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Effects of endophyte infection and methyl bromide on surface-dwelling and edaphic arthropods in tall fescue

Cindy L. Williver

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To the Graduate Council:

I am submitting herewith a thesis written by Cindy L. Williver entitled "Effects of endophyte infection and methyl bromide on surface-dwelling and edaphic arthropods in tall fescue." I have examined the final electronic 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.

Ernest C. Bernard, Major Professor

We have read this thesis and recommend its acceptance:

Henry Fribourg, Kimberly Gwinn, Charles Pless

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Ernest C. Bernard, Major Professor

We have read this thesis
and recommend its acceptance:

Kimberly D. Gwinn

Accepted for the Council:

CW Minkel

Associate Vice Chancellor
and Dean of the Graduate School

EFFECTS OF ENDOPHYTE INFECTION AND METHYL BROMIDE ON SURFACE-DWELLING AND EDAPHIC ARTHROPODS IN TALL FESCUE

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Cindy L. Williver
August 1996

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ABSTRACT

Tall fescue is a common, cool-season pasture grass in the United States. When infected with the endophytic fungus, *Acremonium coenophialum,* it is associated with tall fescue toxicosis. Studies have been conducted to determine the effects of this grass/endophyte interaction on mammals as well as herbivorous arthropods. There has been little previous work done to determine the effects of the interaction on predators and decomposers. The objective of this study was to determine weather the presence of *Acremonium coenophialum* changed the community structure and population dynamics of mesoarthropods in tall fescue fields. The effects of previous methyl bromide treatment in half of the fields were also examined.

Collembola (springtails), Acari (mites), and Carabidae (ground beetles) were collected with pitfall traps for at least six weeks per season for one year. An alcohol mixture was used as a preservative in the field. Pitfall contents were collected twice weekly. Soil cores were taken once a month and arthropods were extracted either with a Crossley-Blair high-gradient soil extraction or by means of a heptane flotation technique.

The effects of endophyte-infected tall fescue on Collembola were species-specific. *Sphaeridia pumilis, Sminthurus fitchi, Sminthurinus henshawi,* and *Isotoma viridis* often had significantly higher populations in endophyte-infected (E+) tall fescue fields. *Homidia socia* and *Pseudosinella violenta* were more abundant in endophyte free (E-) fields. *Lepidocyrtus cinereus* had similar population densities in both E+ and E- fields. Association measures for Collembola indicated that E+ tall fescue selected a particular community composition. Carabids were collected in relatively low numbers in all fields and did not seem to be affected by the presence of the endophyte. Acari were more abundant in E- fields and therefore may have been adversely affected by the endophyte.

The effects of previous methyl bromide treatment were more apparent on truly edaphic species such as *Parajapyx isabellae* and *Epilhommania* sp. However, *Isotoma viridis, S. henshawi, S. fitchi, Sminthurinus elegans,* and *Sphaeridia pumilis* were most abundant in methyl bromide treated (MB+) fields. *Lepidocyrtus cinereus* and *P. violenta* were more common in fields not treated with methyl bromide (MB-). *Homidia socia* showed no preference for MB+ or MB- fields. Carabid populations were also lower in MB+ fields. Groups of Acari acted differently with respect to methyl bromide. *Galumna* sp. and other Oribatida populations were higher in MB+ fields, but *Epilohmannia* sp. was higher in MB- fields.

Additional studies on mesofauna in E+ and E- tall fescues would benefit from monitoring the alkaloid concentrations in the field. Together the information could bring about a better understanding of how the alkaloids produced by the grass/endophyte interaction affect organisms within the field. A different method of soil core extraction should be used to give a better estimate of the soil fauna present.

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Introduction

Tall fescue, *Festuca arundinacea* Schreb. (Poaceae) is the predominant cool season perennial pasture grass in the eastern United States. It often is infected with an endophytic fungus, *Acremonium coenophialum* Morgan-Jones and Gams (Morgan-Jones & Gams, 1982). This association usually results in the production of compounds toxic to many other organisms. Endophyte-infected (E+) tall fescue is more resistant to many insects, nematodes, plant disease fungi, drought, and grazing animal damage than is endophyte-free (E-) tall fescue (Clay et al. 1985, Siegel et al. 1987, Arachevaleta et al. 1989, West 1994). Annual losses due to endophyte in Tennessee alone are estimated between $60 million and $85 million (Fribourg et al. 1991b).

Insect herbivores of tall fescue have previously been studied in some detail (reviewed in Popay & Rowan 1994). The effect of E+ tall fescue on soil and ground-dwelling mesofauna has been studied in less detail. Japanese beetle larvae may be deterred from endophyte-infected grass (Oliver et al. 1990). Chloropid flies are negatively affected by E+ tall fescue (Vogt 1992). Members of the predatory and decomposer food webs have not been previously studied with respect to effect of endophyte presence.

The general objective of this research was to determine whether *A. coenophialum*-infected tall fescue affected the population dynamics and community structure of resident Collembola and Carabidae. Collembola primarily feed on soil microorganisms and dead plant tissue, and most Carabidae are predators. Each of these groups has many species that fill numerous surface and soil niches.

Chapter 2

Literature Review

Tall Fescue

Tall fescue, *Festuca arundinacea* Schreb., is a cool season perennial pasture grass (Buckner & Bush 1979). An estimated 14.2 million ha are planted in the United States (Fribourg et al. 1991b). In Tennessee alone there are more than 1.42 million ha of pasture (Fribourg et al. 1991a, Long & Hilty 1985). Tall fescue grows actively in the fall, winter, and spring, and therefore is good for winter grazing (Thompson & Thompson 1974). Although tall fescue can grow from Canada to Florida, it grows best in the north-south transition zone of North America, between 33° and 40° N latitude and between 95° longitude and the eastern edge of the Piedmont area (Bush & Buckner 1973). Tall fescue has an abundant root system, produces a strong sod, and is resistant to drought. Tall fescue can also survive on hillsides where most other crops cannot (Buckner et al. 1979). Tall fescue is one of the top ten grasses planted in the United States for soil conservation, recreational areas, general purpose turf, and livestock forage (Buckner et al. 1979, Cowan 1956). Although more suitable for pasture, tall fescue can often produce an acceptable yield of hay (Cowan 1956).

Seed was imported into the United States from Europe and established by William Suiter in Menifee County, Kentucky in about 1890 (Cowan 1956). Seed from this hillside in 1931 became the stock seed for the Kentucky 31 cultivar of tall fescue that was released by the University of Kentucky in 1943 (Fergus & Buckner 1972). Kentucky 31 increased in popularity and proved to be drought-tolerant, resistant to many insect pests and diseases, and to have a long grazing season (Fribourg et al. 1991).

Endophytes

Endophytic fungi within the tribe Balansiae of the Clavicipitaceae are fungi that usually live almost entirely within their host plant. The first recorded host grass associated with a fungus dates back 4,400 years to seeds found in the tomb of a Fifth Dynasty Egyptian Pharaoh (Lindau, G. 1904 in Siegel et al. 1987). Endophyte mycelium in the seed of a grass (*Lolium temulentum* L.) was recorded in 1898 by Vogl (Siegel et al. 1987). Endophytes and their grasses are thought to have evolved together (Clay 1993). Sixteen species of fescue have been reported infected with an endophyte (White & Cole 1985). Endophytic fungi may or may not produce infectious symptoms.

The endophytes that are symptomatic often cause choke disease or host sterilization in warm-season grasses (Clay 1994). Sexually reproducing endophytes often produce ascospores and fruiting bodies on their hosts, which usually reduces the fitness of the host. This relationship is parasitic.

In contrast to warm-season grasses, cool-season grasses may possess intercellular endophytes that inhabit the leaf sheaths and stems of plants. These endophytes never invade the cells or become pathogenic. They are passed from generation to generation through the seeds in a clonal form and reproduce only asexually (Hill 1994). Genetic differentiation thus occurs only through random mutation (Clay 1993). High percentages of endophyte infection suggests a mutualistic association between the host and its seed-borne endophyte (Clay 1993). Seed-borne endophytes are found only in the Pooideae and are much more species-specific than sexually reproducing endophytes. Fungal endophytes in this group often coexist in a mutualistic relationship with their plant host. The plant provides the nutrients needed for the growth of the fungi and a means of transmission or dissemination, while the fungus produces secondary compounds that defend its food supply (Popay & Rowan 1994). Endophytes are concentrated in the basal leaf sheath area of the plant, but mycelium may extend through the aerial parts of the plant. Some secondary compounds may be translocated to the roots (Popay & Rowan 1994).

The Tall Fescue Endophyte

Taxonomy

The endophyte associated with tall fescue is a clavicipitaceous fungus in the tribe Balansiae. It was originally described as the asexual stage of *Epichloë typhina* (Pers.), the fungus responsible for choke disease in warm season grasses. In 1982, Morgan-Jones and Gams redescribed the endophyte as *Acremonium coenophialum*. Endophytic species of *Acremonium* are obligatorily host-dependent and asexual (Hill 1994). Although in nature these endophytes are host species-specific, artificial associations between grasses and endophyte survive (Siegel et al. 1990, Latch & Christensen 1985). *Acremonium* endophytes often increase the fitness of their hosts. However, tiller formation, biomass production, specific leaf weight, and drought stress of an individual plant may or may not be enhanced by the endophyte (Hill 1994). Secondary compounds produced by *A. coenophialum* may reduce herbivory of insects and mammals. Endophyte-infected tall fescue is more tolerant to drought than endophyte-free tall fescue (Bates & Joost 1990).

Endophyte Life Cycle

The fungal endophyte produces hyphae between the cells of its tall fescue host. Infected and uninfected plants are indistinguishable by sight. Microscopic or chemical analysis is necessary to determine the status of an individual plant. The endophyte is concentrated in the leaf sheath, the seed, and the inflorescence of a mature plant. Hyphae are rarely found in the leaf blades or roots. *A. coenophialum* is disseminated only in the tall fescue seed and can become genetically differentiated by the accumulation of random mutations (Clay 1993).

Mutualism

Tall fescue and its endophyte, *A. coenophialum,* have evolved together and have a mutualistic relationship (Clay 1993). The endophyte lives its entire life within the grass and receives all its nutrients from the tall fescue. In return, the endophyte gives some plant genotypes the ability to tolerate drought, insect damage, and grazing damage. The endophyte is concentrated in the basal leaf sheath of the vegetative plant (Neill 1941). When the crown and basal leaf sheath are injured, the plant becomes less productive. However, when these portions of the plant are infected by the endophyte, animals and insects can be deterred from feeding on them. Endophyte infection results in reduced animal feeding on the crown and basal leaf sheath of the plant and thus reduces the likelihood of plant injury. Endophyte infection keeps the stand much healthier even in adverse conditions. The viability of the endophyte in the seed declines rapidly after one year (Bacon et al. 1986).

Alkaloids

Tall fescue and *A. coenophialum,* together produce a variety of alkaloids that cause toxic effects in livestock. Insects and other arthropods may also be detrimentally affected. Endophytes produce different combinations of alkaloids, including ergot alkaloids, lolines, and peramine, when paired with different plant genotypes.

Bacon & Siegel (1988) listed seven ergot alkaloids isolated from E+ tall fescue. Ergot alkaloids include clavines, lysergic acid, lysergic acid amide, pyrrolopyrazine alkaloids, and ergopeptine alkaloids (Porter 1995). Ergovaline is the major ergot alkaloid produced *in vitro* by the fungus (Porter et al. 1979, 1981) and is responsible for 10 to 50% of the total ergot concentration in endophyte-infected tall fescue (Dalman et al. 1991). Ergovaline accounts for 84-97% of the total ergopeptine alkaloid content (Lyons et al. 1986). Thompson et al. (1993), Solomons et al. (1989), and Strickland et al. (1989) strongly suggest that ergot alkaloids synthesized by *A. coenophialum* may be responsible for livestock poisoning. In greenhouse-grown tall fescue, ergovaline concentrations peak in spring and fall (Agee & Hill 1994).

N-acetylloline and N-formylloline concentrations may be up to 1,000 times greater than other alkaloids in endophyte-infected tall fescue. Other lolines or pyrrolizidine alkaloids (N-methylloline, norloline, acetylnorloline and N-formylnorloline) are found in low concentrations and have not been studied to any great extent. The presence of the endophyte is necessary for any significant accumulation of pyrrolizidine alkaloids in tall fescue (Bush et al. 1982). There is a direct association between the level of endophyte infection and the level of loline alkaloids in a pasture (Belesky et al. 1987). Lolines have not been isolated from cultures of *A. coenophialum*, nor have lolines been found in tall fescue without the presence of the endophyte. It is possible, however, that the association of the fungus and its host plant together produce loline.

Peramine, an azaindolizine alkaloid, was reported to be a feeding deterrent to the Argentine stem weevil in ryegrass by Gaynor & Rowan (1985). Peramine is produced by *Acremonium lolii* (Latch, Christensen and Samuels), the endophyte associated with perennial ryegrass, in high concentrations.

Alkaloids can be found in a plant in various combinations. Ergot alkaloid and loline concentrations may vary seasonally (Belesky et al. 1987, 1988). Peramine is affected by temperature and plant nutrition (Gaynor & Rowan 1985). Current research is being conducted to try to better define the effects of each alkaloid or group of alkaloids on invertebrate and mammalian toxicity. There is no definitive proof that any of these alkaloids deter mammal or arthropod feeding on tall fescue.

Effects on Livestock

Cattle industry monetary losses of $800 million annually in the United States (Stuedemann as cited in Ball 1987), $26.5 million in Arkansas (Daniels 1989), and $21 million in Tennessee (McLaren 1987) have warranted studies of animals grazing tall fescue. Grazing trials of dairy cattle, beef cattle, sheep, and horses have demonstrated the effects of endophyte-infected tall fescue on animal performance. Three livestock disorders have been associated with endophyte-infected tall fescue pastures: fescue foot, bovine fat necrosis, and summer slump. Although detrimental in some herds, fescue foot and bovine fat necrosis are not nationwide problems (Ball et al. 1991). In regions where tall fescue is grown as the major pasture grass, the widespread occurrence of tall fescue toxicosis has become economically important.

Fescue foot (Cunningham 1949) is characterized by dry gangrene of peripheral tissue, weight loss, and occasional abortion. Usually, fescue foot results in the loss of tips of tails or ears and sometimes the sloughing of hooves or feet. Fescue foot is usually associated with cold weather. Symptoms of fescue foot are similar to chronic ergotism (Lyons et al. 1986).

Bovine fat necrosis is found primarily in 100% endophyte-infected tall fescue fields fertilized with poultry litter for its high nitrogen content. Masses of hard fat in adipose tissue surrounding the intestines of cattle cause digestive and calving problems (Bush et al., 1979). This disease has not been found in female cattle under the age of two, or in bulls or steers. When affected animals were removed from pasture and fed only hay and salt, lesions became smaller (Williams et al. 1969).

The most common and economically devastating endophyte disorder is tall fescue toxicosis. Sometimes referred to as summer slump or summer syndrome, tall fescue toxicosis is characterized by reduced feed intake, poor animal weight gains, intolerance to heat, excessive salivation, higher respiration rates, decreased milk production, nervousness, rough hair coat, more time spent in shade or water, reduced conception rate, less time spent grazing, and reduced reproductive performance. Some or all of these symptoms may occur in livestock suffering from tall fescue toxicosis (Ball et al. 1991, Stuedemann & Hoveland 1988). These symptoms usually, but not always, occur in the summer when heat is most intense. Animals grazing E- tall fescue do not exhibit these symptoms. Bacon et al. (1977) proposed that *A. coenophialum* was involved in tall fescue toxicosis. Ergot alkaloids are suspected to be the primary cause of symptoms leading to tall fescue toxicosis. Other alkaloids in tall fescue are also toxic to livestock.

Effects on Arthropods

Insect resistance in endophyte-infected grasses has been widely studied. Perennial ryegrass, the most important pasture grass in New Zealand, harbors *A. lolii*, which renders the grass resistant to Argentine stem weevil (Prestige et al. 1991). Argentine stem weevil adults exhibit a preference for feeding and ovipositing on endophyte-free ryegrass (Gaynor & Hunt 1983, Barker et al. 1984a). This effect may be due to peramine (Rowan & Gaynor 1986) and/or ergopeptine alkaloids (Popay et al. 1990).

Although the major pasture grass of the eastern U.S. is tall fescue, and its endophyte is *A. coenophialum*, ryegrass and its endophyte and tall fescue and its endophyte are very similar. Endophyte-mediated insect resistance studies have been conducted on both species of grasses and a variety of endophytes associated with them.

