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SEASONAL INCIDENCE AND LABORATORY REARING OF SELECTED CHLOROPID FLIES OCCURRING IN TALL FESCUE IN TENNESSEE

A Thesis

Presented for the

Master of Science

Degree

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James T. Vogt

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ABSTRACT

A two-year study was undertaken to determine seasonal population densities and emergence of chloropid flies (Diptera: Chloropidae) in endophyte-infected (E+) and non-infected (E-) tall fescue in Robertson Co., TN., and aspects of the biology of Rhopalopterum carbonarium, a common chloropid, were investigated. A total of 22 chloropid species or variants were collected in sweep samples and/or emergence traps during 1991-1992.

Rhopalopterum carbonarium (Loew) was the most abundant chloropid collected in both years. Population densities of this and ten other species, were significantly lower in E+ plots than E- plots on several sampling dates; however, on no sampling dates were significantly more specimens collected from E+ plots than E- plots. Fewer chloropids emerged from E+ plots than from E- plots.

Three known pest species of chloropids were collected during this study. Two plant feeders, Oscinella frit (Loew), the frit fly, and Meromyza americana Fitch, the wheat stem maggot, were collected in significantly fewer numbers in sweep samples from E+ plots than from E- plots on at least one occasion. Significantly fewer Liohippelates pallipes (Loew), one of the "eye gnats," emerged from E+ fescue clippings than from E- clippings.

A colony of R. carbonarium was maintained in a growth chamber in 1991. R. carbonarium developed from egg to adult on dead E+ and E- fescue tissues and crabgrass.

These studies revealed that population densities of chloropid flies with different larval habits are negatively affected by the presence of Acremonium coenophialum. Additional studies are needed to determine the possible effects of planting E- pastures on the pest status of chloropids occurring in tall fescue.

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1. INTRODUCTION

Fungal endophytes of grasses (family Clavicipitaceae) have recently been implicated in insect resistance (Funk et al. 1983, Clay et al. 1985), and in toxicity to livestock (Bacon et al. 1986). Tall fescue, Festuca arundinacea Schreb., is infected with such an endophyte, Acremonium coenophialum Morgan-Jones and Gams. Tall fescue is an important forage crop in Tennessee, where ca. 1.4 million ha are grown each year (Long & Hilty 1985). About 75 to 90% of tall fescue is infected with the endophyte. The costs of fescue toxicosis in Tennessee are estimated to be approximately $85 million per year (Fribourg et al. 1991). For a review of current knowledge in Tennessee, see Tennessee Farm and Home Science 160 (Fall 1991).

Several insect species have been studied with regard to feeding behavior and survival on Acremonium-infected grasses (reviewed in Siegel et al. 1987). However, naturally occurring dipterans in tall fescue have, for the most part, been ignored in this research area. Lewis and Clements (1986) found no relationship between frit fly, Oscinella frit (Loew) (Chloropidae) infestation and endophyte infection in perennial ryegrass. Cole (1991) developed a bioassay for toxic factors in endophyte-infected tall fescue using the common laboratory fruit fly, Drosophila melanogaster Meigen (Drosophilidae).

Chloropid species have diverse habits and can be very abundant in grasses and low vegetation (Sabrosky 1935). This research was undertaken to investigate the effects of A. coenophialum on members of the family Chloropidae in Robertson Co., Tennessee by comparing seasonal emergence and population densities of selected species in Acremonium-infected and non-infected tall fescue. The biology of a common species, Rhopalopterum carbonarium (Loew), was also investigated.

2. LITERATURE REVIEW

Tall Fescue

Festuca arundinacea Schreb., tall fescue, is a cool-season perennial grass (Graminae) that is grown on an estimated 12-14 million ha in the United States (Siegel et al. 1987). Tall fescue was introduced from Europe (Cowan 1956) and discovered growing on a farm in Menifee Co., Kentucky, in 1931. This grass rapidly met with acceptance by farmers after it was released as 'Kentucky 31' (K-31) by the University of Kentucky in 1943 (Buckner et al. 1979). The new cultivar was highly adaptable and resistant to environmental stresses, i.e., drought and pest damage (Buckner et al. 1979).

Tall fescue is widely distributed and is grown primarily in the southeastern United States. It is used for erosion control, in recreational areas, and as forage for livestock. More than 1,420,000 ha of tall fescue are grown in Tennessee for livestock forage (Long & Hilty, 1985).

Fescue Toxicosis

As K-31 tall fescue gained popularity, cattle grazing on the cultivar began to show signs of illness. Sloughing of the extremities, particularly the feet (a condition commonly known as "fescue foot"), lowered milk production and reproductive rates, elevated body temperatures and

lowered weight gain all can occur in livestock feeding on tall fescue (Bush & Buckner 1973). These symptoms occur primarily in the summer months (Siegel et al. 1987). Several colloquialisms have been used to describe the diseased condition of cattle feeding on tall fescue; fescue toxicosis is the current accepted term. Losses due to this condition are estimated to be ca. 600 million dollars annually in the U.S. and ca. 85 million dollars annually in Tennessee (Fribourg et al. 1991).

The Fescue Endophyte

Siegel et al. (1987) and Clay (1989) have provided reviews of grass endophytes. Bacon et al. (1977) found an association between fescue toxicosis and an endophytic fungus, Epichloe typhina (Pers.) Tul., which lives within the fescue plant. The fungus was reclassified as Acremonium coenophialum Morgan-Jones and Gams (Clavicipitaceae) (Morgan-Jones & Gams, 1982). Tall fescue with A. coenophialum is referred to as "endophyte-infected" (E+) while tall fescue without the fungus is referred to as "non-infected" (E-).

A. coenophialum is seed-borne only in the seeds of E+ fescue plants. Approximately 8 to 10 days after germination, hyphae infect the seedling leaves. Hyphae invade the seeds during seed head formation (Hinton & Bacon 1985).

