Summary Tennessee 1996).

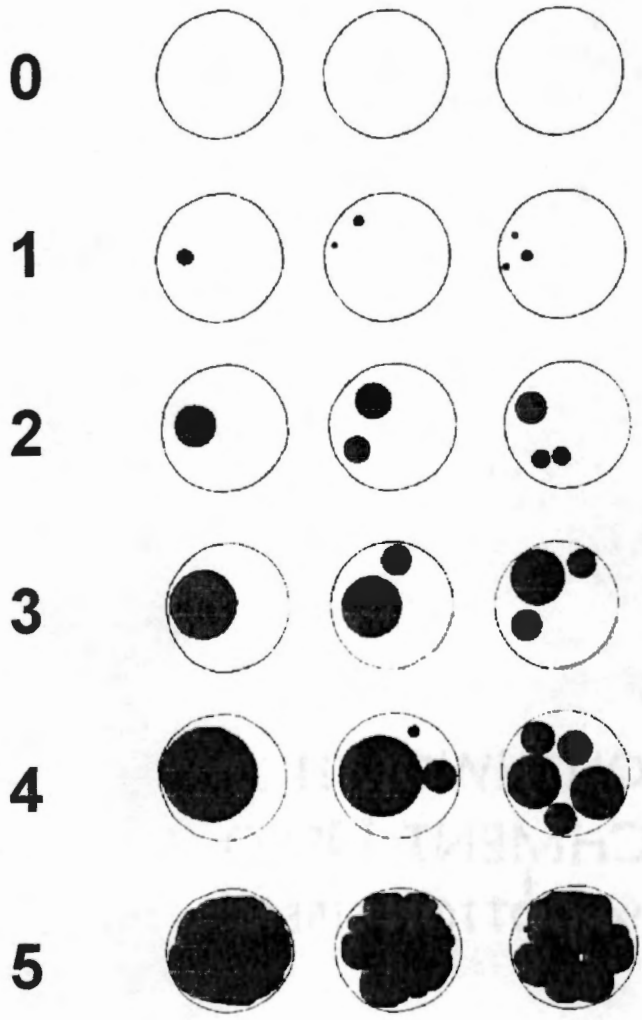


Figure 1. Illustration of scale used to estimate disease severity. Darkened areas represent diseased tissues. The scales ranges from 0 to 5, where 0= no diseased tissues, 1= 1%, 2= 10%, 3= 30%, 4= 60%, and 5= 100% diseased tissues (Canaday 1991).

c. Bacterial Isolation

Diseased broccoli heads were selected randomly for bacterial isolation in both plots for each planting date. Ten plants from each plot were rinsed with tap water and a small section (5 x 5 mm) of diseased tissue was removed from the head with alcohol-sterilized scissors. A bacterial cell suspension was created by agitating each tissue section in 20 ml of sterile water. A sample of the suspension was streaked on King's B medium (Pseudomonas Agar F, Difco). Colonies were evaluated for fluorescence in ultraviolet light and smears were Gram stained.

d. Statistical Analysis

Bacterial soft rot in any degree creates an unmarketable broccoli head. Thus, percentages of plants in test and control treatments displaying symptoms after 10 days in TEST plots were calculated. The number of plants with disease symptoms (disease incidence), rather than the rating of disease (disease severity), was used for the calculations.

The variable soft rot rating was statistically analyzed with the General Linear Model (GLM) procedure ($P = 0.05$) of SAS (SAS Institute, Cary, NC). Since test and control treatments were unequally sampled, type 3 sums of squares were used to determine significant differences in soft rot rating means

among treatments, cultivars, and locations. Means of were separated with the Student-Newman-Keul (SNK) test (P = 0.05).

ii. RESULTS

a. Incidence and Severity of Bacterial Soft Rot

Disease incidence in May was 65.0% in the test treatments, 81.3% in the insect control treatments, and 76.7% in the plant control treatments. In July, disease incidence in test treatments was 24.3%, and 35.0% and 33.3% in insect and plant control treatments, respectively. The percentages of diseased plants in test, insect control, and plant control treatments in August were 40.4, 50.0, and 40.7%, respectively (Fig. 2A).

Bacterial soft rot ratings at day 10 means of treatments at each location were statistically different, but test and control treatment means within each crop were not different. Environmental effects on disease severity were determined by comparing soft rot rating means of all treatments. Means for treatments rated in May were different from July and August means, but July and August means did not statistically differ (Fig. 2B).

Disease severity increased as time progressed. In May, the soft rot rating mean for test, insect control, and plant control treatments increased significantly after 10 days (Fig. 3A). The same pattern occurred in July and August. Test,

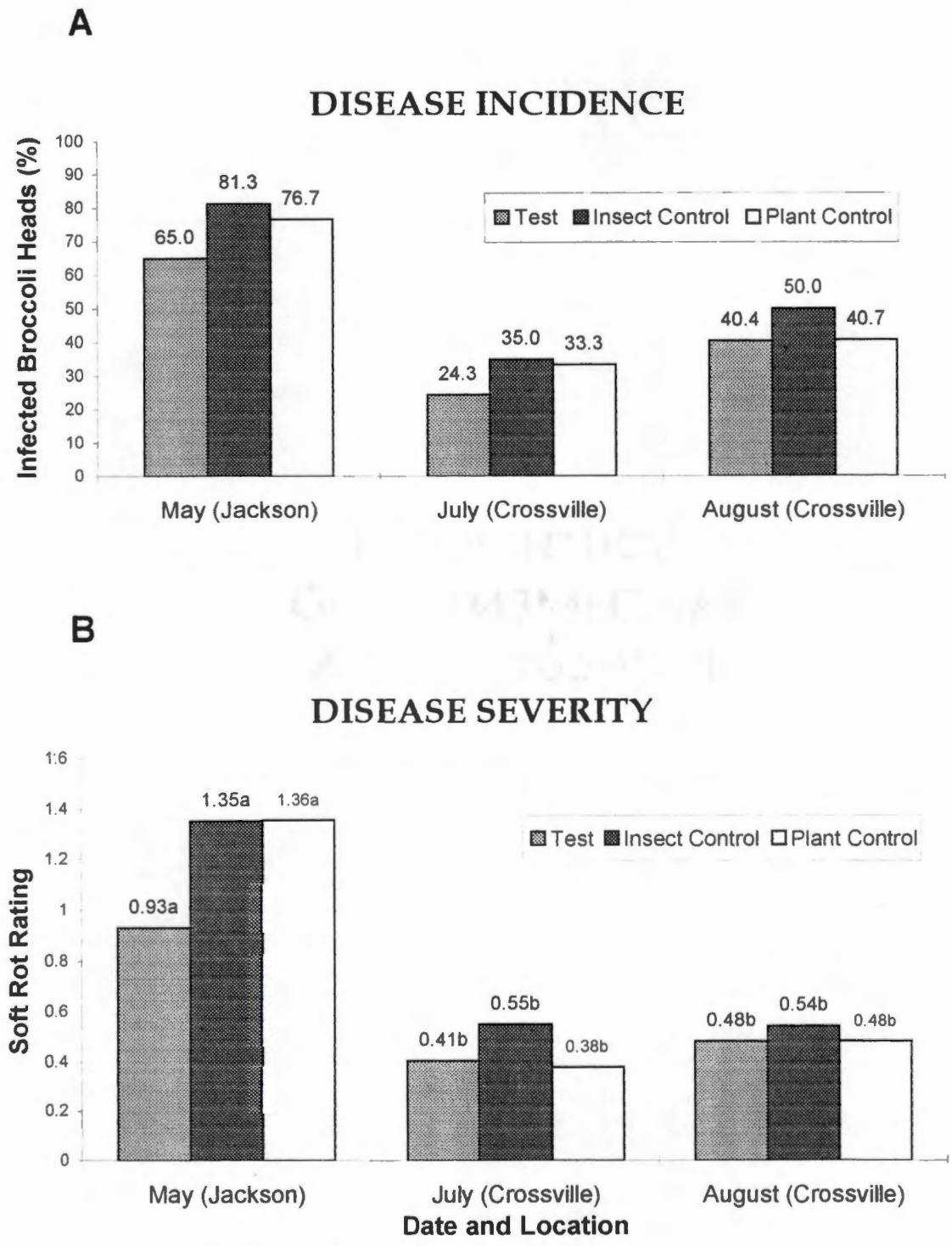
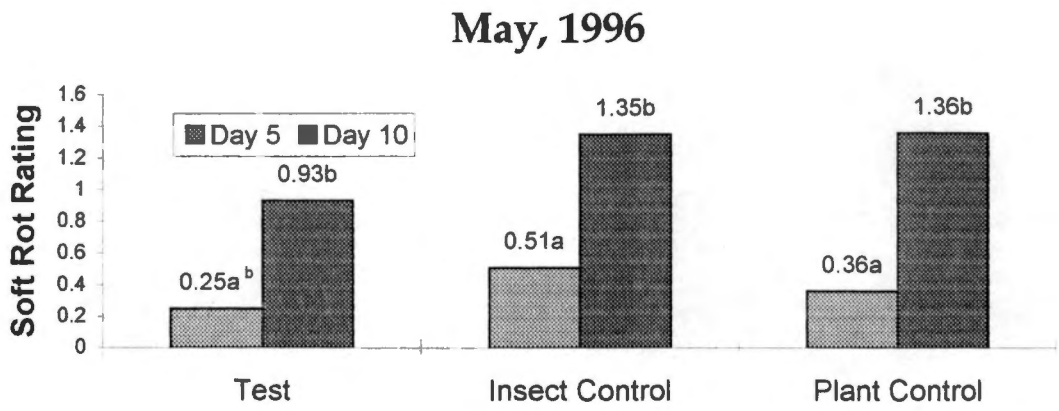