Fall armyworms (*Spodoptera frugiperda* (J. E. Smith)) are generalist feeders and have been found to be sensitive to a range of endophytes (Clay et al. 1985). The larvae show a preference for uninfected grasses (Hardy et al. 1986). Larval weights are significantly higher in groups fed E- ryegrass (Hardy et al. 1985). At high alkaloid concentrations there are drastic declines in larval weights and leaf consumption by fall armyworms (Clay & Cheplick 1989).

Aphid populations often are reduced on endophyte-infected grasses. However, not all aphids are affected by the endophyte in the same way (Popay & Rowan 1994). The oat-bird cherry aphid, *Rhopalosiphum padi* (L.), and the greenbug, *Schizaphis graminum* (Rondani), are deterred by both *Acremonium* endophytes in tall fescue and ryegrass. The English grain aphid, *Stobion avenae* (F.), is unaffected by E+ tall fescue and ryegrass. The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), has lower survival on E+ tall fescue and prefers E- tall fescue. *D. noxia* has lower survival on E+ ryegrass, but feeds equally on E+ and E- ryegrass (Kindler et al. 1991).

Endophyte-infected tall fescue may be resistant to Japanese beetle larvae (*Popillia japonica* Newman) (Oliver et al. 1990). Corn flea beetle *(Chaetocnema pulicaria* Melsheimer) decreased in numbers as percentage of E+ plants in the field increased, suggesting deterrence (Kirfman et al. 1986). Similar effects are not known for ryegrass. Both tall fescue and ryegrass with their respective endophytes are toxic to billbugs *(Sphenophorus* sp.) (Ahmad et al. 1986, Johnson-Cicalese & Funk 1988). Leafhoppers

(*Agallia constricta* Van Duzee, *Exitianus exitious* (Uhler), *Graminella nigrifrons* (Forbes) and four species of *Draeculacephala spp.*) were suppressed by the presence of the endophytes. Some species in E- fields had densities up to 10 times greater than those in E+ fields (Muegge et al. 1990). Popay and Rowan (1994) and Breen (1994) provide tables of some insect species and their response to a variety of endophytes. Some insects are deterred by endophyte presence, whereas others [such as the English grain aphid, *Stobinon avenae* (F.) (Kindler et al 1991), the leaf sheath miner *Ceredontha australis* (Barker et al 1984), and several leafhopper species (Prestidge 1989)] are not affected. Tolerance, toxicity, or deterrence of some major pasture pests may provide a broad means of plant protection to E+ pastures, even though some insects are not affected by the presence of endophytes.

All of the insects previously mentioned feed directly on the foliage or roots of tall fescue. Fewer studies have been conducted to determine if there is an effect of the endophyte on secondary consumers. Presence of E+ tall fescue in the diet of fall armyworms reduced development time of the parasitic wasp *Ecplectrus comstockii* Howard, by six hours, compared to a diet containing E- tall fescue (Bultman et al. 1993). Reproduction of *Folsomia candida* Willam was not reduced when adult females were fed E+ leaf sheaths, root tissue, or a variety of alkaloids (Bernard et al. 1990). Several different diets were tested on *Drosophila melanogaster* (Pless et al. 1993). More larvae developed to pupae when fed E- roots and leaves than when fed on E+ tissues. Studies have not been conducted to determine the effects of endophyte presence on organisms in other trophic guilds.

The objectives of this study were to: 1) determine if the presence of *A. coenophialum* changed the community structure and population dynamics of the mesofauna in four tall fescue fields, and 2) determine if previous methyl bromide fumigation in E+ and E- fields changed the population dynamics and community structure of the mesofauna of four tall fescue fields. Collembola, Carabidae, and predatory Acari were the major groups selected for detailed study.

Chapter 3

Materials and Methods

Research Plots

Four plots were selected at the Knoxville Agricultural Experiment Station Plant Science Field Laboratory. Two fields (A-5 and M-4) were endophyte-infected (E+), and two fields (E-7 and M-3) were endophyte-free (E-). One endophyte-infected plot (M-4) and one endophyte-free plot (M-3) was treated with methyl bromide prior to seeding with Kentucky 31 at 22.4 kg/ha in September, 1990. These fields were designated E+ MB+ and E- MB+. Each of these plots was 1.2 ha. The other two plots (A-5 and E-7) were fallow and treated with Roundup® (N-phosphonomethyl glycine) at 7 L/ha before being seeded with Kentucky 31 at 22.4 kg/ha in September, 1991. These fields were designated E+ MB- and E- MB-. Each of these plots was 0.405 ha. Field designations and soil types are shown in Appendix A. All fields were fertilized in March starting the year following planting with 6-12-12 (NPK) at 1120 kg/ha. Weedmaster® (2,4-D and Bentazon) was applied to all fields in April and September of each year at a rate of 7 L/ha to control broadleaf weeds. All fields were harvested for seed on June 10-15,1995, and straw was cut and removed thereafter.

Pitfall Trapping

All specimens were collected from a 36.6 × 11 m area in each field. Three rows of ten traps each were placed into each of the four fields. Traps were placed approximately 3.7 meters apart. Pitfall traps consisted of a hole dug with a standard bulb planter to a uniform depth of 8.5 cm. Each hole was then filled with two 120 ml sample cups nested one inside the other so that the rim of the inner cup was flush with the ground surface. The bottom cup was perforated to prevent accumulation of water between cups. This system allowed the inner cup to be removed without damaging the soil walls of the holes.

Sixty ml of an alcohol mixture (2 parts 95% ethyl alcohol, 5 parts 99% isopropyl alcohol, 3 parts water) were poured into the top cup to kill trapped specimens and to prevent predation and rapid decay of the collected specimens. The cups were then covered with a Plexiglas MC® (AtoHaas North America Inc., Philadelphia, PA) intercept lid to help prevent rain from flooding the traps and to retard evaporation of the preservative.

The intercept lids were 20 cm square and 0.5 cm thick. Baffles (Hylton et al. 1985) of the same material, approximately 1.2 × 0.5 × 4.0 cm tall, were attached to each square with acrylic solvent cement (Craftics, Inc., Chicago, Illinois) at 90° angles. The baffles aided in guiding small organisms into the trap (Hylton et al. 1985). The outer end of each baffle was placed 1 cm from a corner of the square. The Plexiglas was spray-painted flat white with Krylon® Interior/Exterior Paint (Krylon Division, Sherwin-Williams Company, Solon, OH) to reduce the sun's effect on evaporation of the alcohol mixture. A rock or brick was placed on the top of each cover to prevent it from being turned over by wind, or animals in the field.

The contents of each row of traps were collected, and the preservative was replenished twice a week, by first removing the weight and cover from the trap. The top cup then was removed from the ground and its contents poured through a 150-mm funnel into a 1-L bottle marked for the correct field and row. The insides of the cup and funnel were rinsed thoroughly with a squeeze bottle filled with the alcohol mixture. This rinse was also poured into the larger collection container. The cup was refilled with 60 ml of preservative mixture, placed back into the second cup, and covered with the lid and weight. This process was repeated for each cup in the row. At the end of the row, the 1-L container was capped, another container marked for the following row was opened, and the process was begun for the next row. Samples were collected twice a week. The catches from both days were combined, resulting in data given in terms of numbers of organisms per row per week.

Pitfall traps were monitored for a minimum of six weeks during each season. The spring season collections began on April 11 and ended on June 2, 1995. Between seasons, the top sampling cup was capped and left *in situ* to preserve the holes. Plexiglas covers were removed to protect them from sun damage between each sampling season. On July 20, sampling was resumed for the summer season, with collections lasting until August 28, 1995. Fall sampling took place between October 28, and December 1, 1995. Winter samples were obtained from January 16 to March 1, 1996, at which point the traps were removed from the field.

Collected material was brought to the laboratory and condensed by pouring the contents of each bottle through 2-mm-pore and 106-µm pore sieves. The contents of each sieve were then rinsed into a labeled 120 ml sample cup with 95% ethyl alcohol for storage until the sample could be processed.

Organisms from the 2-mm-pore sieve were separated into ground beetles, spiders, and other arthropods. If the residue from the 106-µm-pore sieve contained excessive mineral matter, organisms in it were separated with a heptane flotation method. The sample was rinsed with distilled water and washed into a 70 ml screw top test tube. A thin layer of heptane was poured into the test tube. The lid was screwed on, and the tube was gently turned upside down to prevent the heptane from separating into bubbles. After the debris on the bottom of the test tube was sufficiently agitated, the test tube was returned to the upright position and the debris was allowed to settle to the bottom. The heptane layer was poured onto a 106-µm-pore sieve and the sides of the test tube were rinsed with distilled water. The process was repeated if necessary. The extract on the 106-µm-pore sieve was rinsed with 100% ethanol to remove the heptane. The contents of the sieve were washed through three nested sieves with 425, 180, and 106-µm-pores. The residue on each sieve was backwashed into a counting dish with 95% ethanol. Debris was checked under a dissection microscope for any organisms not extracted with the heptane (Walter et al. 1987).

Organisms from these sieves were separated as spiders, beetles, and other arthropods. Collembola and mites were further separated and counted at the species, genus, or familylevel.

Soil Cores

Fifteen soil cores (3.2 cm × 30 cm) were taken in each field with a large diameter sampling tube. The cores were taken in a crisscross pattern in the area of the field where the pitfall traps were placed. Cores were taken each month if possible. Soil aliquots were placed into high-efficiency, Tullgren-type extractors, slightly modified from the design of Crossley & Blair (1991). Heptane flotation of fresh soil (Walter et al. 1987) was used to test the efficiency of the high gradient extractor.

High Gradient Extraction

Each extractor unit consisted of four funnels, sample holders, and heaters modeled after Crossley & Blair (1991). Funnels (65-mm diameter) were placed into the holes and sealed with reusable rope caulking (Ace hardware Oak Brook, IL). The soil cores were broken up and mixed, and approximately 165 g of soil were placed in a 177-ml aluminum juice can with the top and bottom cut off. Five ml of Von Torn's solution (an isopropanol, glacial acetic acid, and formaldehyde solution) (Von Torn 1967) were placed in the collection vial. Electrical tape was wrapped around the center of each beverage can to prevent them from falling through the openings. Electrical tape was also used to cover sharp edges. Nine soil aliquots were extracted from each field.

The apparatus was placed in an incubator at 14-16° C for 3 days. After three days, collected specimens were pooled into one sample for each field. Each pooled sample was processed with a heptane flotation method modified from Walter et al. (1987)

to separate specimens from mineral debris. Specimens were stored in 95% ethyl alcohol until they could be sorted.

Heptane Flotation

Heptane flotation was used to separate high gradient-extracted specimens from debris and to determine the extraction efficiency of the Tullgren-type extractor. Only one field on one sampling date was processed by this technique due to the lengthiness of the process. Approximately 165 g of fresh soil from the soil cores were heptane-floated (Walter et al. 1987) twice to ensure adequate specimen extraction. Heptane flotation was also performed on soil previously used in the Tullgren-type extractor. The soil taken from the extractor was dried and therefore weighed less. The average weight of a soil core after extraction was 145 g. This soil was processed in the same manner as the field fresh soil.

Statistical Analysis

Pitfall trapping data were analyzed using a Two-Way ANOVA, Student Newman Keuls Method for all pairwise multiple comparison procedures, and Bonferroni t-test (SigmaStat® Statistical Software, Version 2.0, Jandel Scientific, San Rafael, CA). The Bonferroni t-test is a conservative test for experimentwise error (Schlotzhauer & Littell 1987). Each comparison was tested at a low α-level so that the overall significant results were reported at the $P < 0.05$ level. Collembola and beetle similarities were analyzed among rows and diversity indices were analyzed for means of each field with the Ochiai association measure (Ochiai 1957, Bolton 1991) from Biodiv version 5.1 (Exeter Software, Pensoft, Sofia-Moscow 1995). The Ochiai association measure takes into account both the number of species and the abundance of each species in fields relation to the field being compared.

Chapter 4

Results

Pitfall Traps

Collembola

The relative abundance of each species of Collembola collected in each field is summarized in Table 1. Seven species each accounted for at least 3% of the Collembola collected in at least one field. *Homidia socia* Denis was by far the most abundant species, comprising 45-65% of all specimens collected. *Lepidocyrtus cinereus* Folsom, *Isotoma viridis* Bourlet and *Sminthurinus henshawi* (Folsom) also were frequently collected. Seven species were collected infrequently, each accounting for less than 1% of the total specimens collected (Table 1).

Homidia socia was the most abundant species collected in pitfall traps from April to October, but it was nearly absent in winter. Populations were highest in methyl bromide-treated (MB+), endophyte-free (E-) fields throughout the sampling period (Fig. 1A). The E- MB- field had the fewest specimens in April and May. In July and August, however, the E+ MB+ field had the lowest numbers of *H. socia* (Fig. 1A). Numbers of *H. socia* were higher in E- fields than in E+ fields in spring, but lower in summer. Methyl bromide treatment did not affect *H. socia* populations at their peak (May-July). There was a significant interaction of endophyte infection status and methyl bromide treatment on three of five sampling dates (Fig. 1B).

Table 1. Relative diversity of Collembola collected with pitfall traps in E+ and E- tall fescue fields treated or not treated with methyl bromide.

	Tall Fescue Field*			
	E- MB+	E- MB-	E+ MB+	E+ MB-
Bourletiella sp.	0.15	0.01	0.10	0.03
Cryptopygus sp.	0	0	0	0.03
Folsomia candida Willem	0.06	0.06	0.06	0.02
Homidia sauteri Börner	0.05	0.56	0.01	3.69
Homidia socia Denis	74.51	73.58	55.33	71.83
Hypogastrura sp.	7.09	0.02	3.41	0.03
Isotoma trispinata MacGill.	0	0	0	0.07
Isotoma viridis Bourlet	2.66	7.04	10.14	3.05
Isotomurus bimus Chris. & Bell.	0.30	0	0.36	0.01
Lepidocyrtus cinereus Folsom	5.74	10.98	4.55	10.03
Neanura sp.	0	0.01	<0.01	<0.01
Pseudosinella violenta (Folsom)	0.37	1.07	0.44	0.42
Sminthurides malmgreni (Tullberg)	0.60	0.10	3.90	0.51
Sminthurinus elegans (Fitch)	0	0	1.38	0.74
Sminthurinus henshawi (Folsom)	2.77	2.67	11.19	3.34
Sminthurus fitchi Folsom	1.64	1.70	4.01	2.16
Sphaeridia pumilis (Krausb.)	4.05	2.19	5.01	4.04
Tomocerus sp.	0	0	0	<0.01
Tullbergia sp.	0	<0.01	0	<0.01

*Relative diversity = individuals of one species within a treatment divided by total number of specimens in the treatment × 100. Fields designated with E- were endophyte-free, while E+ fields were endophyte-infected. Fields designated by MB+ were fumigated with methyl bromide in 1990; those with MB- were not fumigated.