The Endophyte-Fescue Relationship

The relationship between tall fescue and A. coenophialum is mutualistic (Arachevaleta et al. 1989). The plant provides shelter, nutrition and a means of dissemination to the fungus (Siegel et al. 1987). Infected plants exhibit increased resistance to drought and herbivory (reviewed by Seigel et al. 1987). Density of Acremonium hyphae in tall fescue plants is influenced by temperature, and density increases with higher temperature (Breen 1992). Plants infected by Acremonium exhibit enhanced growth (Clay 1987, Arachelaveta et al. 1989, Hill et al. 1990). In the field, E+ plants have greater tiller survival and growth than E- plants (Read & Camp 1986). Thus, infected plants are at an advantage and can eventually displace non-infected plants in a stand (Clay et al. 1985, Bacon et al. 1986).

Alkaloids

Several alkaloids produced in E+ tall fescue are absent in E- tall fescue. These alkaloids may be the cause of fescue toxicosis and increased herbivore resistance (Seigel et al. 1987, Clay & Cheplick 1987). Ergovaline (EV) is the primary ergopeptine alkaloid in E+ plants (Lyons et al. 1986). N-formylloline (NFL) and N-acetylloline (NAL) are also found in substantial amounts in infected plants (Siegel et al. 1987).

Alkaloid concentrations in E+ tall fescue can vary according to seasonal and environmental conditions. Peaks in EV concentration in E+ plants have been observed in spring and autumn (Belesky et al. 1988, Rottinghaus et al. 1991, Breen 1992). Water stress and high temperatures greatly increase concentrations of NFL and NAL in a Lolium (ryegrass) X Festuca hybrid (Kennedy & Bush 1983). Loline alkaloid concentrations decrease with leaf age (Hardy et al. 1986, Eichenseer et al. 1991).

Herbivore Resistance

Poor livestock performance on E+ tall fescue has been attributed to grazing deterrence (Schmidt et al. 1982) as well as toxic substances (Stuedemann & Hoveland 1988). E+ fescue is also detrimental to some nematodes (West et al. 1988, Kimmons et al. 1990) and can enhance resistance of infected fescue plants to plant pathogenic fungi (Blank et al. 1991).

In the early 1980's enhanced insect resistance was associated with the clavicipitaceous endophyte ,Acremonium loliae Latch, Christensen and Samuels in perennial ryegrass (Prestidge et al. 1982). Listronotus bonariensis Kuschel (Argentine stem weevil) population densities are lower in E+ perennial ryegrass than E- ryegrass [reviewed by Pottinger et al. (1985)]. Other insects negatively affected by A. loliae include sod webworm, Crambus spp. (Funk et al. 1983),

fall armyworm, Spodoptera frugiperda (J.E. Smith) (Hardy et al. 1985), and hairy chinch bug, Blissus leucopterus hirtus Montandon (Mathias et al. 1990).

Resistance to insect herbivory by E+ tall fescue is well-documented. Negative correlations existed between Acremonium infestation and abundance of three leafhopper species, as well as corn flea beetle, Chaetocnema pulicaria Melsheimer, in Missouri (Kirfman et al. 1986). Other insects exhibiting feeding deterrence, delayed development or mortality when fed E+ plants or alkaloids include the bird cherry-oat aphid, Rhopalosiphum padi (L.) (Latch et al. 1985, Eichenseer et al. 1991), the Argentine stem weevil (Prestidge et al. 1985), the large milkweed bug, Oncopeltus fasciatus (Dallas), and greenbug, Schizaphis graminum (Rondani) (Johnson et al. 1985), the Japanese beetle, Popillia japonica Newman (Oliver et al. 1990), the fall armyworm (Clay & Cheplick 1989), and the Russian wheat aphid, Diuraphis noxia (Mordvilko) (Clement et al. 1990). The mode of action of the complex array of alkaloids present in E+ fescue is not fully understood, but insects may be affected by reduced growth, deterrent properties or antibiosis (Clay 1989).

Very little is known about the effects of clavicipitaceous endophytes on dipteran species. Published field studies have generally disregarded less conspicuous insects such as small dipterans. Cole (1991) investigated

toxic properties of E+ fescue tissue and alkaloid extracts using the common laboratory fruit fly, Drosophila melanogaster Meigen. Significantly fewer Drosophila developed on E+ root- and leaf-amended diets than on diets containing E- tissue. The highest concentration of ergonovine tested caused reduced pupation. Ergotamine and ergocryptine both caused significant mortality. Larvae restricted to the highest concentration of NFL tested failed to pupate.

No significant difference was found in the number of frit fly, Oscinella frit (Loew) (Chloropidae) larvae infesting E+ perennial ryegrass and E- ryegrass in the United Kingdom (Lewis & Clements 1986). The frit fly is a minor pest of small grains and lawns in the U.S. and a more serious pest in the U.K. The family Chloropidae (frit flies or grass flies) are common in sweep samples from low vegetation. The larvae of some species are stem miners, some are gall formers, many are scavengers and a few are predaceous on other arthropods. Some are pests of man and livestock (Sabrosky 1936). This research was undertaken in order to investigate factors influencing population densities of chloropid flies occurring in tall fescue at the Highland Rim Experiment Station (HRES) in Middle Tennessee. Goals of this research were (I) to determine seasonal incidence and emergence of selected chloropids in E+ and E-

tall fescue, and (II) to examine aspects of the biology of Rhopalopterum carbonarium (Loew), a common chloropid in Tennessee.