Figure 2. Effect of location and planting date on incidence and severity of bacterial soft rot in broccoli. Statistical calculations (SAS) by General Linear Model (GLM) and means separated by Student-Newman-Keul (SNK) test (P=0.05).

A



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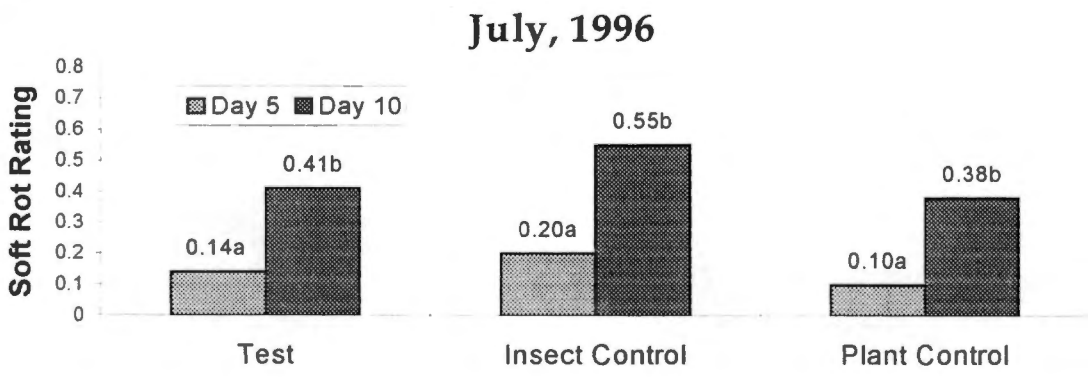

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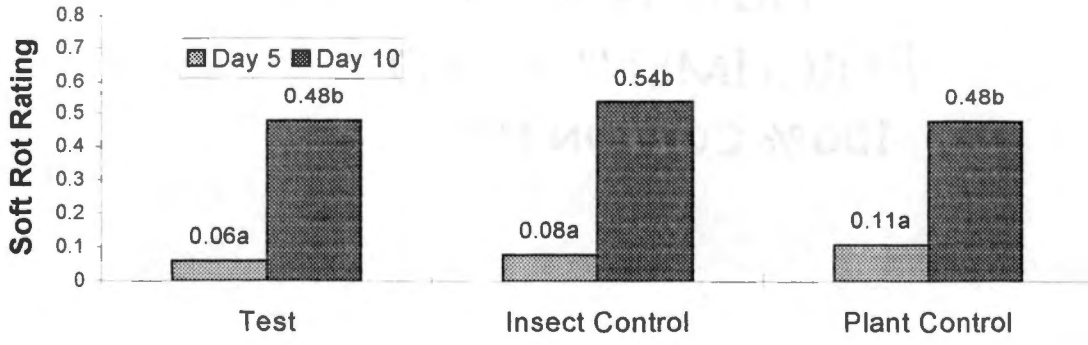

Figure 3. Bacterial soft rot rating means of 5 and 10 day observations. A) West Tennessee Experiment Station, Jackson, TN, B and C) Plateau Experiment Station, Crossville, TN.

insect control, and plant control treatment rating means were significantly higher in the tenth day observation (Fig. 3B, 3C).

Rainfall and temperatures at WTES were above normal. Rainfall was 8.0 cm above average, and temperatures were 1.2°C greater than average during caging of treatments. July rainfall and temperatures at PES were below normal (-2.16 cm and -.3°C). August rainfall departed -.61cm from normal, and temperature slightly increased above normal (+.4°C). High daytime and low nightly temperatures in late August resulted in higher relative humidity, leading to morning fogs enhanced conditions for disease formation. Thus, plants in the SRN developed disease symptoms without overhead irrigation. Plants in the TEST plot, however, did not develop symptoms of disease, probably because of the straw mulch.

b. Bacterial Isolation

Mixed cultures of fluorescent and non-fluorescent bacterial colonies developed on plates inoculated with suspensions from infected broccoli heads. In May samples, 19 of the isolates produced fluorescent colonies, and 3 produced non-fluorescent colonies. Eighteen of the isolates from July produced fluorescent colonies; 3 of the 20 cultures produced non-fluorescent colonies. August plots produced fluorescent colonies in all cultures. Four of the cultures contained non fluorescent colonies. Non-fluorescing colonies stained as Gram negative rods.

A total of 95% of the bacterial cultures produced fluorescent colonies, which were probably a biovar of *P. fluorescens*, or *P. marginalis*. Non-fluorescent colonies, likely *E. carotovora*, were isolated in 17.5% of the cultures.

iii. DISCUSSION

Statistical differences were not found among test and control treatments in each environment. Each treatment behaved independently and did not interact with other treatments. Means of soft rot ratings of Green Comet and Premium Crop cultivars, were not significantly different. Thus, in this study, the presence of TPB did not appear to promote the formation of bacterial soft rot in broccoli .