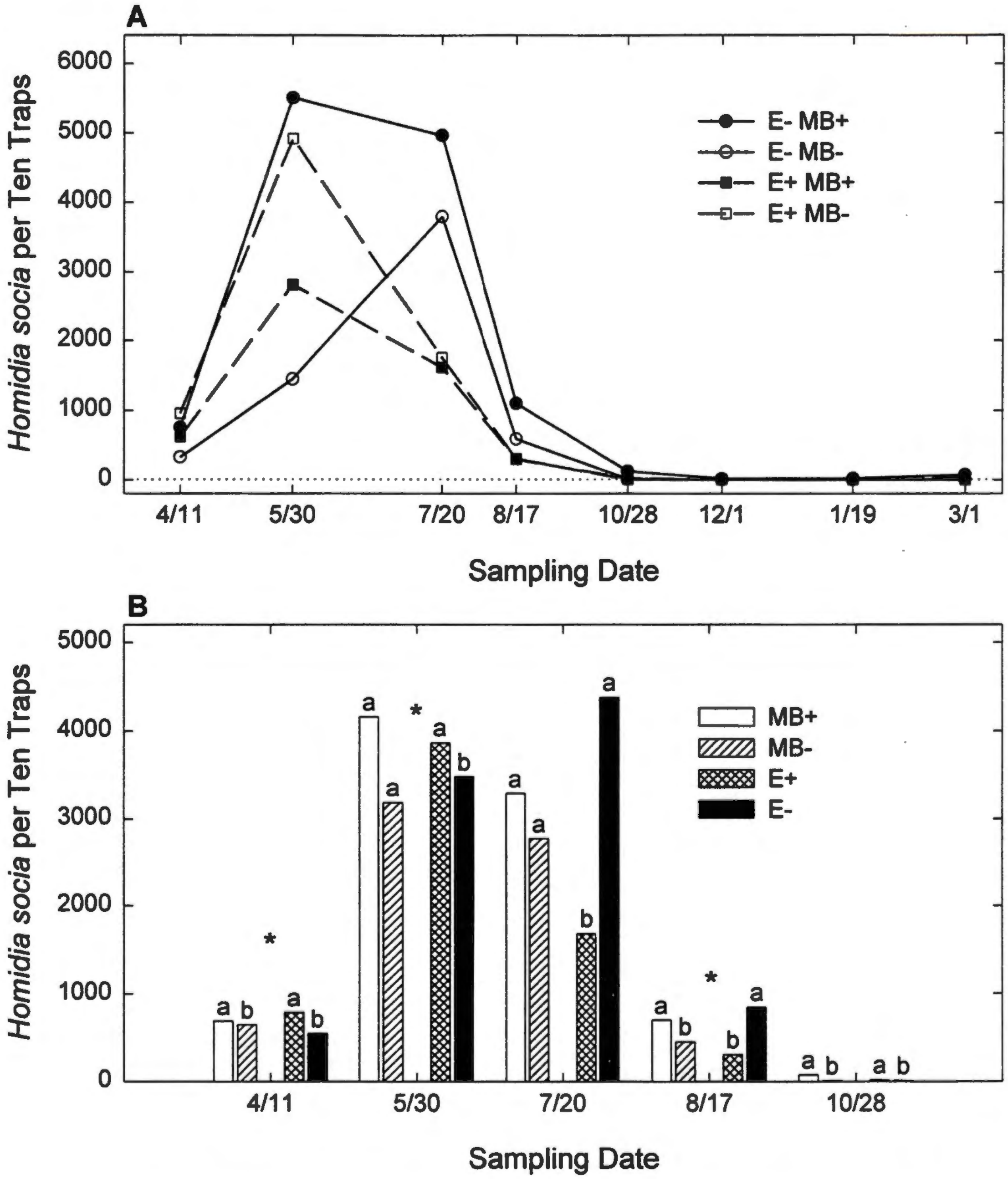

Figure 1. Effects of endophyte infection status and methyl bromide treatment on *Homidia socia.* A) Population dynamics of *H. socia* collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. B) Effects of methyl bromide treatment and endophyte status on *H. socia* populations, as determined with two-way ANOVA and Bonferroni t-test. Only dates with significant differences are plotted. Bars in a pair with different letters are significantly different ($P < 0.05$). Dates with an asterisk indicate methyl bromide/endophyte interaction ($P < 0.05$).

Isotoma viridis was most abundant in the winter and spring samples. Populations of *I. viridis*, at their peak (January-March), were highest in the E+ MB+ field (Fig. 2A). *Isotoma viridis* numbers in March were lower in the E+ MB- field than any other field. Interactions between endophyte infection status and methyl bromide treatment for *I. viridis* occurred on three of four dates (Fig. 2B).

The greatest numbers of *L. cinereus* were found in May and August. Populations were higher in E- fields in August and higher in E+ fields in May. Methyl bromide treatment or non-treatment did not have a consistent effect on *L. cinereus* populations during the sampling period (Fig. 3A). Interactions of endophyte infection status and methyl bromide treatment status for *L. cinereus* occurred on three of seven dates (Fig. 3B).

Pseudosinella violenta (Folsom) was most abundant in August, with the greatest number collected in the E- MB- field. Few were collected in fall and winter. The E+ MB- field contained the highest number of *P. violenta* in April and May (Fig. 4A.). Interactions between endophyte infection status and methyl bromide treatment occurred for *P. violenta* on two of five dates (Fig. 4B).

Sminthurinus elegans (Fitch) was collected only in E+ fields (Fig. 5A). In general, *S. elegans* was more common in the E+ MB+ field. There were no interactions between endophyte infection status and methyl bromide treatment for *S. elegans* (Fig. 5B).

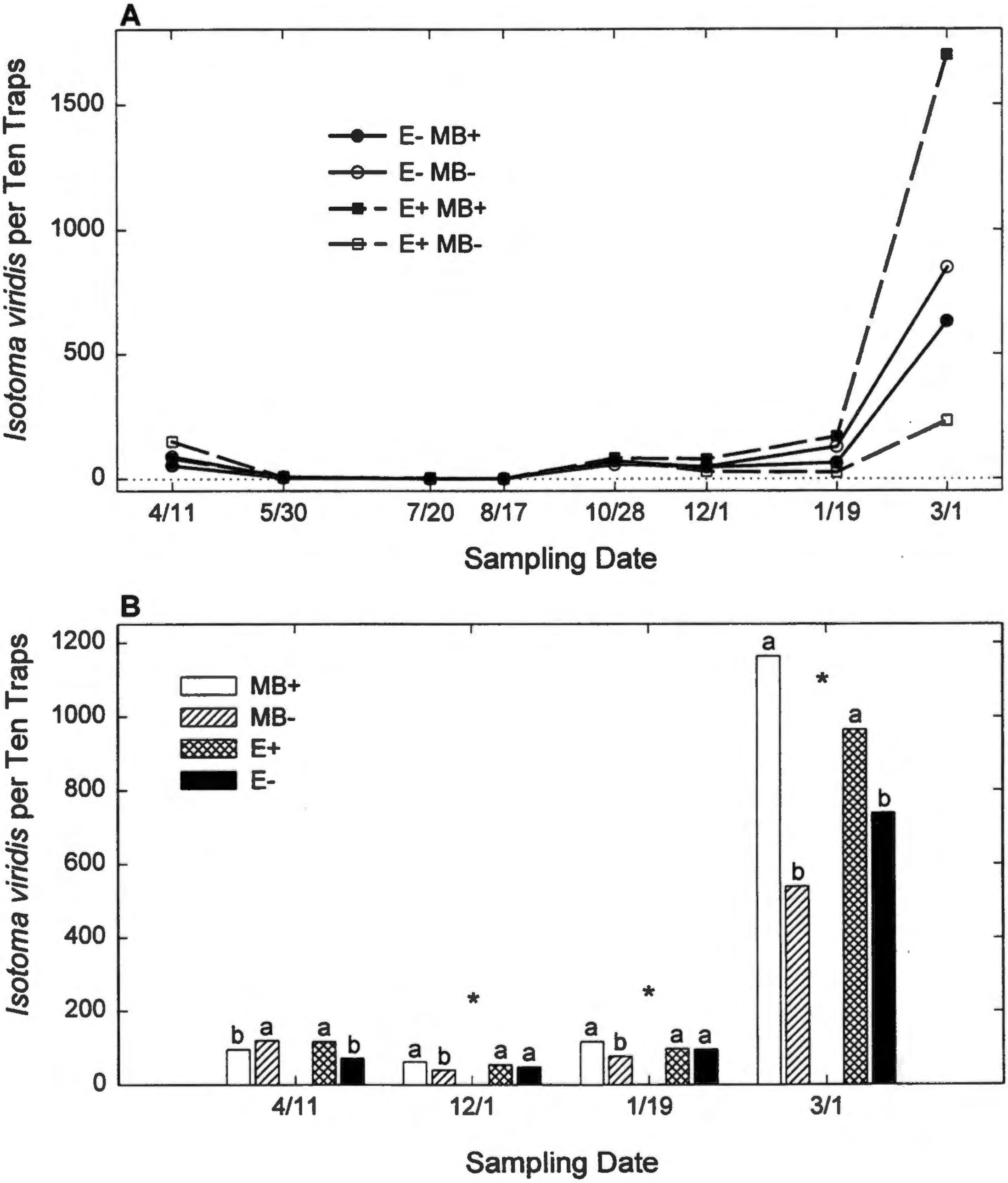

Figure 2. Effects of endophyte infection status and methyl bromide treatment on *Isotoma viridis*. A) Population dynamics of *I. viridis* collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. B) Effects of methyl bromide treatment and endophyte status on *I. viridis* populations, as determined with two-way ANOVA and Bonferroni t-test. Only dates with significant differences are plotted. Bars in a pair with different letters are significantly different ($P < 0.05$). Dates with an asterisk indicate methyl bromide/endophyte interaction ($P < 0.05$).

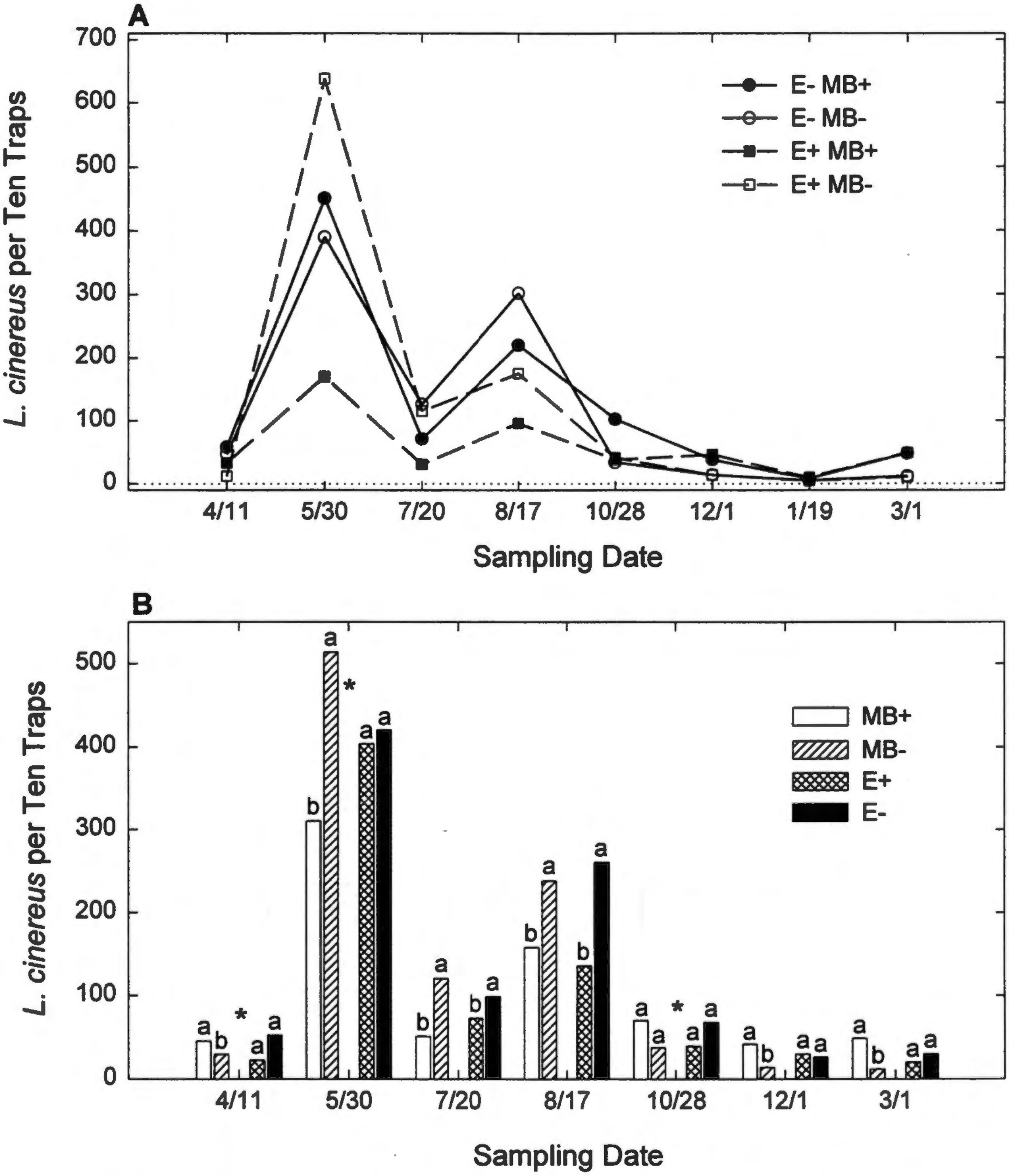

Figure 3. Effects of endophyte infection status and methyl bromide treatment on *Lepidocyrtus cinereus*. A) Population dynamics of *L. cinereus* collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. B) Effects of methyl bromide treatment and endophyte status on *L. cinereus* populations, as determined with two-way ANOVA and Bonferroni t-test. Only dates with significant differences are plotted. Bars in a pair with different letters are significantly different ($P < 0.05$). Dates with an asterisk indicate methyl bromide/endophyte interaction ($P < 0.05$).

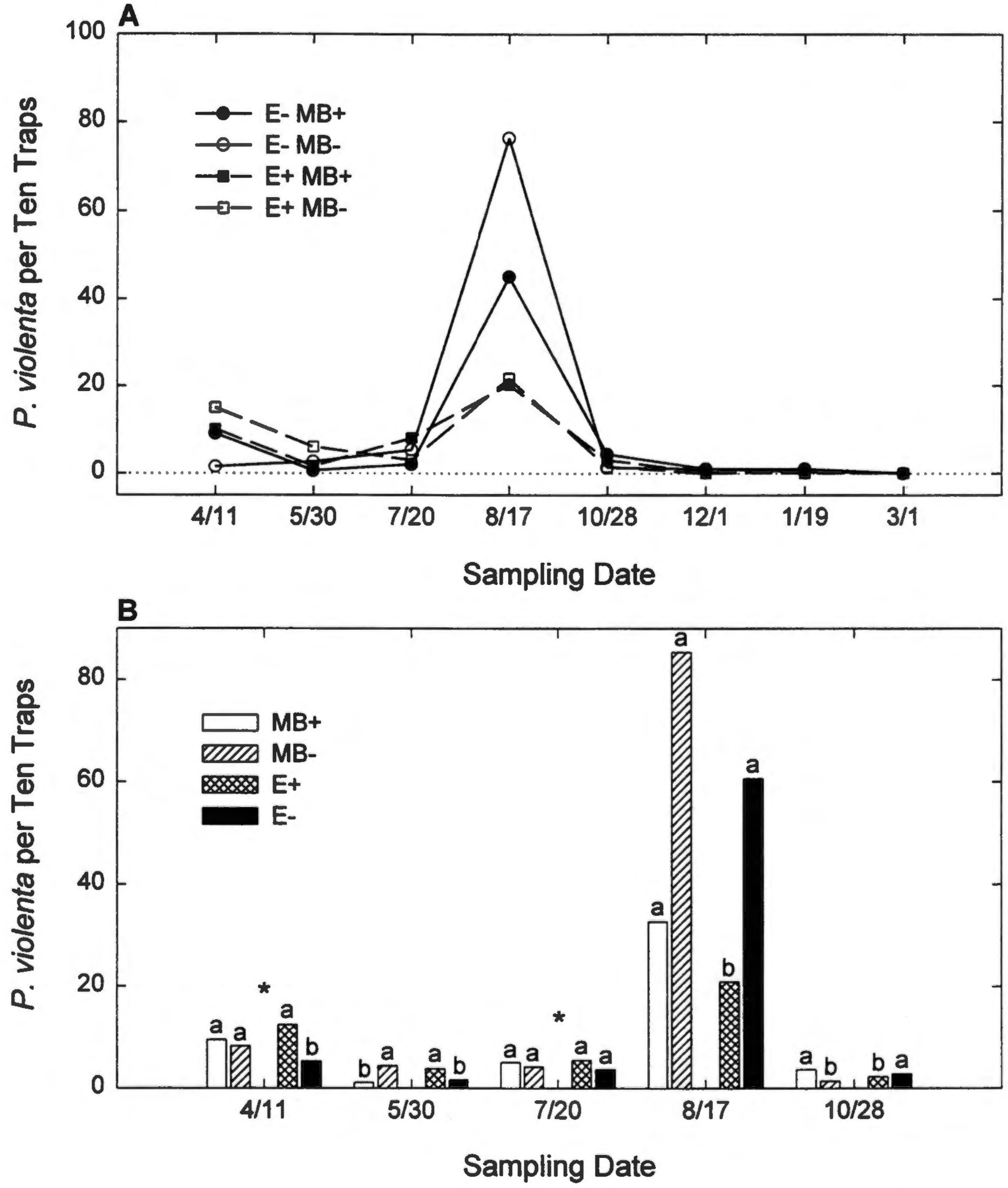

Figure 4. Effects of endophyte infection status and methyl bromide treatment on *Pseudosinella violenta.* A) Population dynamics of *P. violenta* collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. B) Effects of methyl bromide treatment and endophyte status on *P. violenta* populations, as determined with two-way ANOVA and Bonferroni t-test. Only dates with significant differences are plotted. Bars in a pair with different letters are significantly different ($P < 0.05$). Dates with an asterisk indicate methyl bromide/endophyte interaction ($P < 0.05$).