3. MATERIALS AND METHODS

Field Studies

Research plots

Plots (10.6 m X 19.8 m) of high-endophyte 'Kentucky-31' and low-endophyte 'Forager' tall fescue were established at the Highland Rim Experiment Station (HRES), Robertson Co., TN. in spring 1990. The Highland Rim surrounds the Central Basin and is bordered on the east by the Cumberland Plateau and by the Western Valley to the west. Average elevation of the Highland Rim is ca. 1000 feet. Soil type in the plots was Dickson Silt Loam. Field research was carried out from February until October 1991 and 1992. Experimental design was a randomized complete block replicated four times.

Nitrogen was applied to the plots in spring 1991 and 1992 at a rate of 112 kg/ha. Dacthol® was applied (15.7 kg/ha) in the spring of both years for crabgrass control. Plots were clipped periodically to maintain a height of ca. 13 cm.

Endophyte infestation levels were determined. In the spring of both years ten tillers were randomly sampled from each plot, cooled, returned to the laboratory and analyzed using the PAS-ELISA method (Reddick & Collins 1988).

Sweep samples

Sweep samples were collected separately from each plot using a standard sweep net. On each sampling date, 50

pendulum swings were executed while walking randomly through a plot. Sampling was not done in the outer 1.5 m of the plots to avoid overlapping adjoining areas. Sweep samples were transferred to plastic bags, brought to the laboratory, frozen, and sorted. Sampling was done bi-weekly except on a few dates when delayed due to rain. Identifications of chloropids were provided by Curtis W. Sabrosky, Retired Research Entomologist and Cooperating Scientist, Systematic Entomology Laboratory, Agricultural Research Service, U.S. Department of Agriculture.

Emergence traps

Emergence traps were constructed of 19 liter (5 gallon) white plastic buckets painted black on the inside. A 10 cm hole was cut in the bottom of each bucket and covered with fine mesh screen. Sticky trap inserts were constructed of 15 cm long sections of PVC pipe (2.54 cm inner diameter) with a hook attached at one end. Inserts were covered with acetate sheets (15 cm X 10.8 cm) coated with Stiky Stuff® insect trapping compound (Olson Products, Inc., Medina, OH.) Inserts were suspended in the inverted buckets by means of a wire attached directly below the screened hole.

Traps were secured in the field by driving 20 cm nails (2/trap) through the bucket rim and into the ground. The area to be covered was disturbed by hand before placing a trap in order to cause any adult chloropids which might be present in the trap area to fly away. Each trap covered 1520

cm^2. In 1991, 2 traps were placed into each plot; this was increased to 3 traps/plot in 1992. Traps were moved to different areas in the plots on each sampling date in order to avoid sampling the same area twice consecutively. Acetate sheets were replaced with new sheets on each sampling date. Rhopalopterum carbonarium (Loew) and Apallates dissidens (Tucker) were identified and counted in 1991; all species collected were identified and counted in 1992.

Emergence cups

Emergence cups used for grass clipping experiments were constructed of two 2.45 liter paper popcorn containers placed mouth to mouth, one on top of the other. A 7.5 cm^2 hole was cut in the side of each top container and covered with acetate sheets coated on the inner surface with Stiky Stuff®. Plastic bags were placed in bottom containers to help prevent moisture loss.

Grass clipping experiments

Grass clipping experiment I was begun 18 June 1991. Two 40 cm dia. X 15 cm deep grass piles were formed in each plot by collecting clippings with a bagging mower and tightly packing the clippings onto the ground. Clippings were lightly watered every 2-3 days. The piles of clippings were collected individually on 30 June and brought to the laboratory, placed in a bucket and mixed by hand to increase

homogeneity. A sample (2.45 liters) from each pile was placed into an emergence cup (n=16 cups). Fifty ml of tepid water was added to each cup. Cups were kept on a benchtop in an insectary room (14:10 L:D, 28° C). Acetate sheets were changed every three to four days, and trapped chloropids were identified and counted until no more emerged.

Grass clipping experiment II was begun 30 June 1991. On 15 July a 90° wedge including soil to a depth of 2.5 cm was collected from each pile. Piles were not watered. Other methods were identical to those described for grass pile experiment I.

Rainfall data for 1991-1992 were obtained from HRES.

Insect Culture

Larval feeding study

Larval feeding studies were conducted using dishes similar to oviposition dishes described by Tolman et al. (1985), except that wicks were constructed of 10 cm X 20 cm cheesecloth strips. Tops measured 9 cm X 1 cm.

Adult R. carbonarium were collected at HRES on 23 April 1991 and brought back to the laboratory. Approximately 25 flies were placed into each of three plexiglass cages (30.5 cm X 30.5 cm X 30.5 cm) with a small cup of moistened Ward's Instant Drosophila Medium® (Ward's Natural Science Establishment Inc., Rochester, NY) and a water source.

Autoclaved soil was placed into each of six oviposition dishes along with greenhouse-grown live fescue tillers, fescue leaves or ball clover roots. Each cage received two dishes of one treatment. Cages were placed into a growth chamber (14:10 L:D, 28° C, ca. 60% R.H.) and dishes were checked daily for larvae.

Colony maintenance

A colony of R. carbonarium was maintained in a plexiglass cage in a growth chamber (same type cage and conditions as above) from May 1991 until November 1991. Autoclaved sand was placed into the bottom of a 23 cm X 9 cm round plastic container to a depth of ca. 2 cm and saturated with distilled water. E- fescue tissue collected from HRES was frozen (-20° C), surface sterilized by brief immersion in a 10 % Clorox® solution and rinsed with distilled water. The treated fescue was placed onto the sand and tightly packed to a depth of ca. 2 cm. The round container was replaced approximately every month. Oviposition dishes containing E+ or E- fescue tissue were used periodically to observe the feeding habits of R. carbonarium larvae. Crabgrass tissue was placed in oviposition dishes and offered to adult flies to determine whether it was a suitable larval medium.

Statistical Analysis

Sweep sample data were log-transformed and analyzed using a T-test ($P < 0.05$) (SAS Institute 1987). Emergence trap and emergence cup data were combined for each plot, log-transformed and analyzed using a T-test ($P < 0.05$) (SAS Institute 1987).