Although TPB did not appear to influence disease formation, a statistical difference occurred in the severity of bacterial soft rot at WTES and PES. The soft rot rating means of the spring treatments (WTES) were significantly higher than those in crops at PES. The rating means of the crops at PES were not significantly different. Environmental conditions, including prolonged periods of rainfall and warmer temperatures, may have influenced formation of bacterial soft rot. Caging of treatments at WTES occurred between May 22 and June 15, 1996, and disease incidence and severity was greatest in this environment. Rainfall was above normal (+8.03 cm) during this period, and the average temperature was 23.3ºC. These conditions may have enhanced disease occurrence and bacterial dispersal.

Crops in July and August environments had lower soft rot rating means than in May. Ratings and rainfall were lowest in July. Temperatures in both environments were near (± 1°C), and in the optimal temperature range for disease formation (21. to 29.°C). However, lack of moisture may have limited pathogen invasion.

In August, symptoms of bacterial soft rot developed in the SRN before overhead irrigation was initiated. Increased humidity and fogs probably provided sufficient moisture for pathogen invasion. The plants in the TEST plot did not develop symptoms, and appeared to be protected by the straw. Ground covers or mulches may reduce incidence of bacterial soft rot in broccoli fields. Further investigation is warranted.

Bacterial soft rot in broccoli results from invasion of *Pseudomonas* spp. that have of pectolytic and surfactant activity (Hildebrand 1989). Several fluorescence-producing biovars of *P. fluorescens*, and *P. marginalis*, have been isolated from soft-rotted broccoli tissues (Hildebrand 1989). Other pseudomonads, such as *P. aeruginosa* and *P. syringae,* also produce fluorescent colonies. Although these organisms are not typically associated with soft-rotting bacteria of broccoli, they may exist on plant surfaces. Non-fluorescent, pectolytic *E. carotovora* was isolated from diseased tissues only in the presence of pectolytic, fluorescent pseudomonads (Hildebrand 1989). In this study, most cultures of diseased tissues produced fluorescent colonies, indicating that biovars

of *P. fluorescens,* or *P. marginalis*, may be responsible for primary disease formation.

CHAPTER III

ARTHROPODS ASSOCIATED WITH BROCCOLI IN TENNESSEE

i. INTRODUCTION

Identification of beneficial and pest arthropods is necessary for determining suitable management approaches toward pest species. These approaches include the use of different tillage systems, proper planting locations, planting of trap crops, and variation in planting times to avoid major pest problems during the growing season.

Economic thresholds play an important role in crop management. Utilization of these thresholds provides a guide to proper timing of chemical application. Identification of beneficial and pest insects, combined with knowledge of life cycles, reproduction, and feeding habits, determines if pesticides are needed, and when densities of beneficial insects may be least affected.

Sampling methods for faunistic studies are diverse. Sweep nets, drop cloths, and suction, are most commonly used (Southwood 1978). Traps using chemoattractants, stimulants, pheromones, and attractive colors have been developed for specific insects. Sweep nets may be lightweight for aerial insect capture, or made of heavier cloth for ground level sweeping, and for sampling

shrubs and grasses. Drop cloth sampling consists of shaking plants over a cloth laid between the rows and collecting insects that have fallen on it. It is best used with row crops such as soybeans. With the suction method, in which an engine-driven partial vacuum (D-Vac) is created, insects and arthropods are aspirated from the plant and trapped in a bag within the device. This method is most useful in weed-free row crops with spaced plants.

The fauna of broccoli is usually diverse, and several important pest insects comprise a significant proportion. Major pests of broccoli include cabbage and turnip aphids, harlequin bugs, flea beetles, and caterpillars (Hill 1987). Lepidopteran larvae damaging to broccoli and other crucifers include diamond-back moths, cutworms, and imported cabbage worms (Hill 1987). Other pests include stink bugs, thrips, and plant bugs (Cleveland 1982, Hill 1987). The objective of this study was to identify and quantify insects present in broccoli fields in Jackson and Crossville, TN.

ii. MATERIALS AND METHODS

The insect fauna of broccoli was determined in broccoli fields at the West Tennessee Experiment Station (WTES), Jackson, TN, in May-June 1996, and the Plateau Experiment Station (PES), Crossville, TN, in August 1996. Two broccoli fields at each location were sampled. The first field, the soft rot nursery (SRN), consisted of broccoli planted in tilled soil, with high potential for bacterial

soft rot. The second field consisted of broccoli plants in a non-tilled (WTES) or straw-covered (PES) field. This field, the test field (TEST), had a lower potential for bacterial soft rot. Fields were not treated with insecticides prior to sampling.

In the WTES study, D-Vac aspirated samples were obtained from the SRN and TEST plots on May 31, June 6, and June 11, 1996. At PES, sampling was performed on August 23, 26, and 30, 1996. Each sampling consisted of 60 broccoli heads chosen at random from alternating rows, and suctioned for 10 seconds. The bag was changed after 10 heads were aspirated, and placed in glass jars containing paper towels dampened with ethyl acetate to kill the insects. Bags then were emptied into metal dissecting pans, plant materials were removed, and collected insects were stored in 20ml scintillation vials containing 70% ethyl alcohol. The insects from 10-head sampling were identified to family, and counted with the aid of a stereo microscope.

iii. RESULTS AND DISCUSSION

Thirty-five families of arthropods were evaluated in this study (Table 1). In TEST plots, 32 insect families at WTES and 29 insect families at PES were identified (See Appendix). In SRN plots at WTES and PES, 31 and 27 insect families were identified, respectively. Pest families identified at WTES included aphids (Aphidae), leaf beetles (Chrysomelidae), leafhoppers (Cicadellidae), plant bugs (Miridae), lepidopterans (Pieridae, Plutellidae, and several unidentified

Table 1. Arthropods associated with broccoli in May-August, 1996, at the West Tennessee Experiment Station, Jackson, TN, and Plateau Experiment Station, Crossville, TN.

ORDER	FAMILY	INSECT	SPECIES
Coleoptera	Carabidae	---	---
	Chrysomelidae	Corn flea beetle	*Chaetocnema pulicaria* Melsheimer
		Tobacco flea beetle	*Epitrix hirtipennis* Melshimer
		Striped flea beetle	*Phyllotreta striolata* (Fabricius)
		Cabbage flea beetle	*Phyllotreta cruciferae* (Goeze)
		Spotted cucumber beetle	*Diabrotica undecimpunctata howardi* Barber
		Striped cucumber beetle	*Acalymma vittata* (Fabricius)
	Coccinellidae	Pink lady beetle	*Coleomegilla maculata lengi* Timberlake
		7-spotted lady beetle	*Cocinella septempunctata* (Linnaeus)
		Convergent lady beetle	*Hippodamia convergens* Guerin-Meneville
	Lampyridae	---[a]	---
	Melyridae	---	---
Diptera	Cecidomyiidae	---	---
	Chloropidae	---	---
	Dolichopidae	---	---
	Empididae	---	---
	Muscidae	---	---
	Mycetophilidae	---	---
	Otitidae	---	---
	Phoridae	---	---
	Piophilidae	---	---
	Syrphidae	---	---
	Tachinidae	---	---
	Tipulidae	---	---

Table 1. (Continued)