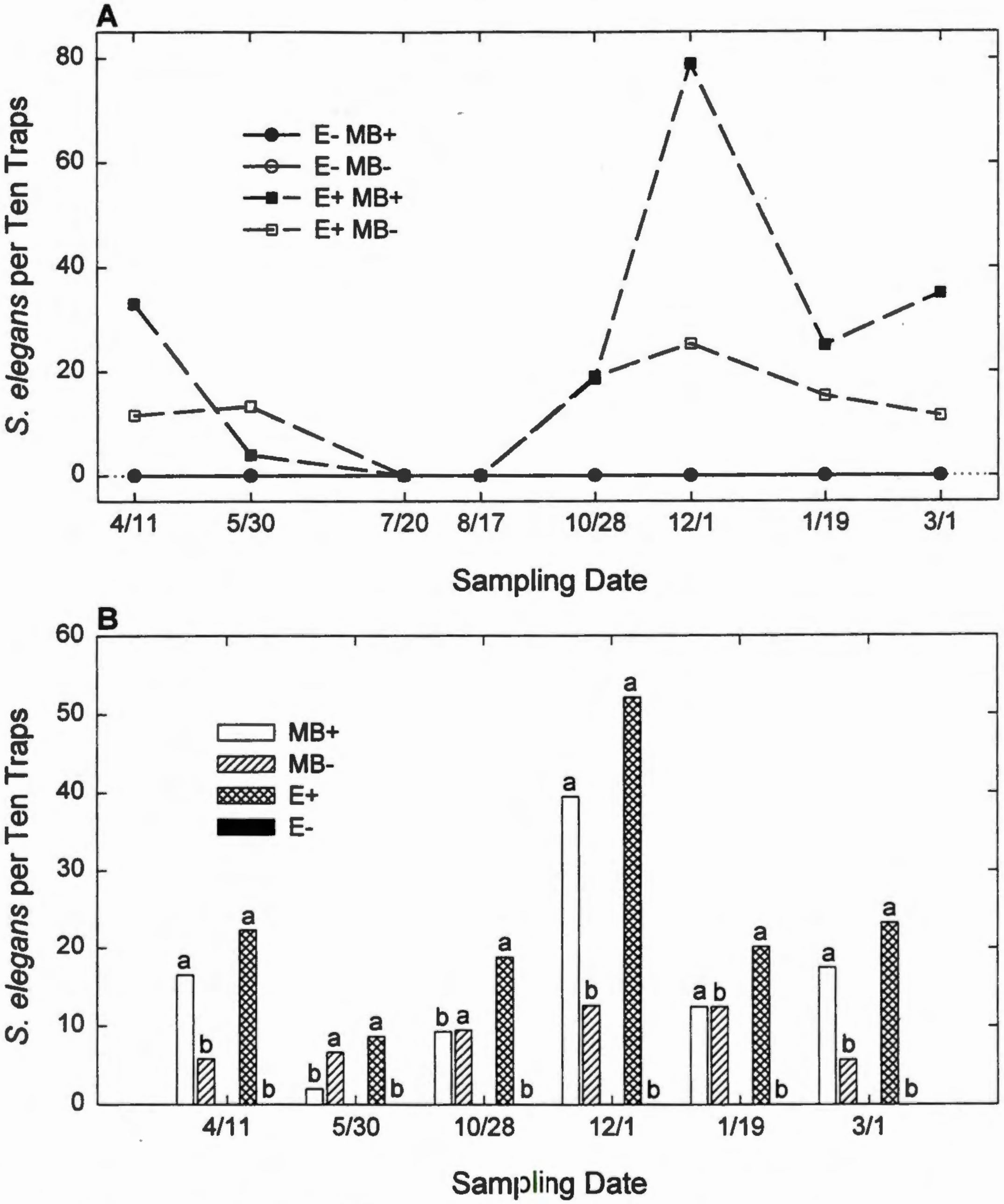

Figure 5. Effects of endophyte infection status and methyl bromide treatment on *Sminthurinus elegans.* A) Population dynamics of *S. elegans* collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. B) Effects of methyl bromide treatment and endophyte status on *S. elegans* populations, as determined with two-way ANOVA and Bonferroni t-test. Only dates with significant differences are plotted. Bars in a pair with different letters are significantly different ($P < 0.05$). Dates with an asterisk indicate methyl bromide/endophyte interaction ($P < 0.05$).

Sminthurinus henshawi (Folsom) was collected almost exclusively in fall and winter. Populations were higher in MB+ fields than in MB- fields. The E+ MB+ field carried the highest population of *S. henshawi*. Lowest numbers of *S. henshawi* were collected in the E- MB- field (Fig. 6A). There were no significant interactions of endophyte infection status and methyl bromide treatment for *S. henshawi* (Fig. 6B).

Sminthurus fitchi Folsom was found throughout the sampling period but was more abundant in April, October and March. The fewest numbers were collected in May. *Sminthurus fitchi* was usually more common in E+ fields (Fig. 7A). The interaction of endophyte infection status and methyl bromide treatment was significant on four of eight dates (Fig. 7A).

Sphaeridia pumilis (Krausbauer) was most frequently collected in July, October, and March. At other times numbers were very low. There were no trends associated with methyl bromide treatment or endophyte status (Fig. 8A). Interactions of endophyte infection status and methyl bromide treatment were significant on three of six dates (Fig. 8B).

Collembola assemblages in the E+ fields were very similar from July to October regardless of methyl bromide treatment, but similarity of assemblages in the E- fields was more variable (Fig. 9). The similarities of assemblages were much more variable in winter. Similarities between E+ and E- fields were usually lower than E+/ E+ similarities, and were comparable to E-/ E- similarities.

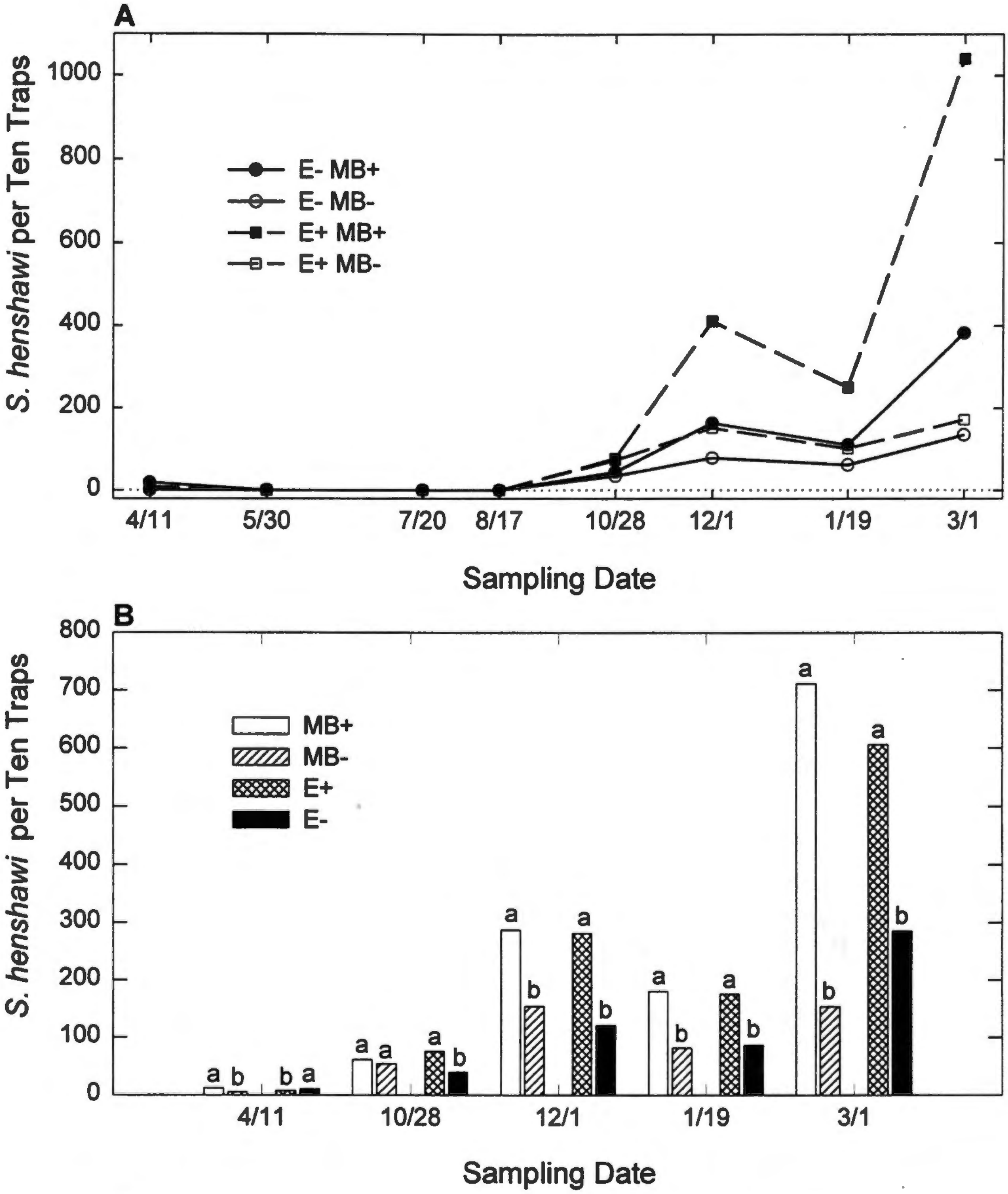

Figure 6. Effects of endophyte infection status and methyl bromide treatment on *Sminthurinus henshawi.* A) Population dynamics of *S. henshawi* collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. B) Effects of methyl bromide treatment and endophyte status on *S. henshawi* populations, as determined with two-way ANOVA and Bonferroni t-test. Only dates with significant differences are plotted. Bars in a pair with different letters are significantly different ($P < 0.05$). Dates with asterisk indicate methyl bromide/endophyte interaction ($P < 0.05$).

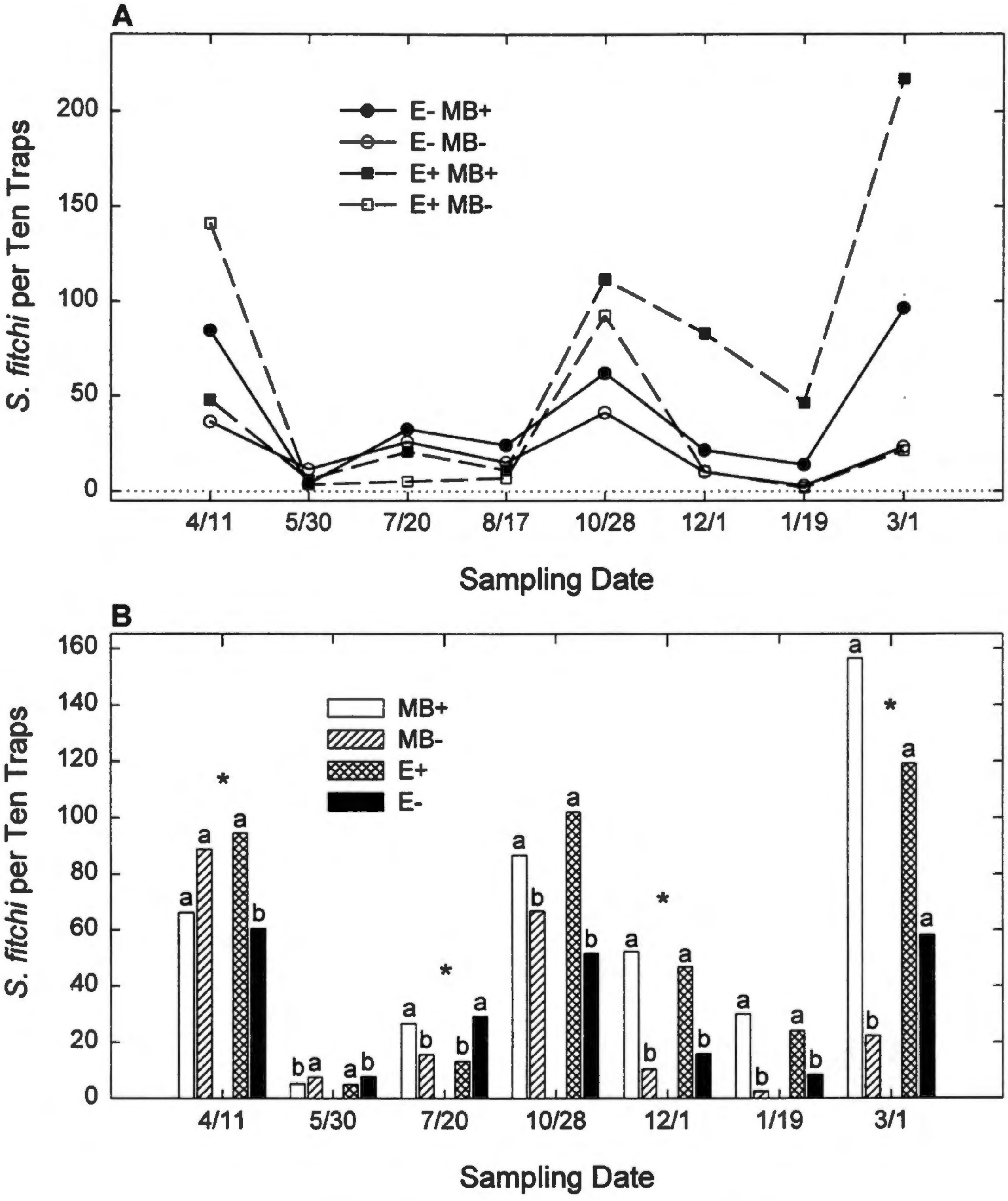

Figure 7. Effects of endophyte infection status and methyl bromide treatment on *Sminthurus fitchi.* A) Population dynamics of *S. fitchi* collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. B) Effects of methyl bromide treatment and endophyte status on *S. fitchi* populations, as determined with two-way ANOVA and Bonferroni t-test. Only dates with significant differences are plotted. Bars in a pair with different letters are significantly different ($P < 0.05$). Dates with asterisk indicate methyl bromide/endophyte interaction ($P < 0.05$).

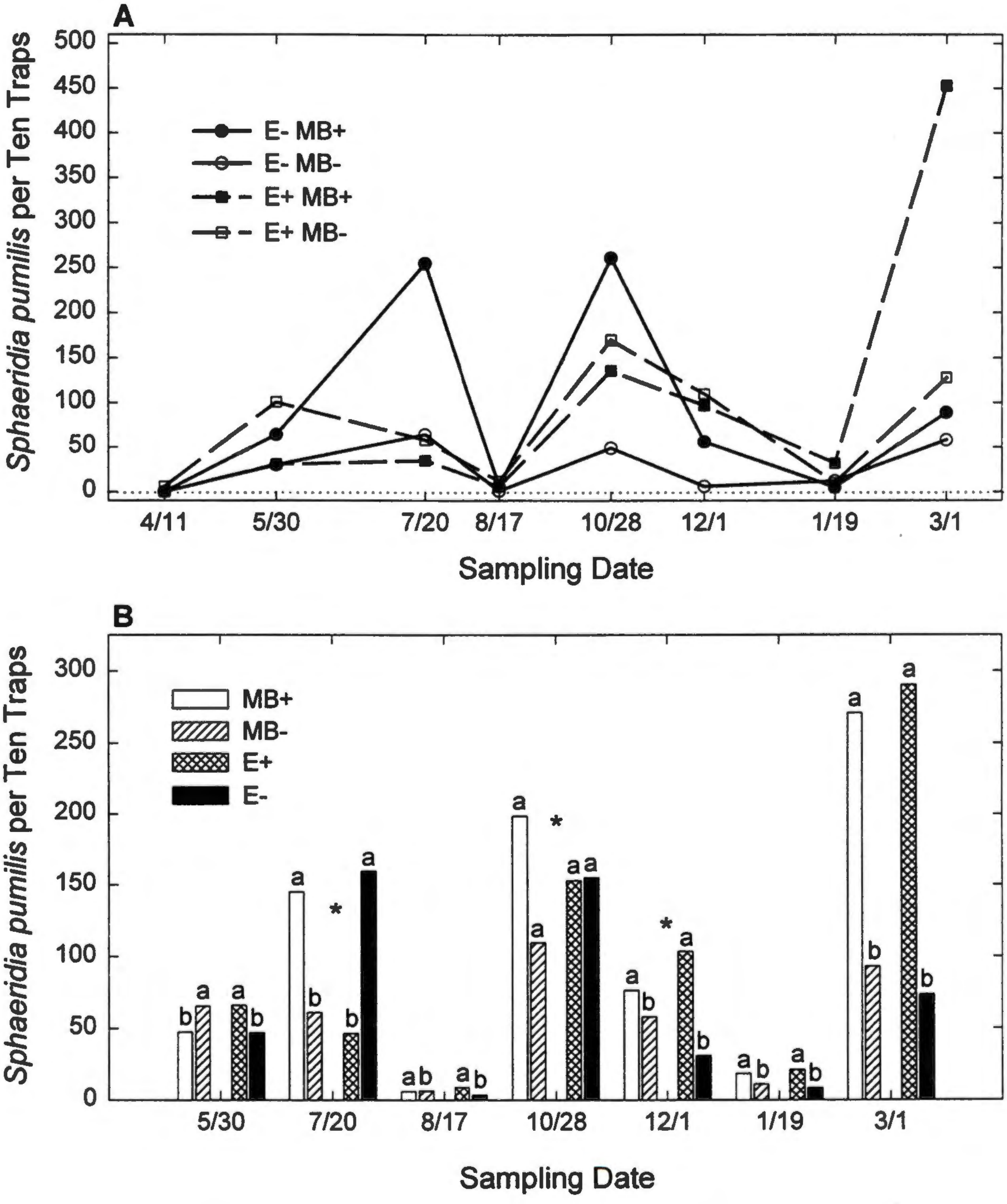

Figure 8. Effects of endophyte infection status and methyl bromide treatment on *Sphaeridia pumilis*. A) Population dynamics of *S. pumilis* collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. B) Effects of methyl bromide treatment and endophyte status on *S. pumilis* populations, as determined with two-way ANOVA and Bonferroni t-test. Only dates with significant differences are plotted. Bars in a pair with different letters are significantly different ($P < 0.05$). Dates with asterisk indicate methyl bromide/endophyte interaction ($P < 0.05$).