4. RESULTS AND DISCUSSION

Field Studies

Endophyte infestation

Endophyte infestation levels (Fig. 1) in E+ plots ranged from 70% to 100% in 1991, and only one tiller from one E- plot tested positive for endophyte. Endophyte infestation levels in 1992 ranged from 90% to 100% in E+ plots, and infestation levels of 40% and 10% were noted for E- replications two and three, respectively. E+ tall fescue has a competitive advantage over E- tall fescue (Clay et al. 1985). Endophyte infestation increased in half the plots in my study from 1991 to 1992. This is indicative of the competitive advantage conferred to the plant by the fungus.

Sweep samples

Over the two-year period of my study, 15,637 chloropids were collected in sweep samples from E+ plots, and 27,488 were collected in sweep samples from E- plots. At no time during this study were significantly more chloropids collected in E+ plots than E- plots. A total of 22 chloropid species or variants were identified (Table 1). More chloropids were collected in 1992 than 1991. An apparent association between chloropid population densities and rainfall has been observed in Kansas (Wilbur & Sabrosky 1936). Lower population densities of chloropids in 1991 may

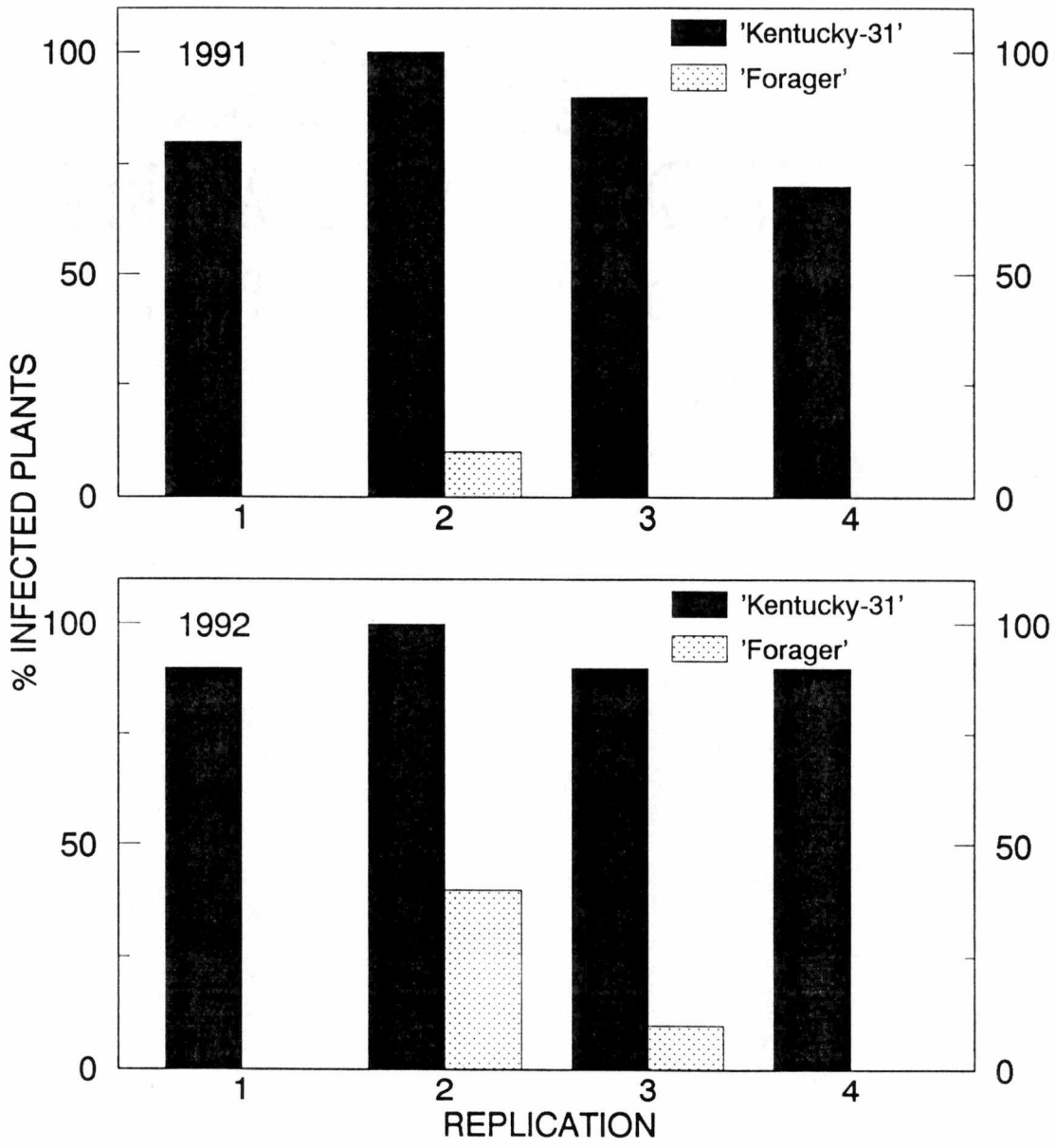


Fig. 1. Acremonium-infestation levels in research plots of tall fescue, Robertson Co., TN.

Table 1. Chloropids collected in sweep samples from tall fescue, Robertson Co., TN.