ORDER	FAMILY	INSECT	SPECIES
Hemiptera	Anthocoridae	Minute pirate bug	*Orius* sp.
	Lygaeidae	Big-eyed bug	*Geocoris* sp.
	Miridae	Tarnished plant bug	*Lygus lineolaris* (Palisot de Beauvois)
		Plant bug	*Lygus* sp.
	Nabidae	Damsel bug	---
	Pentatomidae	Harlequin bug	---
		Green stink bug	*Murgantia histrionica* (Hahn)
Homoptera	Aphidae	---	---
	Cicadellidae	Leafhopper	---
Hymenoptera	Braconidae	---	---
	Chalcidae	---	---
	Formicidae	---	---
	Halictidae	---	---
	Ichneumonidae	---	---
	Pteromalidae	---	---
	Vespidae	---	---
Lepidoptera	Pieridae	Imported Cabbage Worm	*Pieris rapae* (Linnaeus)
	Plutellidae	Diamondback Moth	*Plutella xylostella* (Linnaeus)
Orthoptera	Tettigoniidae	---	---
Thysanoptera	Thripidae	Flower Thrips	*Frankliniella* spp.

a) Specimens of family not identified further

larvae), and thrips (Thripidae) (Figs. 4,5). Of the pest families, leaf beetles (Chrysomelidae) were most abundant, including several species of flea beetles, and spotted and striped cucumber beetles. Among beneficial insects were parasitoid wasps (Braconidae and Pteromalidae), and lady beetles (Coccinellidae) (Fig. 6). Predatory hemipterans included minute pirate bugs (Anthocoridae), big-eyed bugs (Lygaeidae), and damsel bugs (Nabidae). At PES, most of the same common pest species of WTES were observed (Figs.7-9). Stink bugs (Pentatomidae) were more common at PES than at WTES, and diamondback moths were less common.

At both locations, insect numbers were variable. In the survey at WTES, numbers of insects per plant often increased in successive samples (See Appendix). In contrast, at PES, decreases often occurred in the second samples, followed by increases in the final samples (See Appendix). These changes presumably were caused by differing weather conditions, variable plant growth, and seasonal effects, but were not further correlated in this study to any of these parameters.

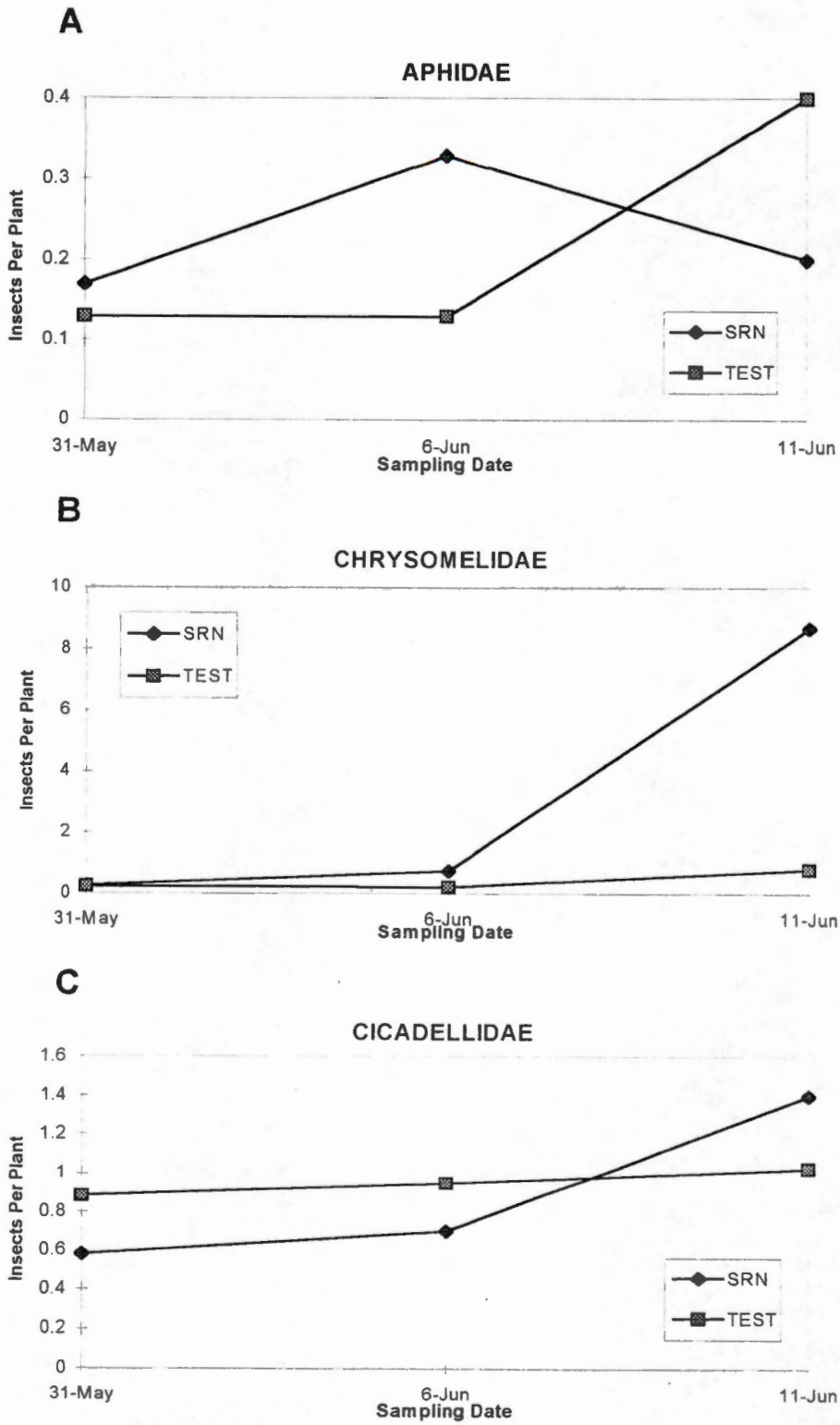

Figure 4. Means per plant of pest insects A) aphids, B) leaf beetles, and C) leafhoppers at the West Tennessee Experiment Station, Jackson, TN, 1996.