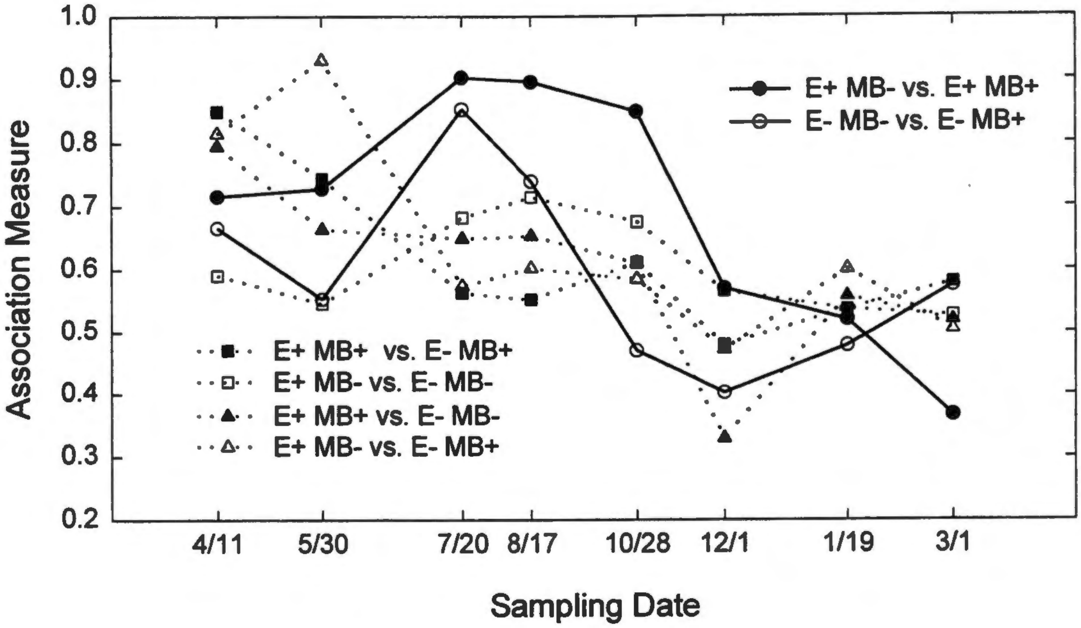

Figure 9. Effects of endophyte infection status and methyl bromide treatment on similarities of Collembola assemblages between all possible pairs of fields. Values for each point were calculated with the Ochiai association measure, where 0 = no similarity between species assemblages, and 1 = identical species assemblages.

Beetles

Seven species of Carabidae were found in tall fescue fields. *Abacidus atratus* Newman and *Agonum punctiforme* Say were the most abundant species (Table 2). Beetles were collected in insufficient numbers for a statistical analysis. Similarities among ground beetle communities were highly variable and not correlated with endophyte status or methyl bromide treatment (Table 3). However, The E+ MB+/E- MB+ comparisons were identical on November 4 and November 10. The E+ MB-/E- MB- comparison was also identical on January 19. Three of the six comparisons were totally dissimilar on August 17, January 19, and March 1. *Abacidus atratus* was most abundant in May and October. MB- fields had the highest populations of *A. atratus*. Populations were lower in MB+ fields throughout the season (Fig. 10A). Populations of *Agonum punctiforme* were highest in May and December in the E+ MB- field. The MB+ fields followed the same pattern throughout the season except for March (Fig. 10B).

Acari

Galumna sp. were collected in greatest numbers in the E- MB+ field throughout the sampling period. Populations were highest in August. *Galumna* were more abundant in MB+ fields than MB- fields (Fig. 11A). There were no interactions between endophyte infection status and methyl bromide treatment (Fig. 11B).

Oribatida were collected in greatest numbers in May and were found in low numbers in the fall and winter. The MB+ fields showed similar patterns of Oribatida

Table 2. Relative diversity of ground beetles (including Cicindelidae) collected in pitfall traps in E+ and E- tall fescue fields treated or not treated with methyl bromide.

	Tall Fescue Field*			
	E- MB+	E-	E+ MB+	E+
Abacidus atratus Newman	31.25	40.88	27.06	33.33
Agonum punctiforme Say	17.19	9.49	21.18	15.76
Amara sp.	3.12	2.19	2.35	0
Harpalus pensylvanicus DeGeer	10.94	29.20	23.53	27.27
Megacephala virginica L.	1.56	0	5.88	8.48
Scarites subterraneus Fabricius	35.94	18.25	20.00	15.15

*Relative diversity = individuals of one species within a treatment divided by total number of specimens in the treatment × 100.

Table 3. Association measures for ground beetle assemblages in E+ and E- tall fescue fields treated or not treated with methyl bromide.

	Beetle assemblages*					
Sample date	E+ MB+ vs E+ MB-	E- MB+ vs E- MB-	E+ MB+ vs E- MB+	E+ MB- vs E- MB-	E+ MB+ vs E- MB-	E+ MB- vs E- MB+
4/11	0.77	0.89	0.77	0.67	0.71	0.60
4/18	0.87	0.83	0.71	0.61	0.71	0.62
4/25	0.91	0.57	0.84	0.74	0.74	0.75
5/2	0.67	0.59	0.44	0.87	0.62	0.71
5/19	0.46	0.52	0.45	0.85	0.50	0.62
5/23	0.75	0.68	0.92	0.72	0.71	0.82
5/30	0.62	0.84	0.78	0.64	0.65	0.63
7/20	0.68	0.73	0.57	0.38	0.35	0.52
8/3	0.55	0.82	0.85	0.64	0.78	0.65
8/10	0.74	0.67	0.61	0.67	0.91	0.45
8/17	0.57	0.00	0.00	0.48	0.71	0.00
8/24	0.82	0.45	0.78	0.71	0.58	0.63
10/28	0.69	0.09	0.10	0.72	0.14	0.20
11/4	0.45	0.29	1.00	0.52	0.29	0.45
11/10	0.52	0.28	0.63	0.39	0.33	0.65
11/17	0.71	0.22	1.00	0.24	0.22	0.71
11/24	0.85	0.39	0.45	0.30	0.35	0.38
12/1	0.78	0.20	0.87	0.14	0.18	0.68
1/19	0.45	0.00	0.00	1.00	0.45	0.00
3/1	0.63	0.00	0.00	0.82	0.64	0.00

*Similarities of beetle assemblages between all possible pairs of fields. Values for each point were calculated with the Ochiai-Barkman association measure, where 0 = no similarity between species assemblages, and 1 = identical species assemblages.

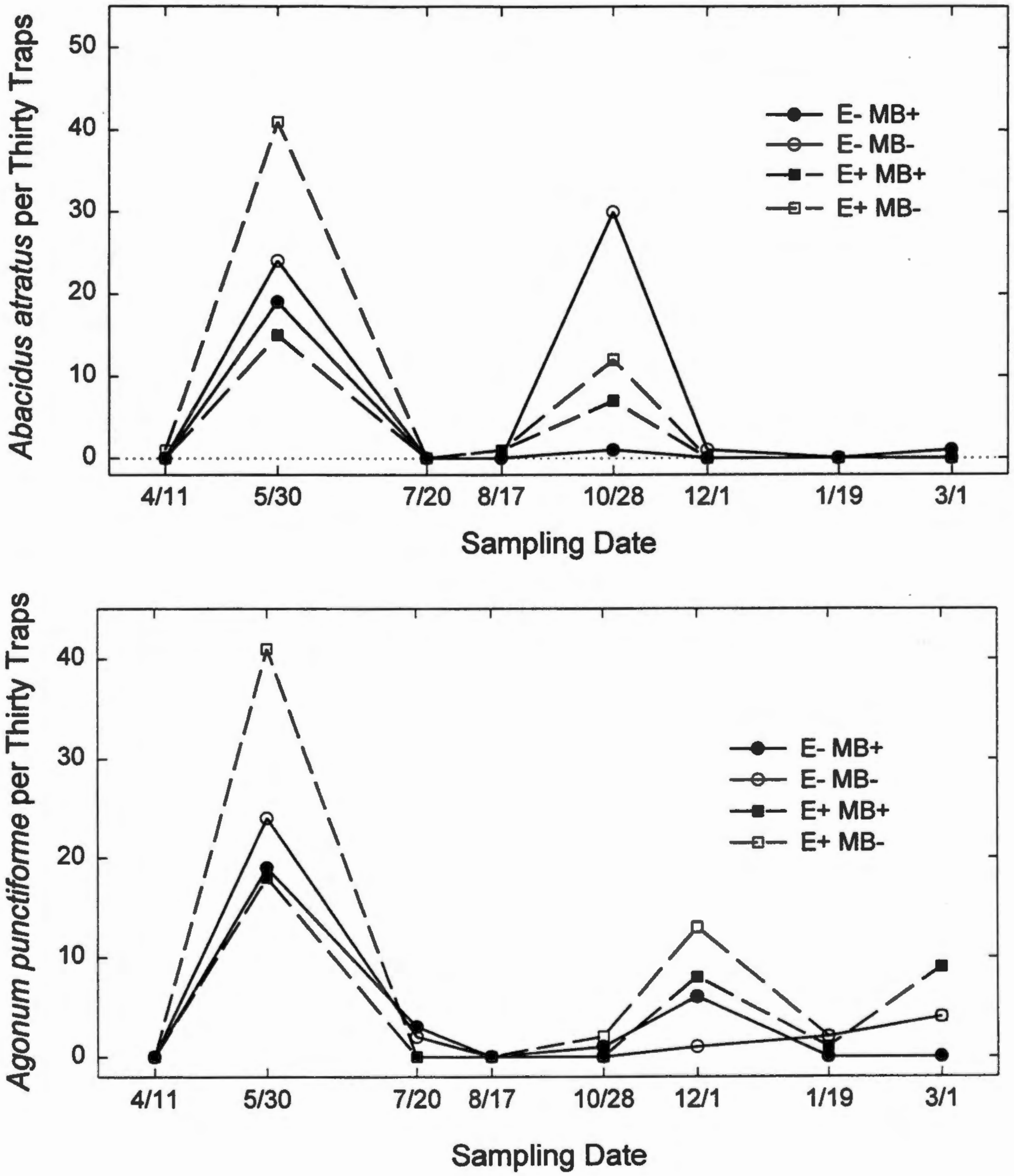

Figure 10. Effects of endophyte infection status and methyl bromide treatment on population dynamics of ground beetles collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- are endophyte-free and E+ are endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. A) *Abacidus atratus* B) *Agonum punctiforme*.

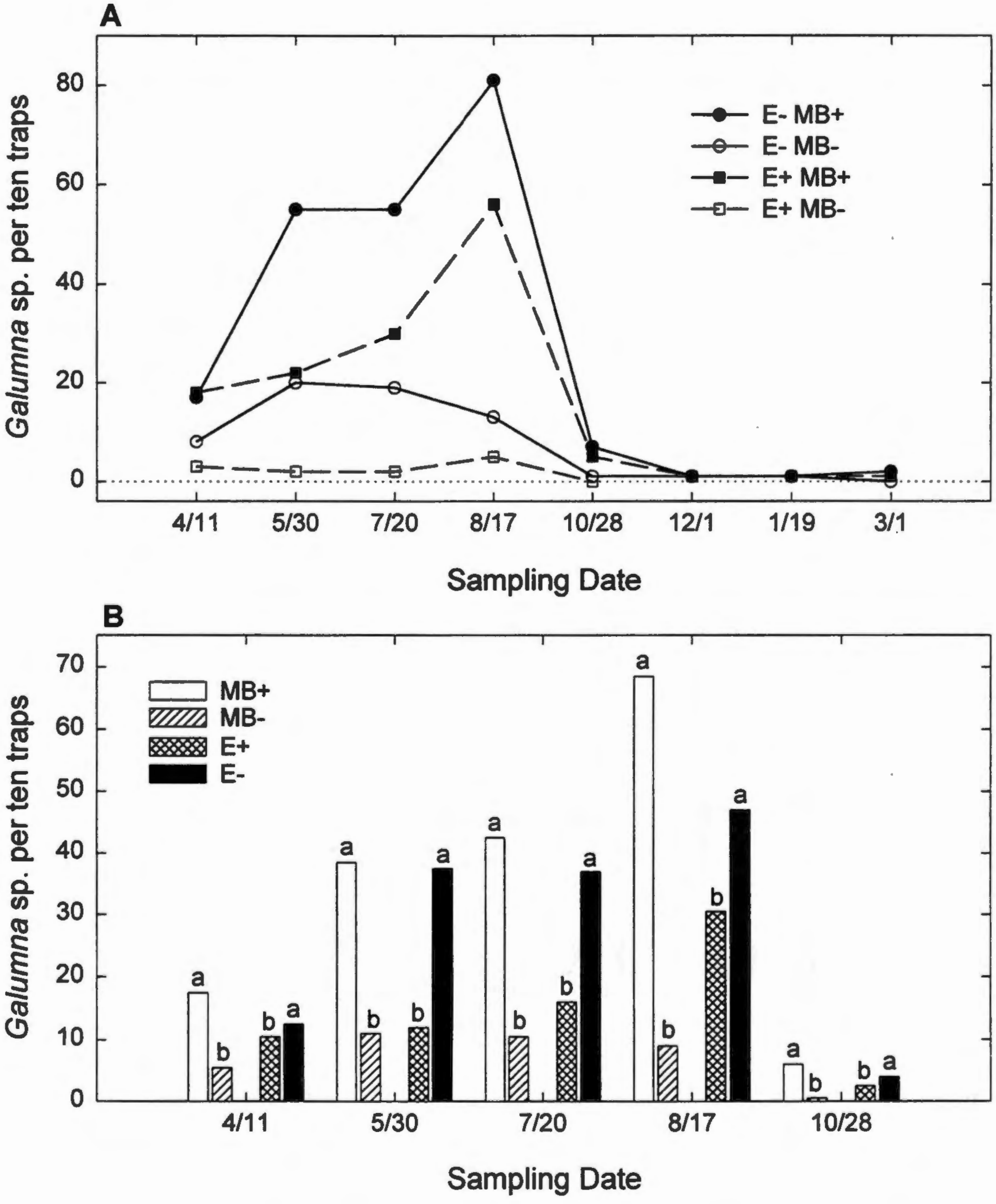

Figure 11. Effects of endophyte infection status and methyl bromide treatment on *Galumna* sp. A) Population dynamics of *Galumna* sp. collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. B) Effects of methyl bromide treatment and endophyte status on *Galumna* populations, as determined with two-way ANOVA and Bonferroni t-test. Only dates with significant differences are plotted. Bars in a pair with different letters are significantly different ($P < 0.05$). Dates with asterisk indicate methyl bromide/endophyte interaction ($P < 0.05$).

collection but the MB- fields were different than each other (Fig. 12A). There were significant interactions between endophyte infection status and methyl bromide treatment on all four dates (Fig. 12B).