SPECIES	TOTAL NO. COLLECTED 1991 E+	1991 E-	1992 E+	1992 E-	Months Collected
Rhopalopterum carbonarium (Loew)	3,370	4,965	2,742	4,814	Apr-Oct
Apallates dissidens (Tucker)	2,758	3,844	627	1,946	Mar-Oct
Rhopalopterum umbrosum (Loew)	272	683	1,303	2,581	May-Oct
Incertella minor (Adams)	455	932	858	1,362	Apr-Oct
Monochaetoscinella nigricornis (Loew)	274	607	817	1,868	May-Oct
Apallates particeps (Becker)	69	222	474	1,120	May-Oct
Apallates neocoxendix (Sabrosky)	116	177	589	729	May-Oct
Olcella trigramma (Loew)	136	185	347	385	Apr-Oct
Oscinella frit (Loew)	37	89	82	247	May-Oct
Thaumatomyia glabra (Meigen)	112	219	10	57	May-Oct
Meromyza americana Fitch	30	106	25	116	Apr-Oct
Conioscinella? poss. new species	21	34	44	38	May-Oct
Thaumatomyia pulla var. *punctum* (Becker)	26	57	9	43	May-Oct
Ceratobarys eulophus (Loew)	5	10	6	16	Apr, Jun, Aug-Sep
Olcella cinerea (Loew)	7	5	6	0	Jun, Aug
Apallates coxendix (Fitch)	4	11	1	0	Jun, Jul
Diplotoxa versicolor Loew	-	-	1	8	May
Rhopalopterum criddlei (Aldrich)	-	-	4	2	May
Elachiptera erythropleura Sabrosky	0	3	0	1	May-Jun
Chlorops sp.	-	-	0	2	May
Oscinella frit var. or n. sp.	-	-	0	2	Jul
Liohippelates pallipes (Loew)	0	1	0	1	Jun
TOTALS:	7,692	12,150	7,945	15,338	

have been a result of decreased rainfall at HRES during the summer of 1991 (Fig. 2).

Rhopalopterum carbonarium was the most abundant chloropid collected in both years. R. carbonarium has been reported from several plant species (Sabrosky, personal communication) and feeds on dead and decaying plant tissue (Vogt, unpublished data). Significantly fewer R. carbonarium were collected from E+ plots than from E- plots on several sampling dates (Fig. 3). The spring population peak was higher in 1991 than in 1992, possibly due to increased rainfall during April and May 1991. A drop in the population density of R. carbonarium occurred during July and August 1991, a period of no rainfall at HRES. The population density of R. carbonarium peaked during the same time period in 1992, when rainfall occurred, resulting in four well-defined peaks in population densities throughout the season.

Apallates dissidens (Hippelates dissidens) has been reported from decaying vegetable matter (Sabrosky, personal communication) and breeds in organic matter above the soil surface (Dow 1970). Significantly fewer A. dissidens were collected in E+ plots than E- plots on several sampling dates (Fig. 4). Population densities of this insect were much higher during summer 1991 than summer 1992, which might indicate that the larvae of A. dissidens are more suited to drier conditions. A. dissidens breeds in greater numbers

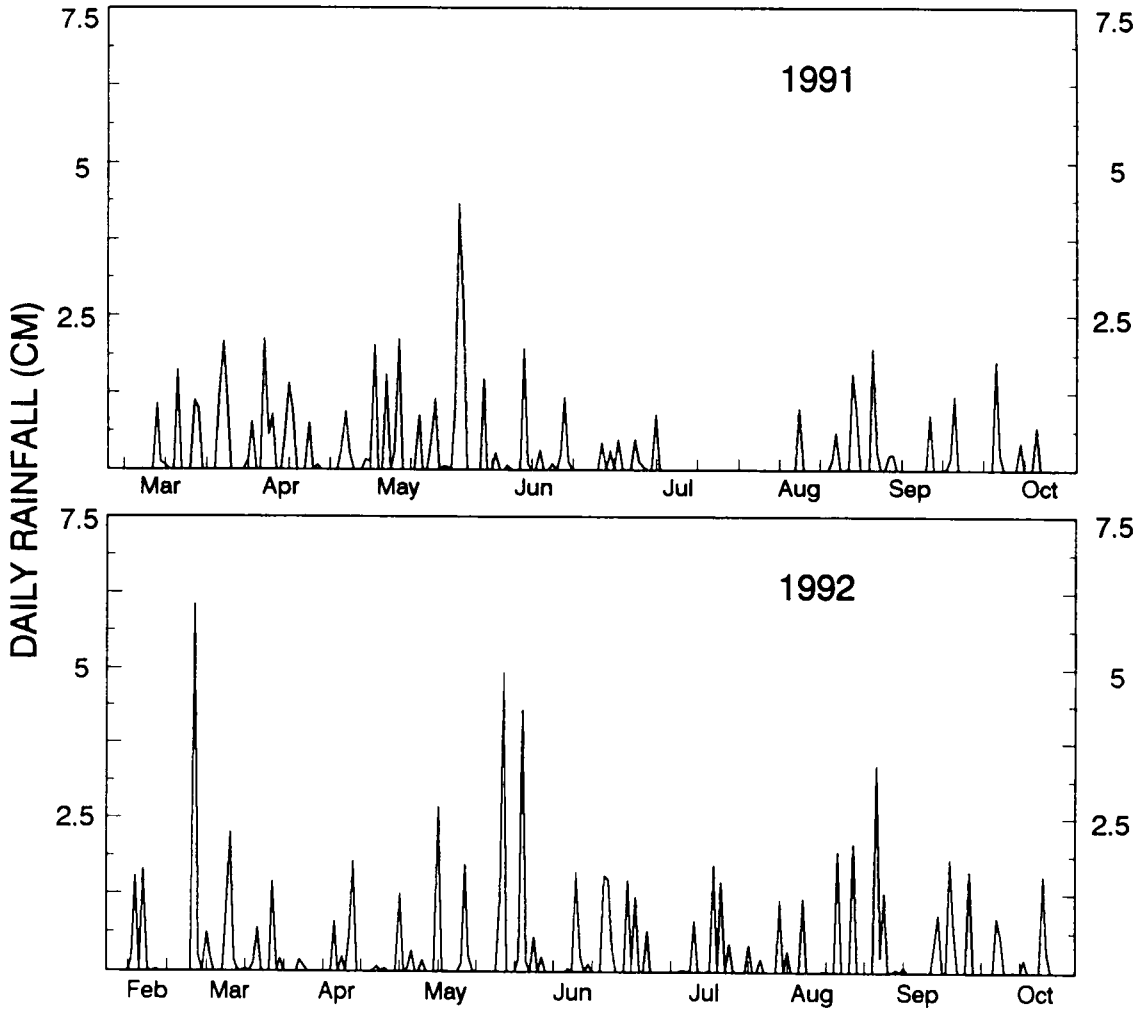


Fig. 2. Daily rainfall at HRES, Robertson Co., TN.