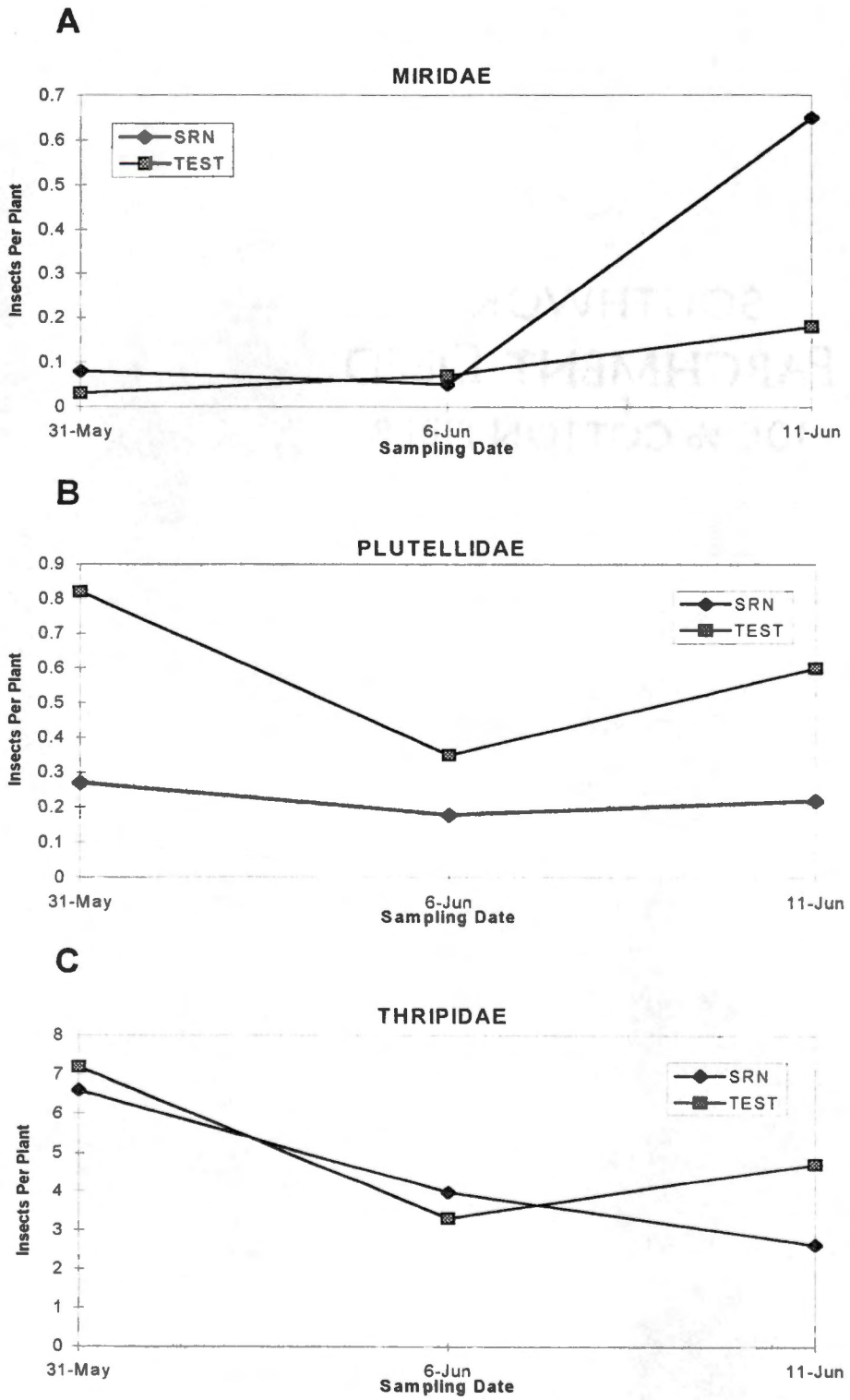

Figure 5. Means per plant of pest insects A) plant bugs, B) diamondback moths, and C) thrips at the West Tennessee Experiment Station, Jackson, TN, 1996.

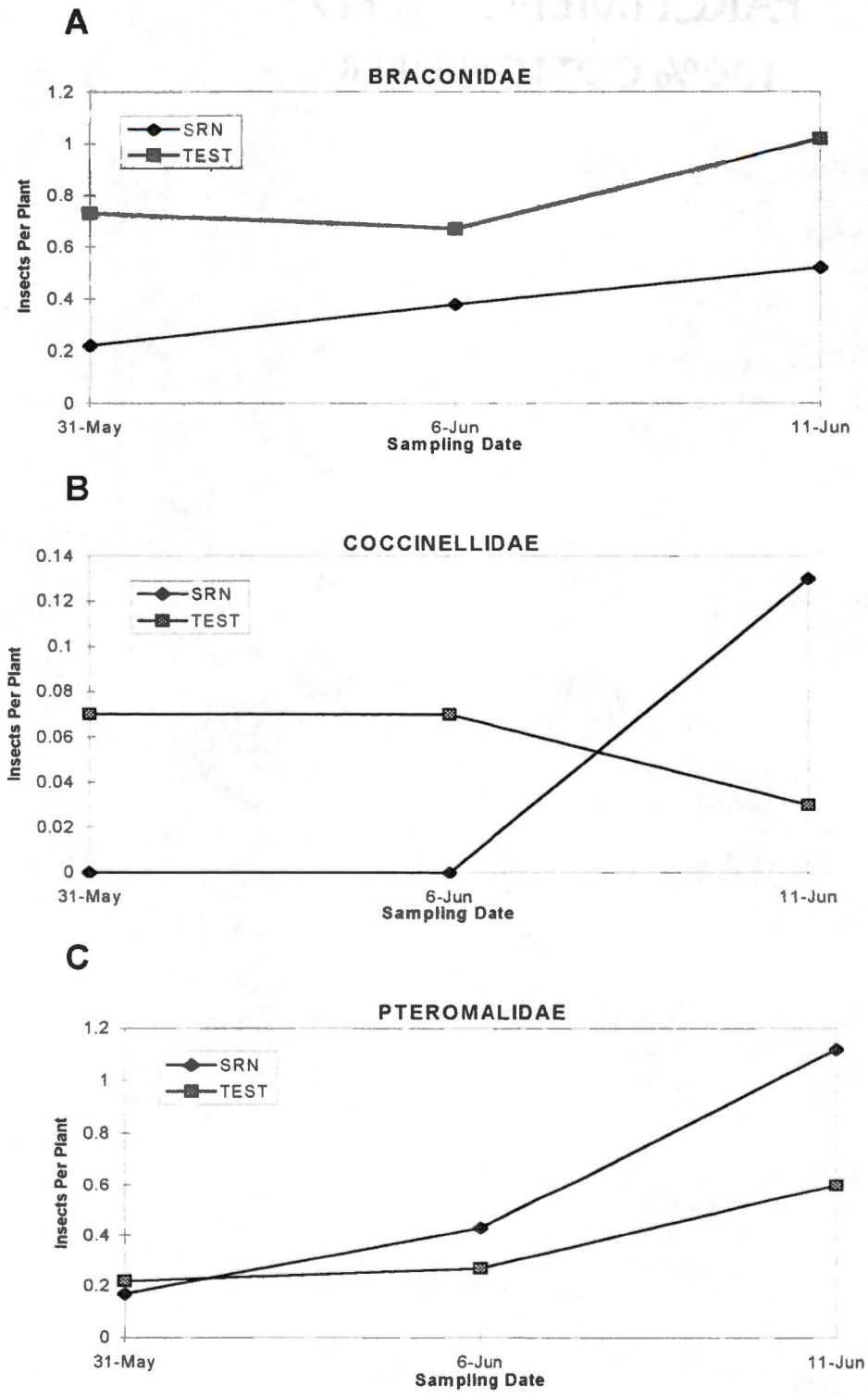

Figure 6. Means per plant of beneficial insects A) and C) parasitic wasps, and B) lady beetles at the West Tennessee Experiment Station, Jackson, TN, 1996.