Soil Cores

Nineteen species of Collembola were identified from soil core extractions (Table 4). Japygidae and many Acari were also collected from soil cores. *Tullbergia* spp. were the most common springtails extracted with this method. *Isotoma viridis, Folsomia candida* Willem, *P. violenta*, and *Hypogastrura* sp. were other Collembola collected in substantial numbers. The japygid, *Parajapyx isabellae* (Grassi) was often common. Three groups of mites, *Epilohmannia* sp., *Galumna* sp., and other Oribatida, were also differentiated.

Folsomia candida was most numerous in the winter. The largest numbers were collected in MB+ fields. On three out of six dates the E- MB+ field yielded the largest population of *F. candida*. Both E- fields followed the same basic pattern. Populations of *F. candida* were not clearly affected by methyl bromide treatment (Fig. 13).

Hypogastrura sp. was collected infrequently in E+ fields and the E- MB- field, but were higher in the E- MB+ field. *Hypogastrura* sp. was most abundant in October and February (Fig. 14).

Isotoma viridis Bourlet was most prevalent in the October soil core extraction. The E+ MB+ field carried the largest population of *I. viridis*. The lowest population

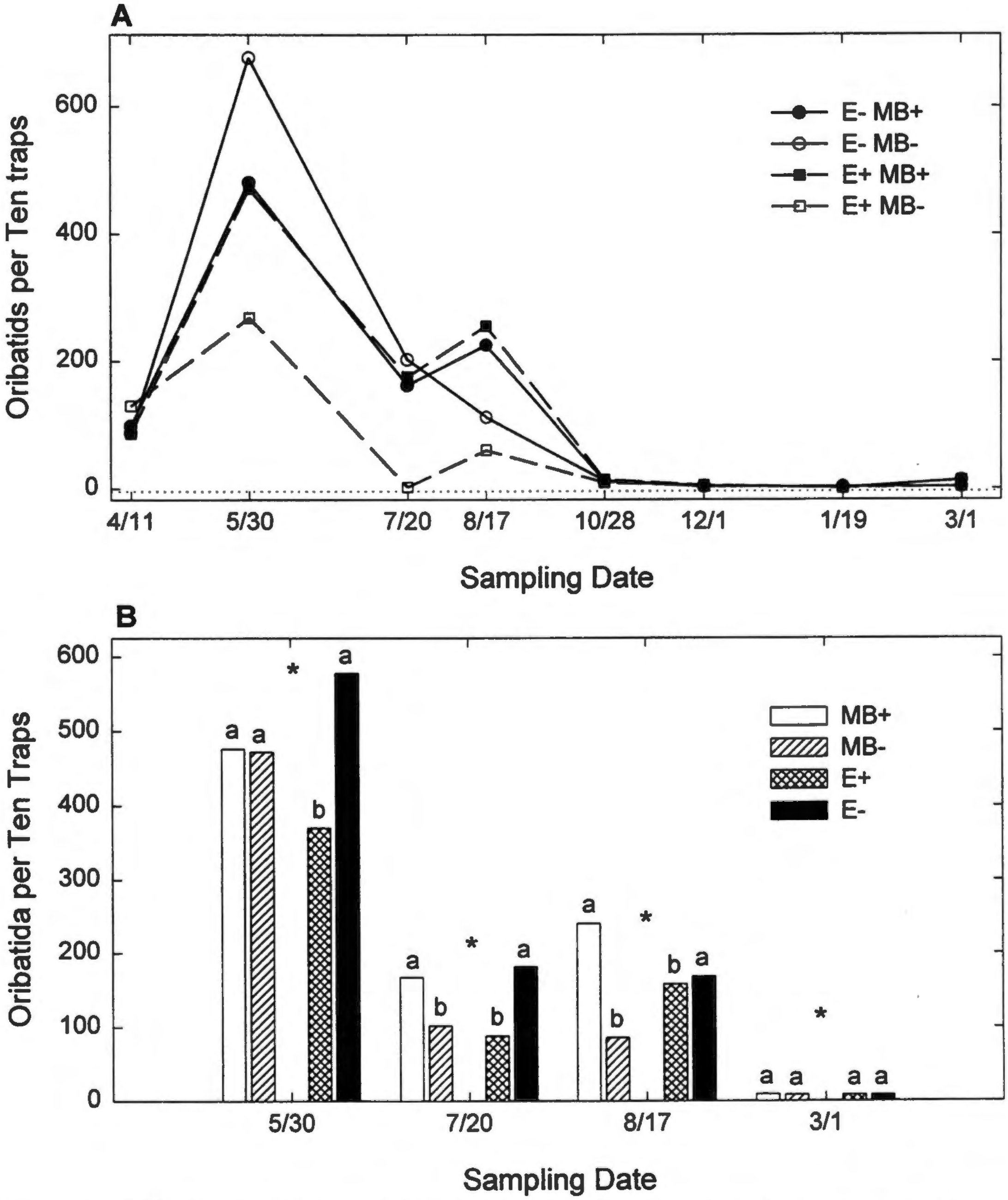

Figure 12. Effects of endophyte infection status and methyl bromide treatment on Oribatida (excluding *Galumna* sp.). A) Population dynamics of Oribatida collected in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide. B) Effects of methyl bromide treatment and endophyte status on Oribatida populations, as determined with two-way ANOVA and Bonferroni t-test. Only dates with significant differences are plotted. Bars in a pair with different letters are significantly different ($P < 0.05$). Dates with asterisk indicate methyl bromide/endophyte interaction ($P < 0.05$).

Table 4. Relative diversity of Collembola collected with soil cores in E+ and E- tall fescue fields treated or not treated with methyl bromide.

	Tall Fescue Field*			
	E- MB+	E- MB-	E+ MB+	E+ MB-
Arrhopalites sp.	1.03	1.90	0.43	0.66
Folsomia candida Willem	25.10	11.68	21.55	1.32
Folsomides americanus Denis	1.14	1.09	3.02	1.32
Folsomides marchicus (Frenzel)	0.38	0	0.43	0
Homidia socia Denis	0.76	2.72	0.86	1.99
Hypogastrura sp.	14.83	0.54	3.02	1.99
Isotoma viridis Bourlet	7.60	4.89	31.47	5.30
Isotomrus bimus Chris. & Bell.	0.38	0.54	0.43	0
Isotomodes sp.	0.38	0	0	0
Lepidocyrtus cinereus Folsom	3.42	0.27	1.29	0.66
Micranurophorus sp.	0	0.27	0	0
Neelidae	0.76	2.17	0.43	0
Orchesella sp.	0	0.27	0	0
Onychiurus sp.	0.38	3.80	0	0.66
Pseudosinella violenta (Folsom)	7.60	5.99	4.31	5.30
Sminthurides malmgreni (Tullberg)	0	0	0.43	0
Sminthurinus henshawi (Folsom)	0.38	0.27	0.43	0
Sminthurus fitchi Folsom	0.38	0	0	0
Sphaeridia pumilis (Krausbauer)	5.32	1.90	3.02	28.48
Tullbergia spp.	29.66	61.68	28.88	31.79

*Relative diversity = individuals of one species within a treatment divided by the total number of specimens in the treatment × 100. Fields designated with E- were endophyte-free, while E+ fields were endophyte-infected. Fields designated by MB+ were fumigated with methyl bromide in 1990; those with MB- were not fumigated.

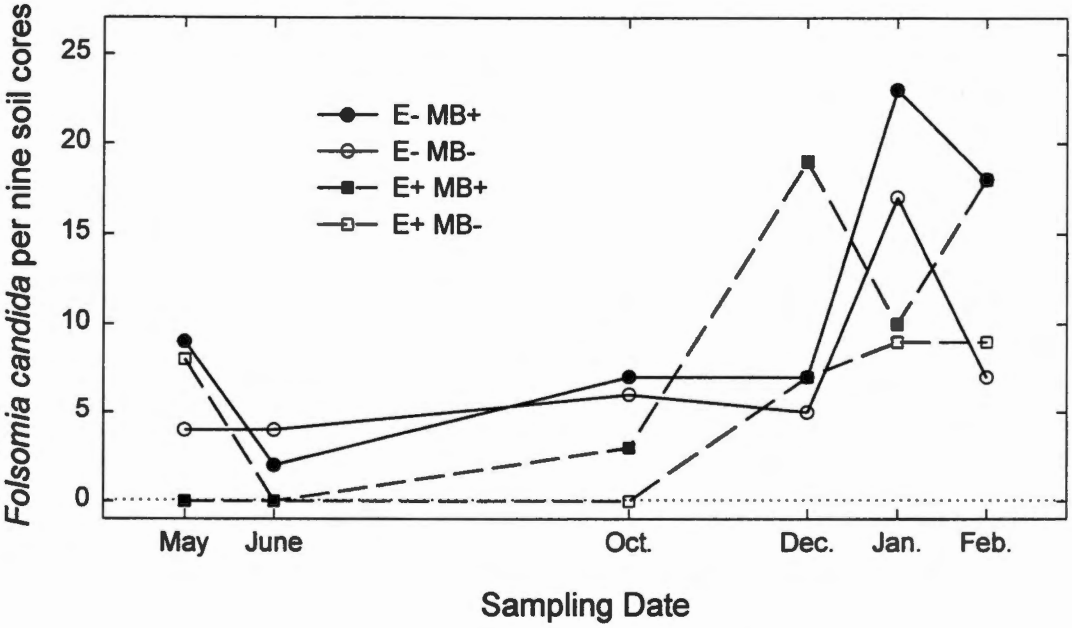

Figure 13. Effects of endophyte infection status and methyl bromide treatment on population dynamics of *Folsomia candida* collected from soil cores in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide.

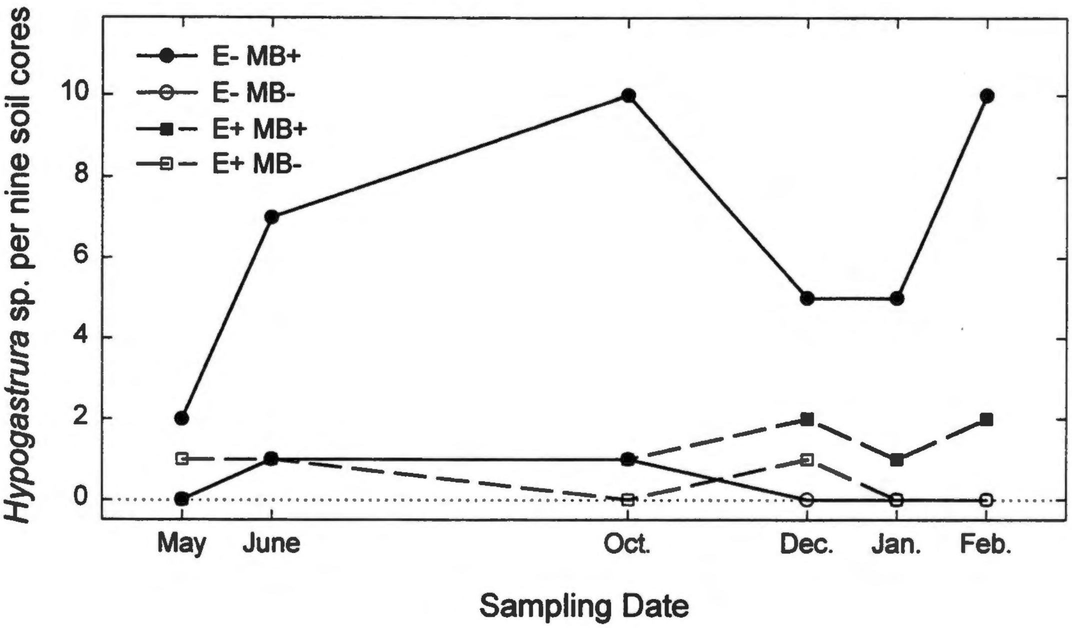

Figure 14. Effects of endophyte infection status and methyl bromide treatment on population dynamics of *Hypogastrura* sp. collected from soil cores in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide.

sampled was obtained from the E+ MB- field. The E- fields followed the same general pattern throughout the collecting period (Fig. 15).

Pseudosinella violenta (Folsom) was found in relatively low numbers throughout the sampling period. The largest numbers were collected in October in the E- MB+ field. Numbers were lowest in the E+ MB- field in October. Numbers of *P. violenta* were higher in E- fields and lower in E+ fields regardless of methyl bromide status (Fig. 16). *Tullbergia* spp. were most prevalent in the E- MB- field and were most abundant in January. *Tullbergia* populations were lower in E+ fields than E- fields and lower in MB+ than MB- fields except in January and February (Fig.17).

Populations of *P. isabellae* were lowest in June and highest in February. MB- fields had much higher populations of *P. isabellae* than MB+ fields. The E- MB- field yielded the highest number of *P. isabellae* (Fig 18).

Galumna sp. was found in greatest numbers in the E- MB- field except in May and January. Populations were lower in E+ fields than E- fields on all dates but December. Populations remained low throughout the sampling period (Fig. 19).

Epilohmanniidae were absent in the E- MB+ field and found only in December, January and February in the E+ MB+ field. Populations were highest in the E- MB- field. (Fig. 20).

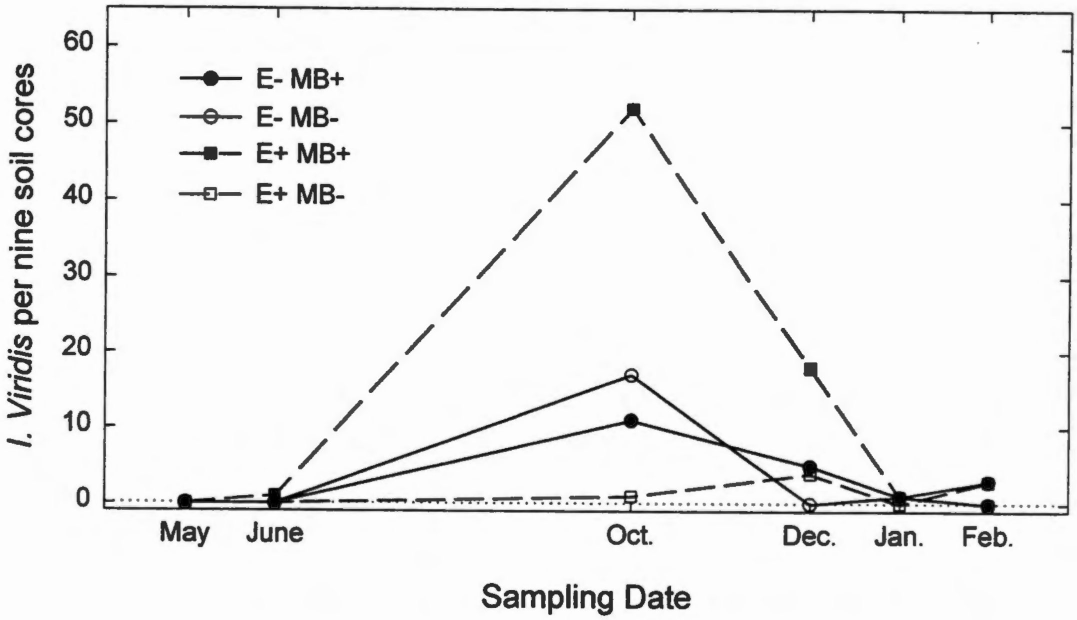

Figure 15. Effects of endophyte infection status and methyl bromide treatment on population dynamics of *Isotoma viridis* collected from soil cores in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide.

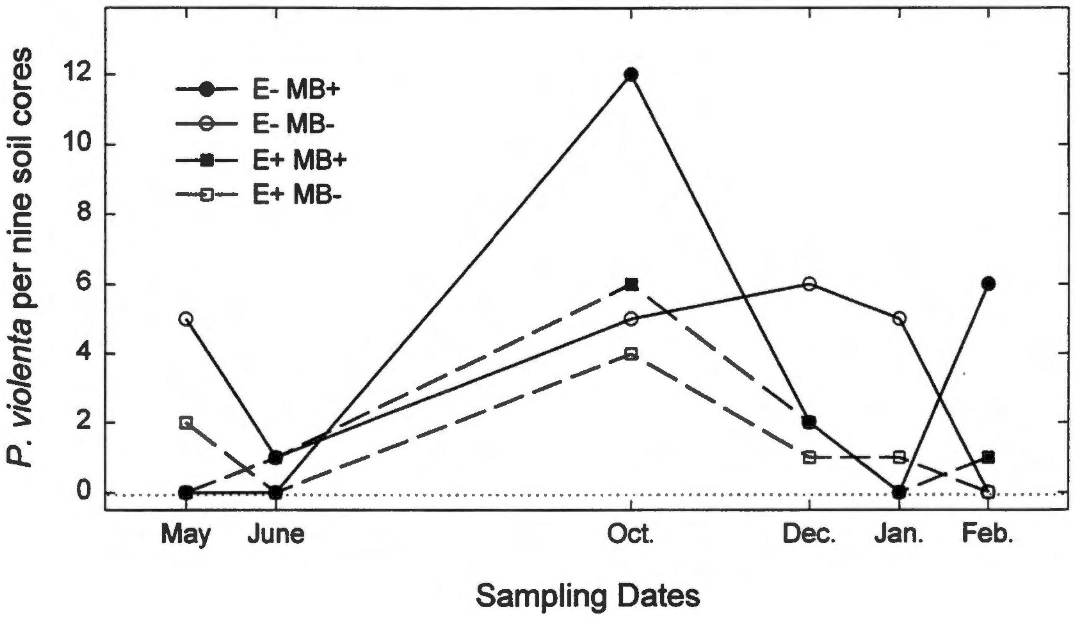

Figure 16. Effects of endophyte infection status and methyl bromide treatment on population dynamics of *Pseudosinella violenta* collected from soil cores in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide.