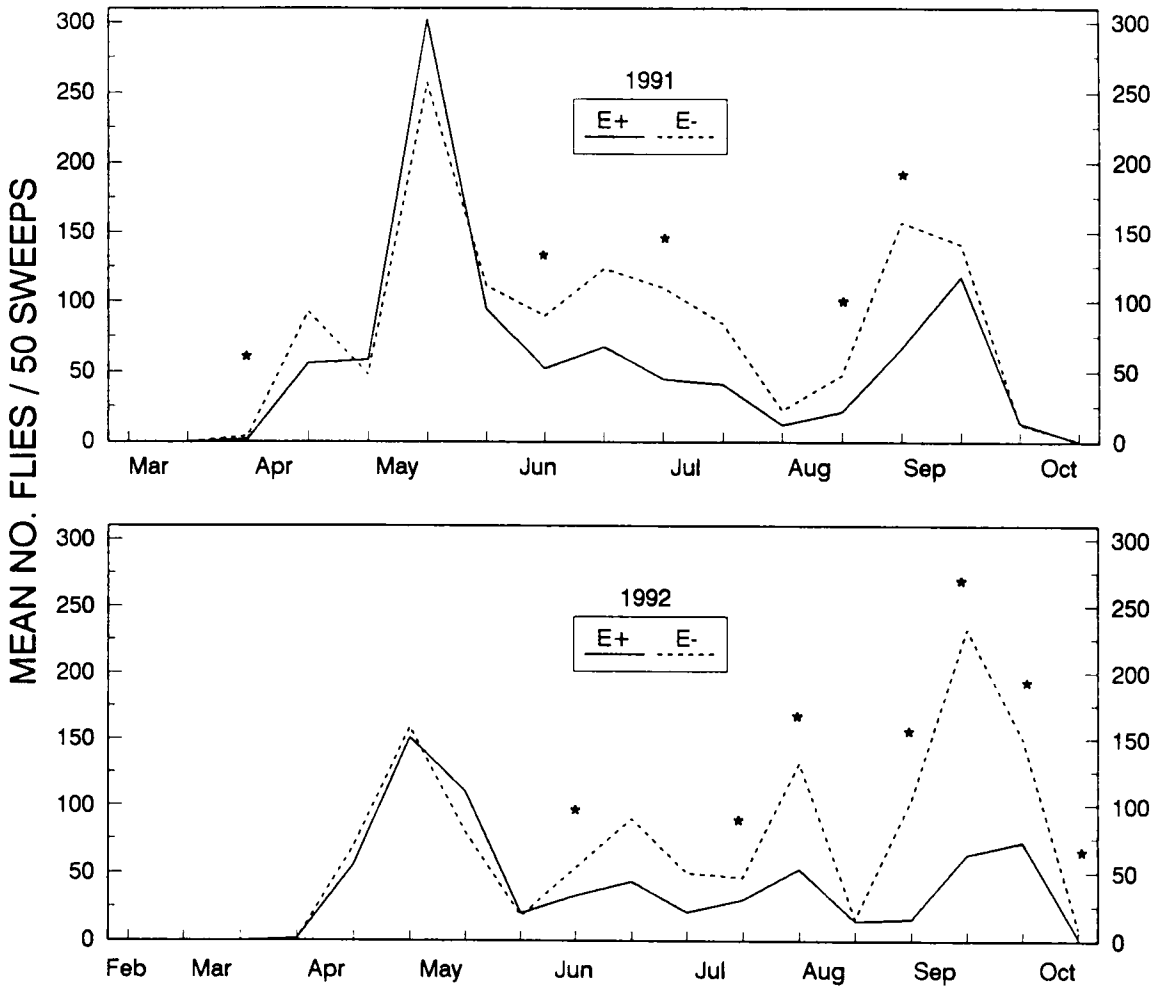


Fig. 3. Seasonal incidence of Rhopalopterum carbonarium in sweep samples from tall fescue plots in Robertson Co., TN., 1991 and 1992. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