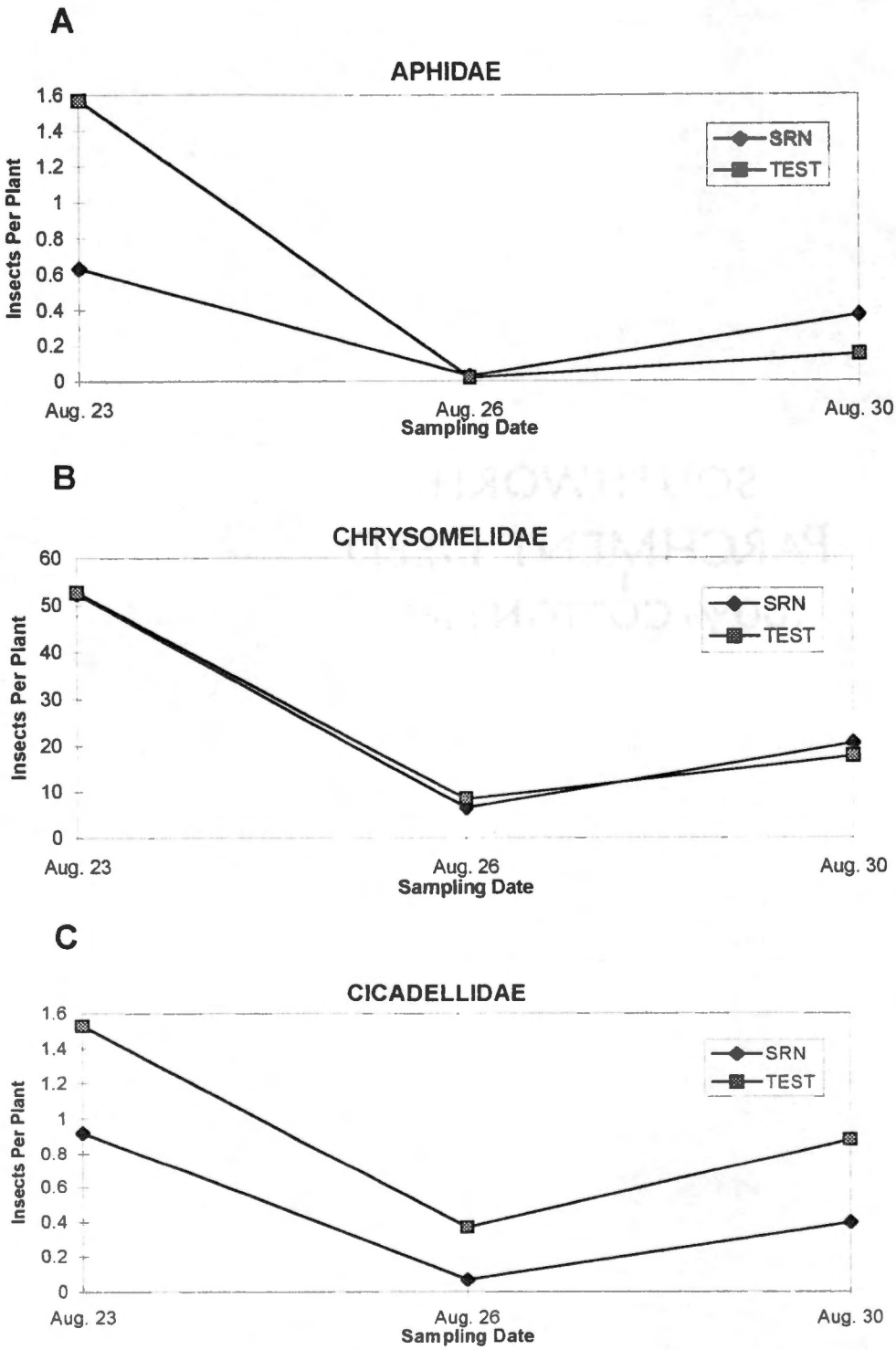

Figure 7. Means per plant of pest insects A) aphids, B) leaf beetles, and C) leafhoppers at the Plateau Experiment Station, Crossville, TN, 1996.

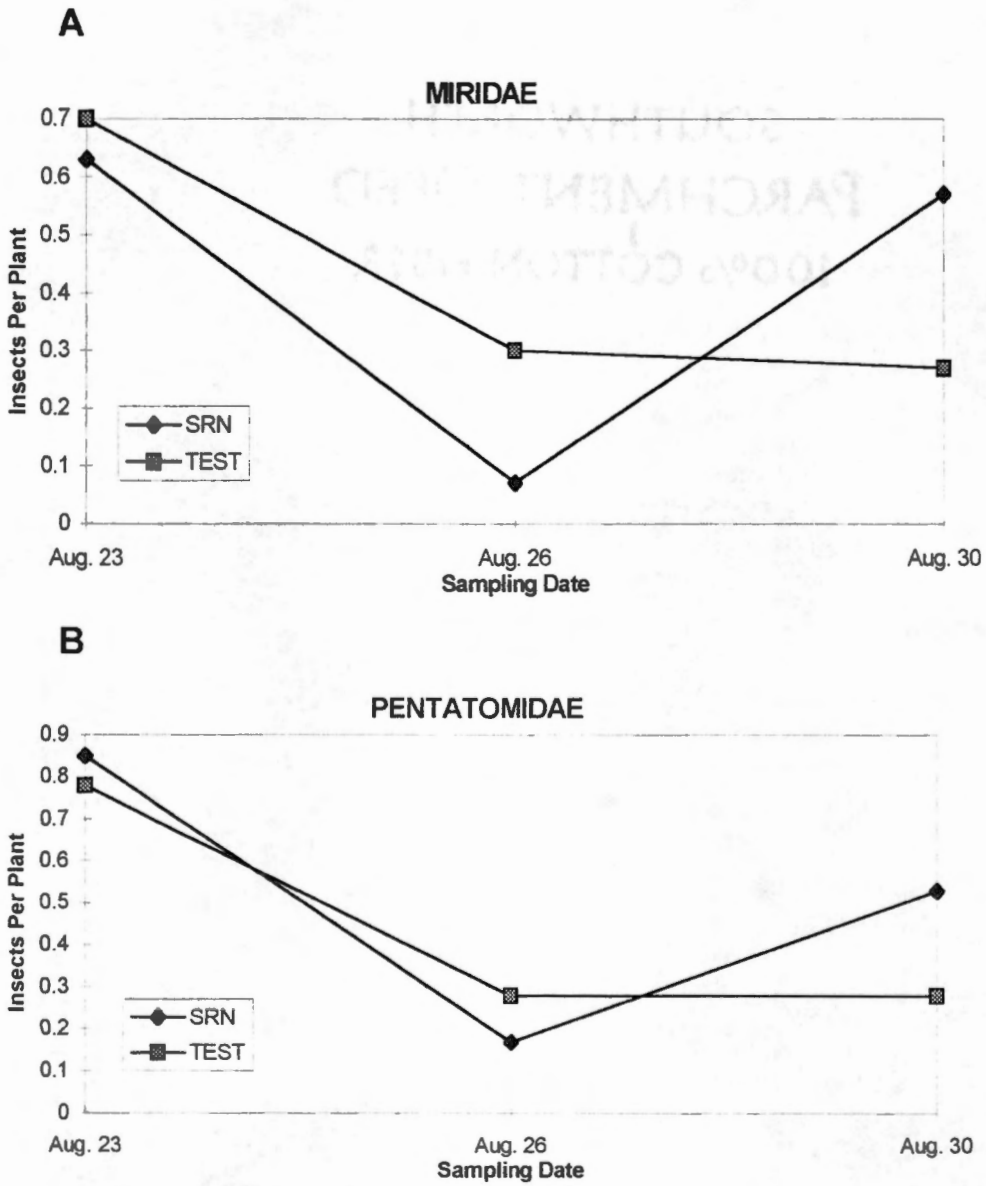

Figure 8. Means per plant of pest insects A) plant bugs, and B) stink bugs at the Plateau Experiment Station, Crossville, TN, 1996.