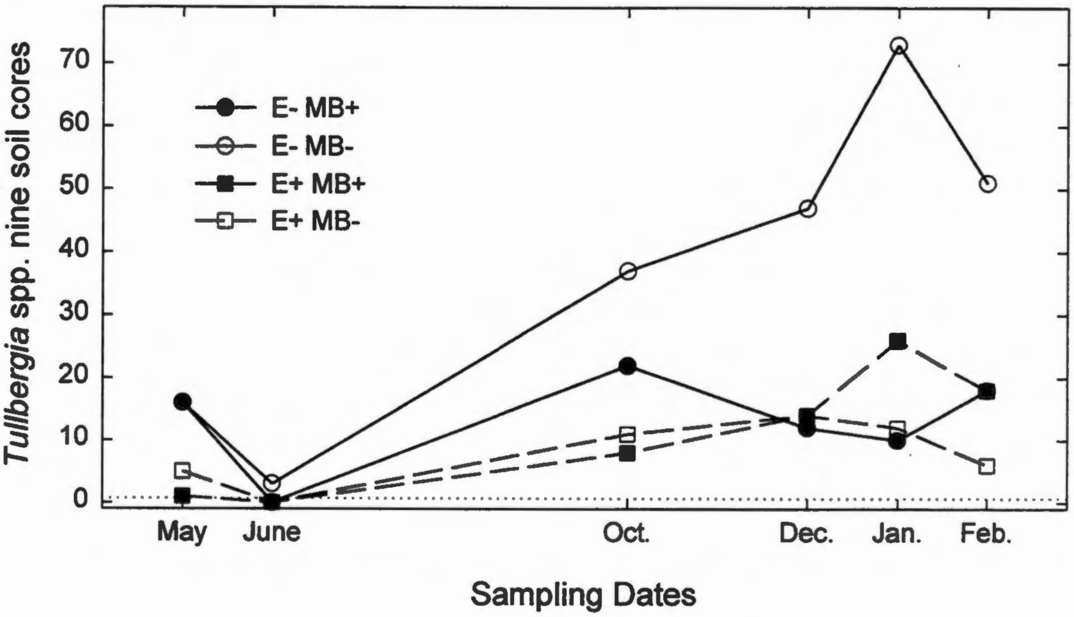

Figure 17. Effects of endophyte infection status and methyl bromide treatment on population dynamics of *Tullbergia* spp. collected from soil cores in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide.

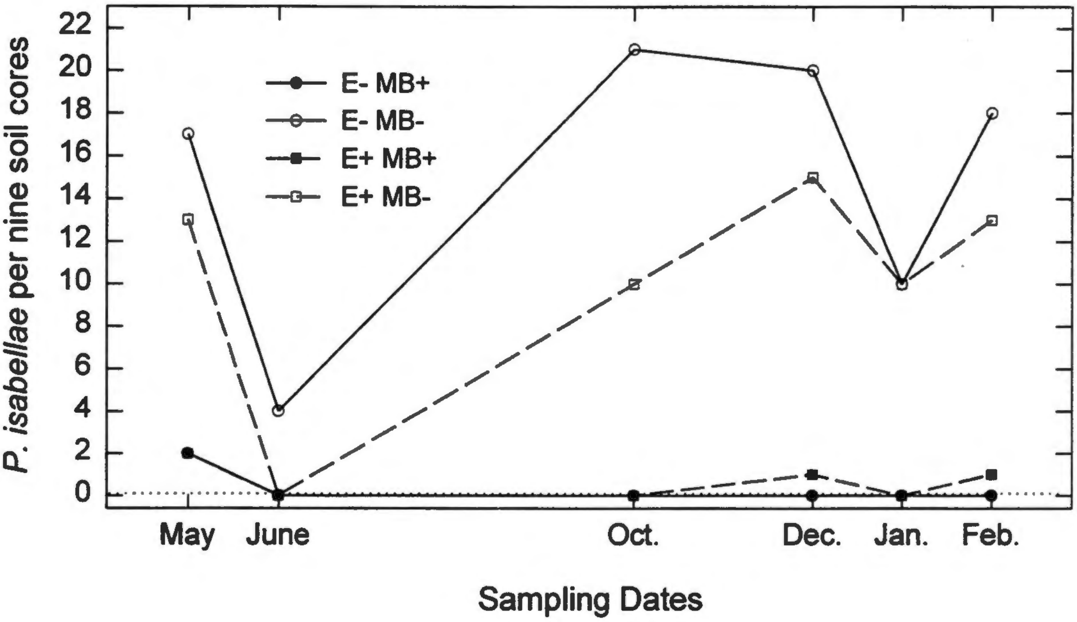

Figure 18. Effects of endophyte infection status and methyl bromide treatment on population dynamics of *Parajapyx isabellae* collected from soil cores in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide.

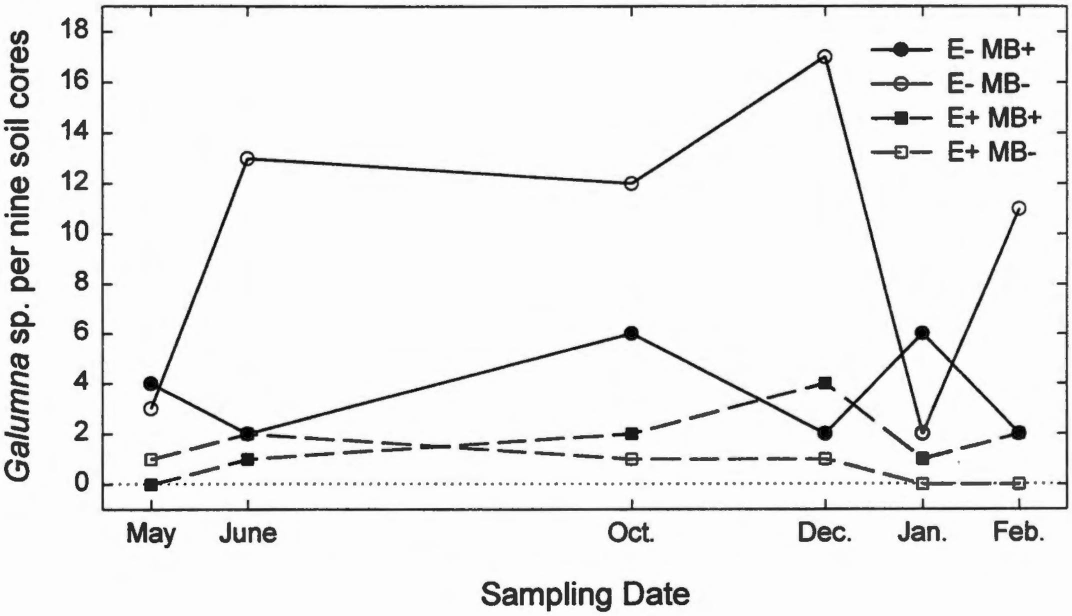

Figure 19. Effects of endophyte infection status and methyl bromide treatment on population dynamics of *Galumna* sp. collected from soil cores in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide.

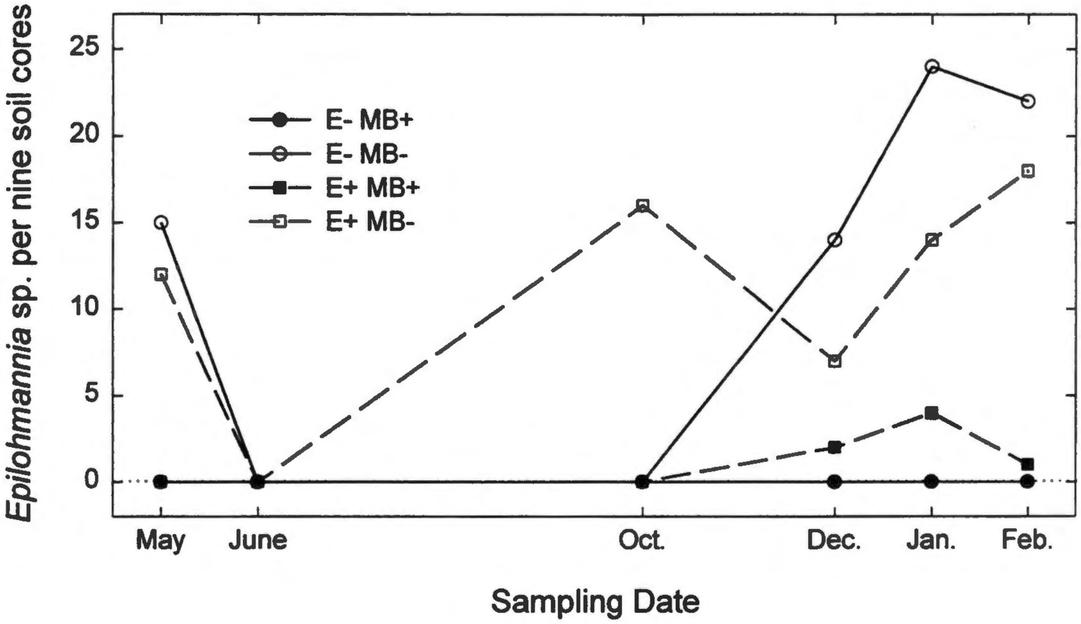

Figure 20. Effects of endophyte infection status and methyl bromide treatment on population dynamics of *Epilohmannia* sp. collected from soil cores in tall fescue fields from April 11, 1995 to March 1, 1996. Fields designated with E- were endophyte-free and E+ were endophyte-infected. Fields designated with MB+ were treated with methyl bromide in 1990. Fields designated MB- were not treated with methyl bromide.

Extraction Efficiency

Extraction of soil cores with the Crossley high-gradient extractor was less efficient than extraction with heptane flotation. Each species counted was extracted with a different efficiency. The Crossley extractor extracted only 3% of *Tullbergia* spp. compared to heptane flotation of a comparable amount of soil. High gradient extraction efficiency of *P. isabellae* was 34% compared to heptane flotation. Extraction efficiencies for other common taxa were between these extremes (Fig. 21).

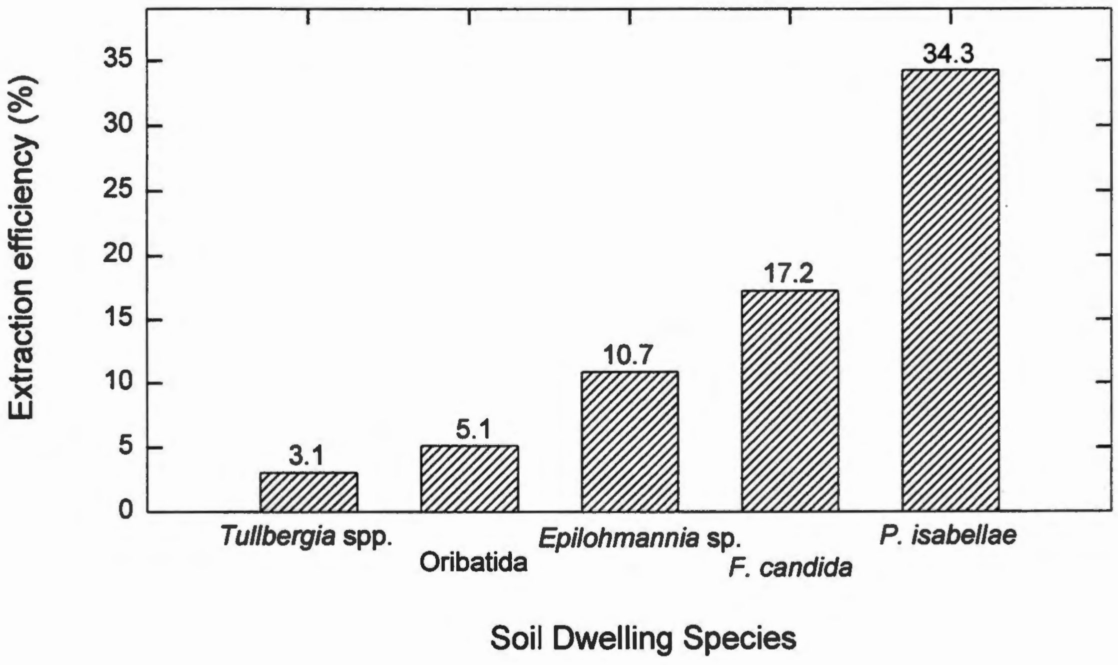

Figure 21. Relative extraction efficiency of the Crossley & Blair high-gradient extractor compared to heptane flotation for organisms in a Sequoia silty clay loam soil.

Chapter 5

Discussion

Collembola may be affected by endophyte-infected grasses in several ways. Herbivorous species of Collembola may directly ingest tall fescue that contains the endophyte. Alkaloids produced by the endophyte could reduce the growth or reproduction of Collembola, as in mammals. Collembolans feeding on fungi or decaying plant material may be affected by residual alkaloids, ultimately changing their demography. However, *F. candida* reproduction was unaffected by endophyte-impregnated diets (Bernard et al. 1990). Population changes in Collembola in the current study appear to be species-specific and not related to changes in Collembola as a whole. Reductions in Collembola densities may reduce available food for their predators and parasites, thus reducing their populations. Conversely, increases in Collembola numbers may increase predator and parasite numbers. Predator and parasite fluctuations can cause instability in overall community structure as well as in individual species.

Endophyte status may play an important role in the number of Collembola collected throughout the year. *Sphaeridia pumilis, S. fitchi, S. henshawi,* and *I. viridis* populations were usually significantly higher in E+ fields. *Homidia socia* and *P. violenta* were more abundant in E- fields. There was no difference in the population sizes of *L. cienereus* in E+ and E- fields. When densities were very low, some species higher in E+ seemed to be reversed, and were higher in E-. This may be due to environmental conditions such as time of year, water stress, and temperature. In addition, reduction in the number of collected specimens tends to make the results less reliable. Populations found in the same relative abundance in both fields may not be affected by the endophyte directly and may feed on organisms also unaffected by the endophyte.

Homidia socia was more abundant in E+ fields on two sampling dates and more abundant in E- fields on two other sampling dates. These contradictory results suggest that endophyte does not play a role in the regulation of *H. socia* populations. *Homidia socia* primarily feeds on fungal spores and hyphae (Bernard, Silas-Parman, and Williver unpublished). These foods probably did not contain compounds harmful to *H. socia. Lepidocyrtus cinereus* reacted similarly.

In contrast to *H. socia* and *L. cinereus*, *S. elegans* was found only in fields containing the endophyte. Little is known of the ecology of *S. elegans.* A reduction in parasites or predators due to the presence of endophyte could enhance *S. elegans* survival, but it seems unlikely to have caused total elimination from E- fields.

Association measures for Collembola for E+ MB-/E+ MB+ comparisons were above 0.7 from April to October, remaining high in summer while measures for other comparisons declined. Endophyte presence appears to select a particular community composition. Similarity indices were uniformly lower in the winter, likely due to reduction in the numbers of specimens collected in winter or to reduction of endophyte influence. The E- MB-/E- MB+ similarities of Collembola associations were usually much lower than the E+ MB-/E+ MB+ similarities, and differed little from the other comparisons.

Although methyl bromide fumigation was performed in 1990, some of its effects were still apparent on individual species. Effects of methyl bromide may be due to initial population reduction and slow recolonization rate, both of Collembola and their predators and parasites, as well as a reduction in food sources. *Isotoma viridis, S. henshawi, S. fitchi, S. elegans* and *S. pumilis* were most abundant in MB+ fields. Reproduction of these species may outstrip that of more slowly colonizing natural enemies. Equilibrium levels may not be reached for many years.