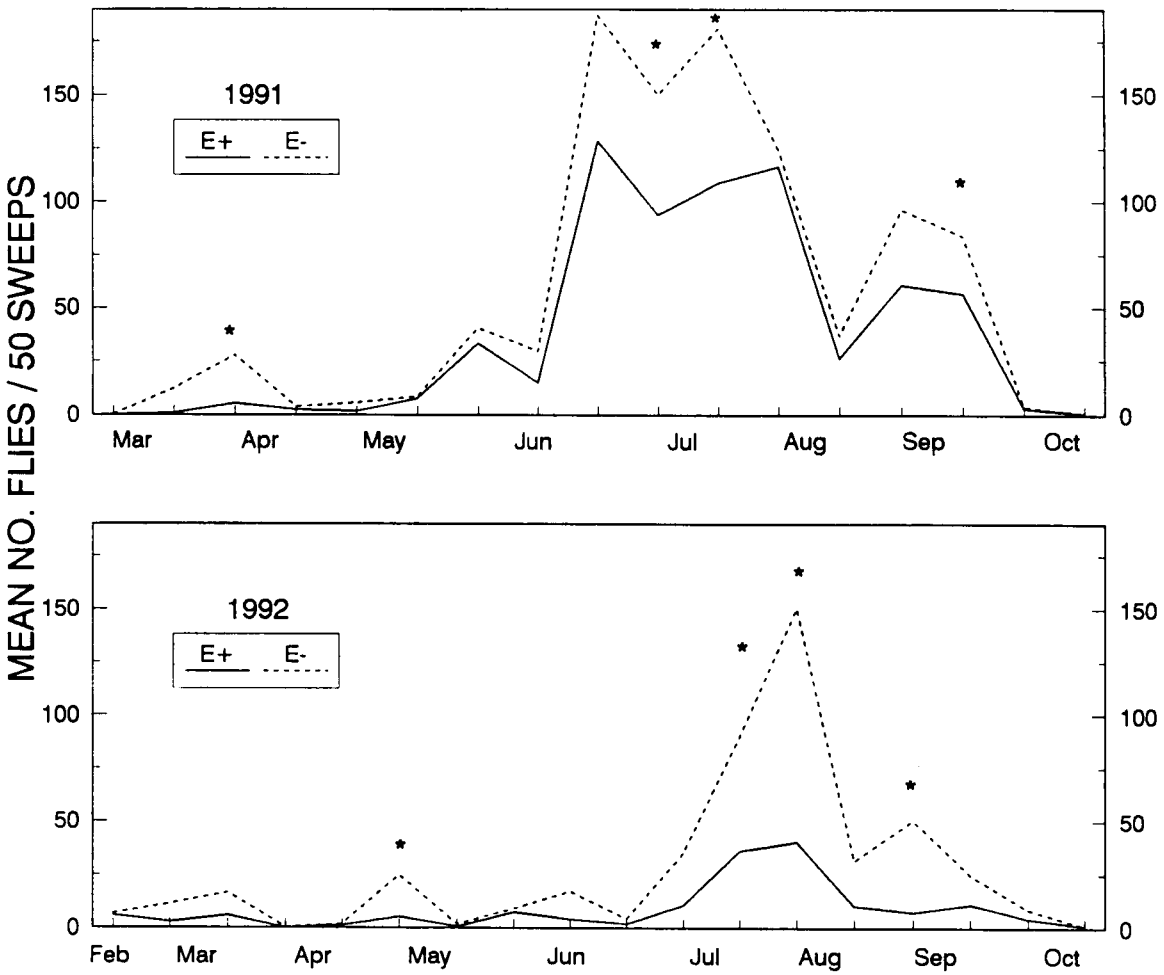


Fig. 4. Seasonal incidence of Apallates dissidens in sweep samples from tall fescue plots in Robertson Co., TN., 1991 and 1992. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

above the soil level (where conditions are apt to be drier) than within organic matter below soil level (Dow 1970).

Rhopalopterum umbrosum ranked third in abundance in my study, and significantly fewer specimens were collected in E+ plots than E- plots on several sampling dates (Fig. 5). Lack of rainfall during summer 1991 appeared to affect population densities of this species drastically, with the number of specimens collected approaching zero during July and August. Population densities were much higher during July-August 1992, with four well-defined peaks of incidence. The larval habits of this species are unpublished.

Incertella minor larvae are scavengers (Sabrosky, personal communication). This species occurred in significantly fewer numbers in E+ plots than E- plots on some sampling dates (Fig. 6), and had three to four poorly-defined population peaks. Seasonal peaks of incidence of I. minor were quite different between years, and no cause is readily evident, although weather may have been a factor.

Monochaetoscinella nigricornis adults frequently appear in emergence cages in the southeast, and larvae are probably scavengers (Sabrosky 1948). Significantly more M. nigricornis were collected in E- plots than E+ plots on several sampling dates (Fig. 7). Population densities of this species peaked in late July both years, and again in late September 1991 and early October 1992. M. nigricornis occurred in much greater numbers in July 1992 than July