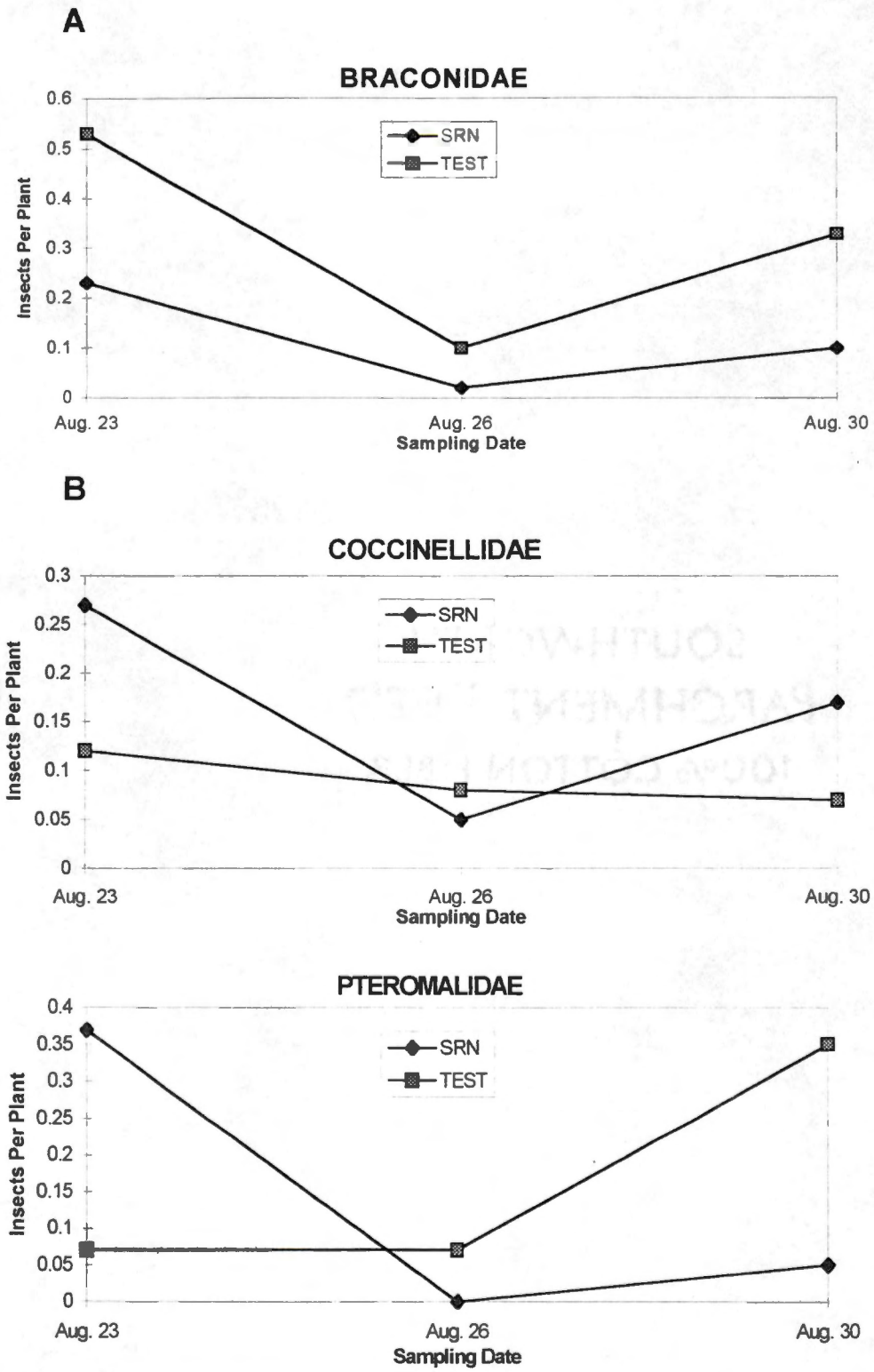

Figure 9. Means per plant of beneficial insects A) and C) parasitic wasps, and B) lady beetles at the Plateau Experiment Station, Crossville, TN, 1996.

CHAPTER IV

CONCLUSIONS

Bacterial soft rot ratings did not differ (P=0.05) among test, insect control and plant control treatments. These results were repeated in the May, July, and August plantings. Therefore, according to this study, broccoli heads caged with bacteria-exposed tarnished plant bugs (TPB) did not develop a significantly greater amount of bacterial soft rot when compared to the two control treatments.

Although the presence of the TPB did not appear to influence the formation of bacterial soft rot in broccoli, significantly less disease developed in treatments of the July and August plantings. Since wet weather conditions affect disease formation (Canaday 1991, Hildebrand 1989,), it is possible that the above average amount of rainfall and increased temperatures may have stimulated disease development in the May plantings WTES. Disease development in the July and August plantings at the PES was not as great as in the May planting at WTES. Rainfall at PES was below normal in July and August. Temperatures were slightly below normal during July and normal in August. Thus, increased head moisture due to rainfall and warmer field conditions may have increased disease formation at WTES.

Significant differences occurred in the 5-day disease ratings. The final soft rot rating of each treatment was significantly higher than the first rating. Thus, once disease developed, it continued to become more severe.

In the August planting at PES, bacterial soft rot developed in the soft rot nursery (SRN) without the aid of overhead irrigation. Fluctuations of temperatures from evening to morning hours probably caused increased relative humidity, which led to condensation on the broccoli heads to create more favorable conditions for disease development. Bacterial soft rot did not develop in the straw-covered TEST plot. The straw appeared to limit disease formation, probably by reducing soil splash during rainfall.

Tarnished plant bugs were abundant in the mustard fields planted at WTES. Approximately 1,000 TPB were used in test and insect control treatments at WTES and PES. Further research should be performed to indicate if mustard plantings may serve as suitable trap crops for TPB.

The insect fauna of broccoli fields in Tennessee is diverse. Numerous potential pest taxa were collected in the fields. Wounds created by insect feeding or piercing of plant parts may lead to infection by fungi, bacteria, and viruses. Nutrient deficiencies from natural insect feeding may lead to reduced yields. Beneficial insects also were a part of the broccoli fauna. Many of these insects were parasitic wasps that parasitize caterpillars or eggs. Others, such as lady beetles and minute pirate bugs, prey upon aphids and other wound-inducing pests.

Although TPB did not increase disease incidence in tested broccoli plants, identification of wound-inducing insects on broccoli may lead to control measures that limit insect damage. Since the bacteria that cause bacterial soft rot may become opportunistic in plant wounds, control measures should be

performed to minimize wounds caused by pest species and reduce sources of bacterial inoculum. Combinations of resistant cultivars, mulches, planting sites in areas of least rainfall, drip or non-aerial irrigation, and control of wound-causing pests may result in less bacterial soft rot in broccoli fields in Tennessee.

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APPENDIX

Table A1. Insect families identified in surveys of TEST and SRN broccoli plots, West Tennessee Experiment Station, Jackson, TN, 1996.