Lepidocyrtus cinereus and *P. violenta* were most prevalent in MB- fields. These species may have been suppressed by methyl bromide. These two species may be good survivors in well-established communities, but poor colonizers. In contrast, there was no difference in *H. socia* numbers in MB+ and MB- fields. *Homidia socia* likely is a strong colonizer of new tall fescue habitats.

Association measures of Collembola assemblages comparing the endophyte infection status within MB+ and MB- fields were low, suggesting that methyl bromide status dose not play a major role in Collembola community structure in these tall fescue fields. *H. socia* was the most dominant species in all four fields, and was not affected by methyl bromide, thus the association measure may have been biased toward finding no differences.

There is little known about the ecology of most of the Collembola collected from the sampled fields. The population dynamics of only two of these species have been previously studied. In this study *I. viridis* was collected most abundantly in winter and early spring in pitfall traps. In soil cores *I. viridis* were found in greatest numbers in

October. *Isotoma viridis* apparently has one generation per year, with its early juvenile stages in the soil and later juveniles and adults living on the surface in winter and spring. These results vary from those of Morris (1920), who found *I. viridis* in the soil throughout the year in Cheshire, UK. Another species in this study, *S. pumilis,* was most numerous in March, July, and October. Blancquaert et al. (1982) observed similar peaks in Belgium in July and October, but not March. The Belgian populations were low in late winter. In both studies *S. pumilis* numbers dropped sharply in August, perhaps due to low rainfall in both places.

The most abundant species of Collembola collected in pitfall traps (*H. socia*) has not previously been reported in Tennessee. *Homidia socia* is also common in tall fescue lawn turf in Knox County (Bernard, personal communication). This species was originally described in Japan, and appears to have colonized in the United States only in the past 25 years (Christiansen & Bellinger 1980).

The Carabid fauna of these fields was remarkably depauperate, consisting of only seven species. Hylton et al. (1985) reported 58 species of ground beetles in pasture and hay fields in Tennessee. Morrill (1992) found 18 species in tall fescue and bermudagrass in Georgia. Carabid beetles in general were not affected by the presence or absence of the endophyte. Although some species of carabids, such as *Harpalus pensylvanicus,* are herbivores (Webster 1900), the majority are predators and therefore would only be affected secondarily by the endophyte-infected tall fesuce. Beetle catches were rather low in all fields. Trap catches are a function of both population density and activity (Morrill et al. 1990). Lowered activity may have resulted in the low numbers of beetles captured in the pitfall traps and, therefore, effects of the endophyte may not have been discernible. However, there is no evidence from our study to indicate lower activity compared to the results of Hylton et al. (1985) or Morrill (1992).

Carabid beetle populations were lower in MB+ fields than in MB- fields, an effect that would be expected shortly after fumigation. Predator populations usually recover more slowly because they require many prey for growth and have a longer reproductive cycle. Without adequate food sources, populations of predators would not rise. Predator populations thus reach equilibrium some time after prey returns to sufficient abundance. Results of this study indicate that Carabid species composition and number probably have not yet stabilized in MB+ fields.

Abacidus atratus had two generations, one peaking in late May and the other in late October. Hylton et al. (1985) also observed a population peak in October, but he made no collections in May. Methyl bromide treatment probably eliminated beetles from treated fields, since few were found in MB+ fields five years later. *Abacidus atratus* appears to be a slow recolonizer.

Agonum punctiforme populations peaked in late May and early December. Populations at their peaks were most numerous in MB- fields, suggesting that *A. punctiforme* may recolonize treated fields slowly. Reddick & Mills (1994) reported *A. punctiforme* as attaching codling moth larvae in laboratory studies, suggesting that they are predatory. However, Johnson & Cameron (1969) found that 11 species in the genus *Agonum* were grass seed and foliage consumers. *Agonum punctiforme* may be an omnivore, eating whatever is available. There may be a drop in the amount of food available to seed-eating beetles because seed is harvested from these fields. Therefore, population densities of smaller prey organisms could be a factor in regulating population densities of omnivorous beetles.

Populations of Oribatid mites were larger in E- fields than E+ fields. In general, Oribatida feed on fungi and decaying plant material. Lower populations of Oribatida in E+ fields would suggest that they are either deterred by the endophyte or that consumption of dead endophyte-infected tall fesuce tissue is toxic to these mites.

Different groups of Oribatid mites were affected differently by the application of methyl bromide. *Galumna* sp. and other Oribatida populations were much higher in MB+ fields, except for *Epilohmannia* sp., which were found only in soil cores and were almost absent in MB+ fields. Larger numbers of truly edaphic *Epilohmannia* in MB- fields suggest that this taxon is slow to disperse and requires more than five years to return to former habitats.

Populations of *Galumna* sp. were much higher in MB+ fields, suggesting that either they were not affected by methyl bromide fumigation, or they were quick to recolonize treated areas. Populations also were higher in E- fields, suggesting that they are affected by the endophyte presence either directly or indirectly. *Galumna* sp. appeared to have one generation during the sampling period. Populations of *Galumna* were collected throughout the sampling period in both pitfall traps and soil cores. They may migrate vertically in the soil or spend only part of their life cycle in the soil.

Pitfall traps were collected twice weekly for at least six weeks during each season. Rain and snow often made it difficult to collect all pitfall traps on a given date, therefore sampling dates were not always evenly spaced in time. The highest or lowest populations may not have been shown on the dates they actually occurred because of the time between each analyzed sampling date. Additional analysis of collected samples may allow for more detailed analysis of population dynamics of each species.

Soil cores were not collected in summer because the soil was too dry and hard. Populations of soil arthropods are usually higher in winter than in summer (Thompson 1924); thus, the absence of summer soil core samples may not have skewed the results. Species collected solely in soil cores, e.g. *Tullbergia* spp., *P. isabellae,* and *Epilohmannia* sp., seemed to be more affected by methyl bromide treatment than organisms living in both soil and surface habitats.

Folsomia candida was found in MB+ fields more frequently than in MB- fields. *Folsomia candida* exhibits high tolerances to many agricultural chemicals, such as DDT (Butcher & Snider 1975) and may have high tolerance for other toxins, as well.

Parajaypx isabellae (Japygidae) was found almost solely in MB- fields, suggesting that *P. isabellae* was suppressed by the methyl bromide treatment. Populations also were higher in E- fields than in E+ fields, suggesting that the presence of the endophyte also may depress populations of *P. isabellae.* This japygid is associated with soil surrounding wheat, barley, peach trees and pine-oak forests (Swenk 1903). It is reported to feed on the root cortex of plants (Zimmerman 1948).

Regardless of treatment, populations of each species in each field peaked at the same times, even though their densities often varied. Overall, community structure in each of the four combinations of treatments varied only because of changes in the numbers of each species.

Collembola populations peaking in March usually were found in greatest numbers in the E+ MB+ field. Endophyte-infected tall fescue begins to grow early in the season and E+ plants produce more vegetation than their E- counterparts (Clay 1987). The effects of increased plant biomass could include reduced solifluction, less extreme fluctuations in moisture and humidity, and increased substrate for fungi. However, population dynamics of each species differed and therefore may have been affected differently at different times by the presence of the endophyte. Alkaloid production and concentrations within plant tissue vary seasonally. In two-year studies, ergovaline peaked in May (Gwinn unpublished) or in June (Rottinghaus et al. 1991). In a one-year study, ergopeptine alkaloids peaked in October (Belesky et al. 1988). In a study in which loline concentrations were monitored for three years, highest concentrations were drastically different in time and intensity (Belesky et al. 1987). Collembola species that normally peak at the same time as a bioactive alkaloid likely will change more than an equally susceptible species peaking when alkaloid concentrations are low. Continuation of this study for another season might show population peaks at different times. Monitoring of alkaloids in fields during collections could answer the question of which alkaloids are possibly involved in soil arthropod population modifications.

The Crossley-Blair high-gradient soil core extractor was less efficient than expected when compared with heptane flotation. This method was originally used to extract microarthropods from forest soils (Crossley & Blair 1991). Forest soils used by

Crossley and Blair were very friable and had a high organic matter content. Soil samples in this study had low organic matter content and low friability. Therefore the extraction efficiency was much lower and varied from species to species. The high-gradient extractor probably is not suitable for quantitative estimates in mineral soils, but may still be usable where proportional results are acceptable. Other means of extraction, such as high-density flotation solutions, should be tried.

Chapter 6

Summary and Conclusions

Endophyte-mediated resistance effects on non-herbivorous arthropods have not previously been studied in tall fescue. The effects of endophyte infection of tall fescue on Collembola appears to be species-specific. Carabids were not affected by the presence of the endophyte in a field, but they had low densities in all tall fescue fields. Populations of Acari were consistently lower in E+ tall fescue fields. Fumigation with methyl bromide depressed populations of soil-dwelling Collembola and Acari for several years. Arthropods living partially in soil and partially on the surface were more abundant in MB+ fields than those that complete their entire life cycle in the soil. Peak population densities of surface-dwelling Collembola varied among species. Populations that peaked in the summer or winter were less affected by the endophyte. Monitoring of decomposer and predator populations along with determination of alkaloid concentrations should give a better understanding of the role of endophyte-induced alkaloids in the population dynamics of arthropods in E+ tall fescue fields.

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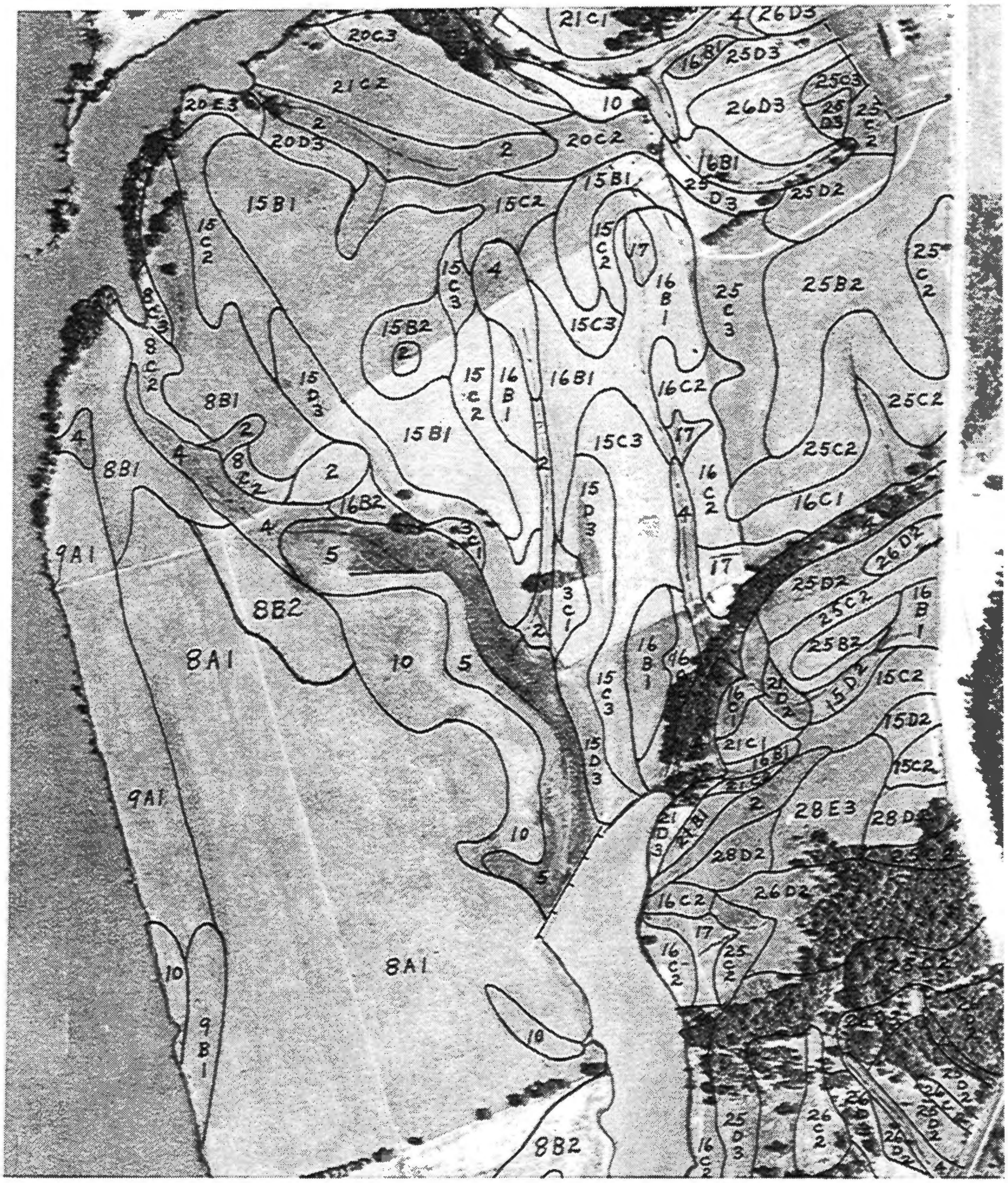
Appendix



0 400 800 1200 Feet

Scale

Soil Map, Plant Science Farm, University of Tennessee, Knoxville, Tennessee. Mapped by J. A. Elder, SCS, and M. E. Springer, 1963. Fields used in this study are outlined on the overlay.

Symbol	Soil Name
2	Huntington loam
3C1	Huntington clay loam, 5-8% slopes
4	Lindside loam
5	Melvin silt loam
8A1	Sequatchie loam, 0-2% slopes
8B1	Sequatchie loam, 2-5% slopes
8B2	Sequatchie loam, 2-5% slopes, eroded
8C1	Sequatchie loam, 5-12% slopes
8C2	Sequatchie loam, 5-12% slopes, eroded
8C3	Sequatchie loam, 5-12% slopes, severely eroded
9A1	Sequatchie fine sandy loam, 0-2% slopes
9B1	Sequatchie fine sandy loam, 2-5% slopes
10	Whitwell loam
15B1	Etowah silt loam, 2-5% slopes
15B2	Etowah silt loam, 2-5% slopes, eroded
15C2	Etowah silt loam, 5-12% slopes, eroded
15C3	Etowah clay loam, 5-12% slopes, severely eroded
15D2	Etowah silt loam, 12-20% slopes, eroded
15D3	Etowah clay loam, 12-20% slopes, severely eroded
16B1	Captina silt loam, 2-5% slopes
16B2	Captina silt loam, 2-5% slopes, eroded
16C1	Captina silt loam, 5-12% slopes
16C2	Captina silt loam, 5-12% slopes, eroded
17	Taft silt loam
20C2	Waynesboro loam, 5-12%, eroded
20C3	Waynesboro clay loam, 5-12%, severely eroded
20D3	Waynesboro clay loam, 12-20%, severely eroded
20E3	Waynesboro clay loam, 20-30%, severely eroded
21B1	Holston silt loam, 2-5% slopes
21C1	Holston silt loam, 5-12% slopes
21C2	Holston silt loam, 5-12% slopes, eroded
21D2	Holston silt loam, 12-20% slopes, eroded
21D3	Holston silt loam, 12-20% slopes, severely eroded
25B2	Sequoia silt loam, 2-5% slopes
25C2	Sequoia silt loam, 5-12% eroded
25C3	Sequoia silty clay loam, 5-12% slopes, severely eroded
25D2	Sequoia silty clay laom, 12-20% slopes, eroded
25D3	Sequoia silty clay laom, 12-20% slopes, severely eroded
26C2	Dandrige silt loam, 5-12% slopes, eroded
26D2	Dandrige silt loam, 12-20% slopes, eroded
26D3	Dandridge shaly silty clay loam, 12-20% slopes, severely eroded
28D2	Tellico loam, 12-20% slopes, eroded
28E3	Tellico clay loam, 20-30% slopes, severely eroded

Map Symbols

Soil
Slope
Erosion
28 B 2

Slope
A - level- Nearly level - 0-2%
B - Gently sloping - 2-5%
C - Sloping- 5-12%
D - Moderately steep - 12-20%
E - Steep - 20-30%

Erosion
1 - Uneroded to slightly eroded
2 - Moderatly eroded
3 - Severely eroded

Vita

Cindy Lynn Williver was born in Dover, New Jersey, on February 1, 1972. She was graduated from Roxbury High School in Succasunna, NJ in 1990. She received a Bachelor of Science degree in Biology from East Carolina University, Greenville, NC, in May 1994. She entered the Department of Entomology and Plant Pathology at The University of Tennessee, Knoxville, in fall 1994 as a Graduate Research Assistant, and graduated with a Master of Science degree in Entomology and Plant Pathology in August 1996.

Cindy Williver is a member of Phi Sigma Pi National Fraternity, Tri Beta National Biological Honor Society, the Tennessee Entomological Society, and the Entomological Society of America.