	TEST			SRN		
FAMILY	**31-May**	**6-Jun**	**11-Jun**	**31-May**	**6-Jun**	**11-Jun**
Anthocoridae	+	+	+	--	+	+
Aphidae	+	+	+	+	+	+
Braconidae	+	+	+	+	+	+
Carabidae	+	+	+	--	+	+
Cecidomyiidae	--	--	--	--	--	+
Chalcidae	--	--	--	--	--	--
Chloropidae	+	+	+	+	+	+
Chrysomelidae	+	+	+	+	+	+
Cicadellidae	+	+	+	+	+	+
Coccinellidae	+	+	+	--	--	+
Dolichopidae	+	+	+	--	+	+
Empididae	+	+	+	+	+	+
Formicidae	+	--	--	--	--	+
Halictidae	--	--	--	--	+	+
Ichneumonidae	+	+	+	+	+	+
Lampyridae	+	--	+	+	--	--
Lepidoptera (larvae)*	+	+	+	+	+	+
Lygaeidae	+	--	+	--	+	+
Melyridae	--	--	--	--	--	--
Miridae	+	+	+	+	+	+
Muscidae	+	+	--	+	+	+
Mycetophilidae	--	--	+	+	--	+
Nabidae	+	+	+	--	--	--
Otitidae	+	--	--	--	--	+
Pentatomidae	+	+	+	+	--	+
Phoridae	+	+	+	+	+	+
Pieridae	--	--	+	--	--	+
Piophilidae	--	+	--	--	+	--
Plutellidae	+	+	+	+	+	+
Pteromalidae	+	+	+	+	+	+
Sisyridae	--	--	+	--	--	--
Syrphidae	--	--	+	--	+	+
Tachinidae	+	--	--	--	+	+
Tettigoniidae	--	+	+	--	--	--
Thripidae	+	+	+	+	+	+
Tipulidae	--	--	+	--	+	--
Vespidae	--	--	--	--	--	--
TOTAL	**25**	**22**	**27**	**17**	**23**	**28**

*Grouping of several unidentified families

Table A2. Insect families identified in surveys of TEST and SRN broccoli plots, Plateau Experiment Station, Crossville, TN, 1996.

	TEST			SRN		
FAMILY	23-Aug	26-Aug	30-Aug	23-Aug	26-Aug	30-Aug
Anthocoridae	+	+	+	+	+	-
Aphidae	+	+	+	+	+	+
Braconidae	+	+	+	+	+	+
Carabidae	+	+	+	+	+	+
Cecidomyiidae	-	-	-	-	-	-
Chalcidae	+	-	+	-	-	-
Chloropidae	+	+	+	+	+	+
Chrysomelidae	+	+	+	+	+	+
Cicadellidae	+	+	+	+	+	+
Coccinellidae	+	+	+	+	+	+
Dolichopidae	+	+	+	+	+	+
Empididae	+	+	+	-	-	-
Formicidae	-	-	-	+	-	-
Halictidae	-	-	-	-	-	-
Ichneumonidae	+	+	+	+	+	+
Lampyridae	-	-	-	-	-	-
Lepidoptera (larvae)	+	+	+	+	+	+
Lygaeidae	+	-	-	+	-	-
Melyridae	+	-	-	+	-	+
Miridae	+	+	+	+	+	+
Muscidae	+	+	+	+	+	+
Mycetophilidae	-	-	-	-	-	-
Nabidae	+	-	+	+	-	+
Otitidae	+	-	-	-	-	+
Pentatomidae	+	+	+	+	+	+
Phoridae	+	-	+	+	-	+
Pieridae	+	+	-	-	-	-
Piophilidae	+	-	+	+	-	+
Plutellidae	-	-	+	+	-	+
Pteromalidae	+	+	+	+	-	+
Sisyridae	-	-	-	-	-	-
Syrphidae	-	-	-	+	-	+
Tachinidae	+	+	-	+	-	-
Tettigoniidae	+	-	-	-	-	-
Thripidae	+	-	+	+	-	+
Tipulidae	-	-	-	-	-	-
Vespidae	+	-	-	+	-	-
TOTAL	28	18	22	26	14	22

*Grouping of several unidentified families

Table A3. Means per plant of selected insects in TEST and SRN broccoli plots, West Tennessee Experiment Station, Jackson, TN, 1996.

	TEST			SRN		
FAMILY	**31-May**	**6-June**	**11-June**	**31-May**	**6-June**	**11-June**
Anthocoridae	0.02	0.07	0.03	0.00	0.03	0.05
Aphidae	0.13	0.13	0.40	0.17	0.33	0.20
Braconidae	0.73	0.67	1.02	0.22	0.38	0.52
Chloropidae	5.10	4.25	4.92	2.87	6.27	8.10
Chrysomelidae	0.23	0.20	0.78	0.25	0.73	8.70
Cicadellidae	0.88	0.95	1.03	0.58	0.70	1.40
Coccinellidae	0.07	0.07	0.03	0.00	0.00	0.13
Ichneumonidae	0.32	0.07	0.40	0.42	0.28	0.78
Lepidoptera (Larvae)*	0.22	0.25	0.37	0.10	0.10	0.12
Lygaeidae	0.03	0.00	0.25	0.00	0.05	0.78
Miridae	0.03	0.07	0.18	0.08	0.05	0.65
Nabidae	0.02	0.02	0.05	0.00	0.00	0.00
Pentatomidae	0.02	0.02	0.00	0.02	0.00	0.12
Plutellidae	0.82	0.35	0.60	0.27	0.08	0.22
Pteromalidae	0.22	0.27	0.60	0.17	0.43	1.12
Thripidae	7.20	3.30	4.73	6.60	3.97	2.63

*Grouping of several unidentified families

Table A4. Means per plant of selected insects in TEST and SRN broccoli plots, Plateau Experiment Station, Crossville, TN, 1996.

	TEST			SRN		
FAMILY	**23-Aug**	**26-Aug**	**30-Aug**	**23-Aug**	**26-Aug**	**30-Aug**
Anthocoridae	0.30	0.02	0.10	0.02	0.03	0.00
Aphidae	1.57	0.02	0.15	0.63	0.03	0.37
Braconidae	0.53	0.10	0.33	0.23	0.02	0.10
Chloropidae	7.62	1.60	2.47	3.08	0.10	1.15
Chrysomelidae	52.67	8.48	17.63	52.45	6.53	20.53
Cicadellidae	1.53	0.37	0.88	0.92	0.07	0.40
Coccinellidae	0.12	0.08	0.07	0.27	0.05	0.17
Ichneumonidae	0.47	0.10	0.47	0.45	0.02	0.13
Lepidoptera (Larvae)*	0.38	0.02	0.07	0.50	0.27	0.25
Lygaeidae	0.08	0.00	0.00	0.03	0.00	0.00
Miridae	0.70	0.30	0.27	0.63	0.07	0.57
Nabidae	0.10	0.00	0.02	0.08	0.00	0.02
Pentatomidae	0.78	0.28	0.28	0.85	0.17	0.53
Plutellidae	0.00	0.00	0.03	0.03	0.00	0.02
Pteromalidae	0.07	0.07	0.35	0.37	0.00	0.05
Thripidae	0.25	0.00	0.72	0.07	0.00	0.05

* Grouping of several unidentified families

VITA

Kelly Silas Parman was born on February 16, 1968, in New Iberia, Louisiana. She attended St. Joseph Elementary School in Jeanerette, Louisiana, and graduated from John McDonogh (High) School, New Orleans, in 1985. She relocated to Knoxville, Tennessee, in 1987. In 1991, she graduated from Roane State Community College with an Associate of Applied Science degree in Medical Laboratory Technology. She received her Bachelor of Arts in Biology from Carson-Newman College, Jefferson City, Tennessee. After working as a medical laboratory technologist for 2 years, she entered the Master's program in Entomology and Plant Pathology, at The University of Tennessee, Knoxville. She received her Master of Science degree in August, 1997.

Mrs. Parman is a member of the Entomological Society of America, Gamma Sigma Delta Agricultural Honor Society, the Tennessee Entomological Society, and the Graduate Student Association of Entomology and Plant Pathology. She is an active member of St. John's Lutheran Church, Knoxville, and frequently provides insect demonstrations at surrounding elementary schools.