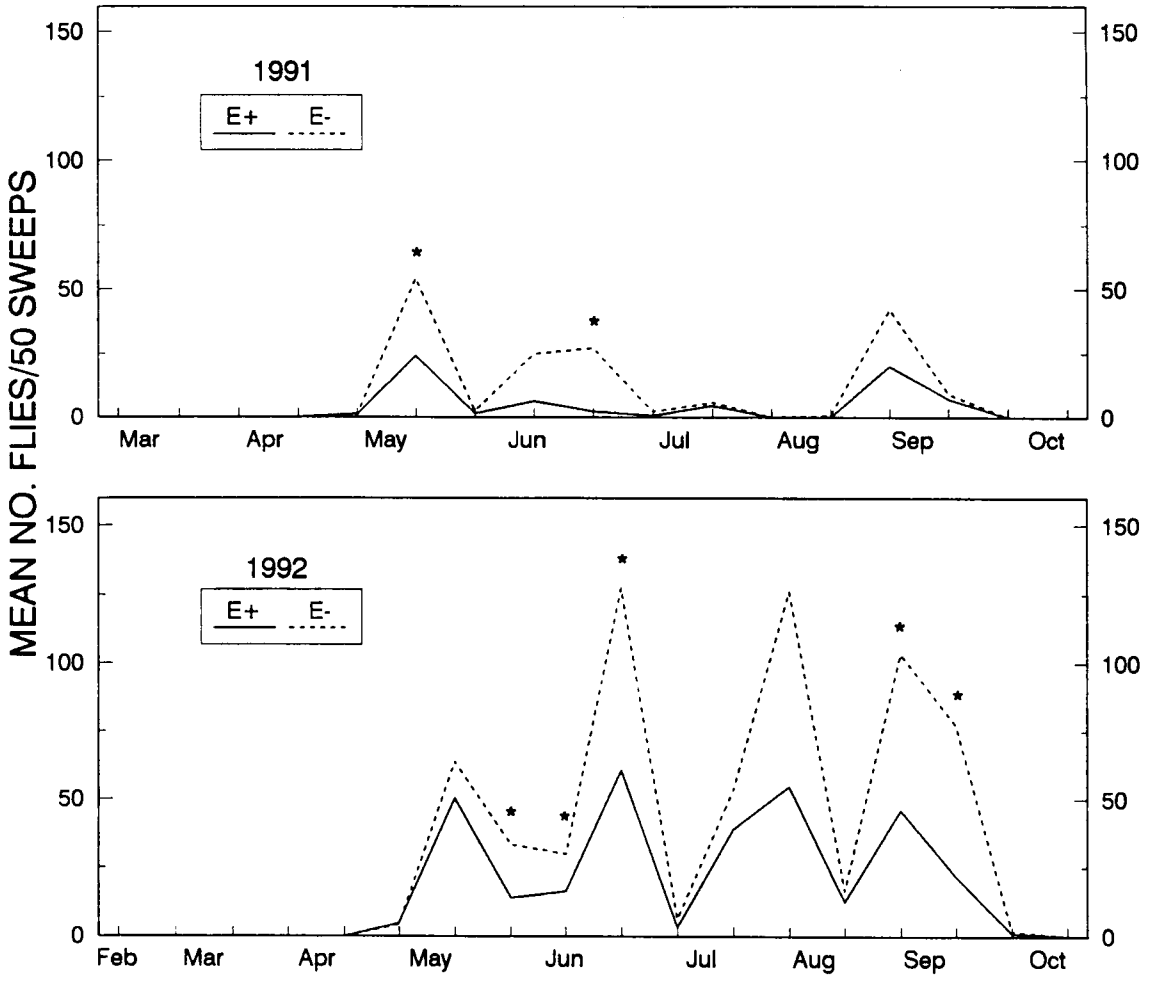


Fig. 5. Seasonal incidence of Rhopalopterum umbrosum in sweep samples from tall fescue plots in Robertson Co., TN., 1991 and 1992. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

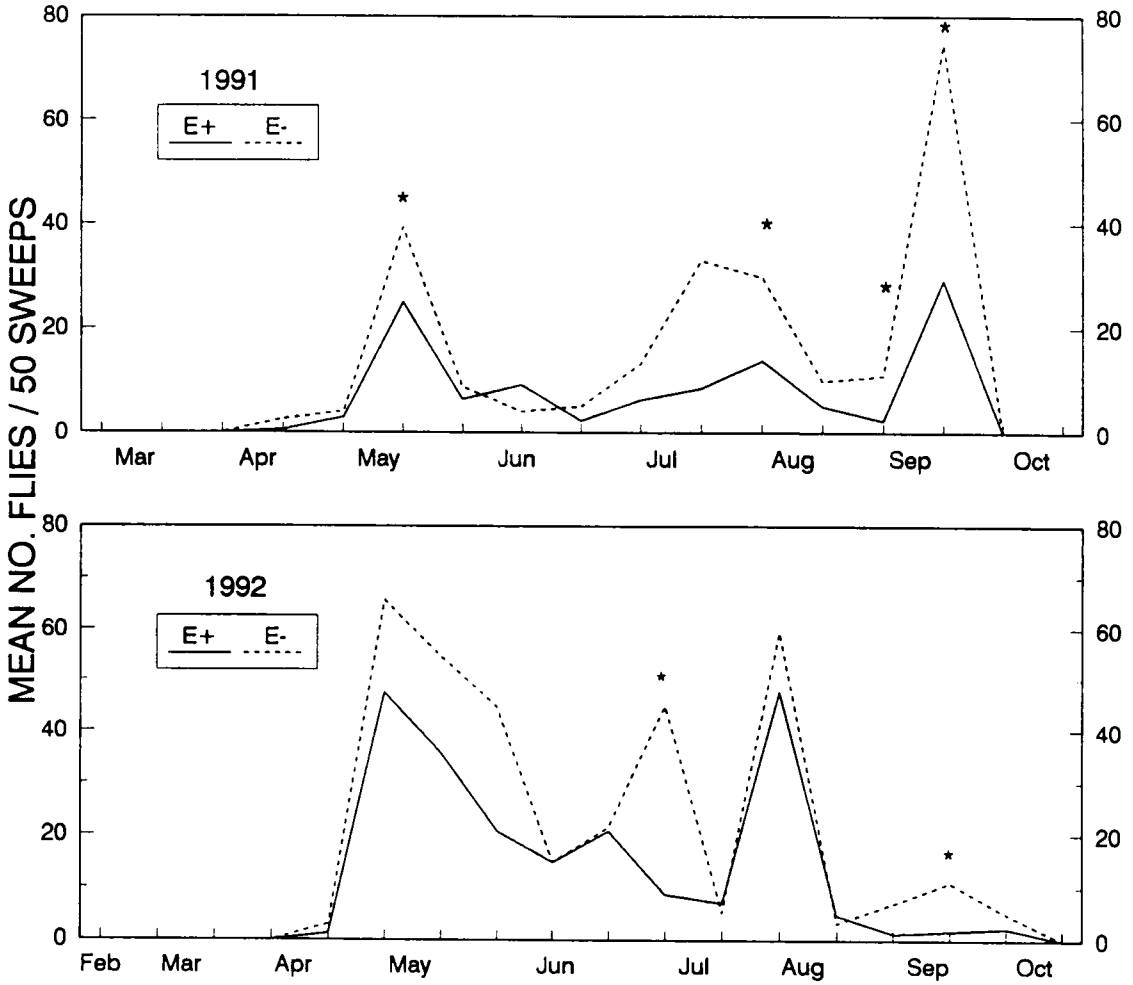


Fig. 6. Seasonal incidence of Incertella minor in sweep samples from tall fescue plots in Robertson Co., TN., 1991 and 1992. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

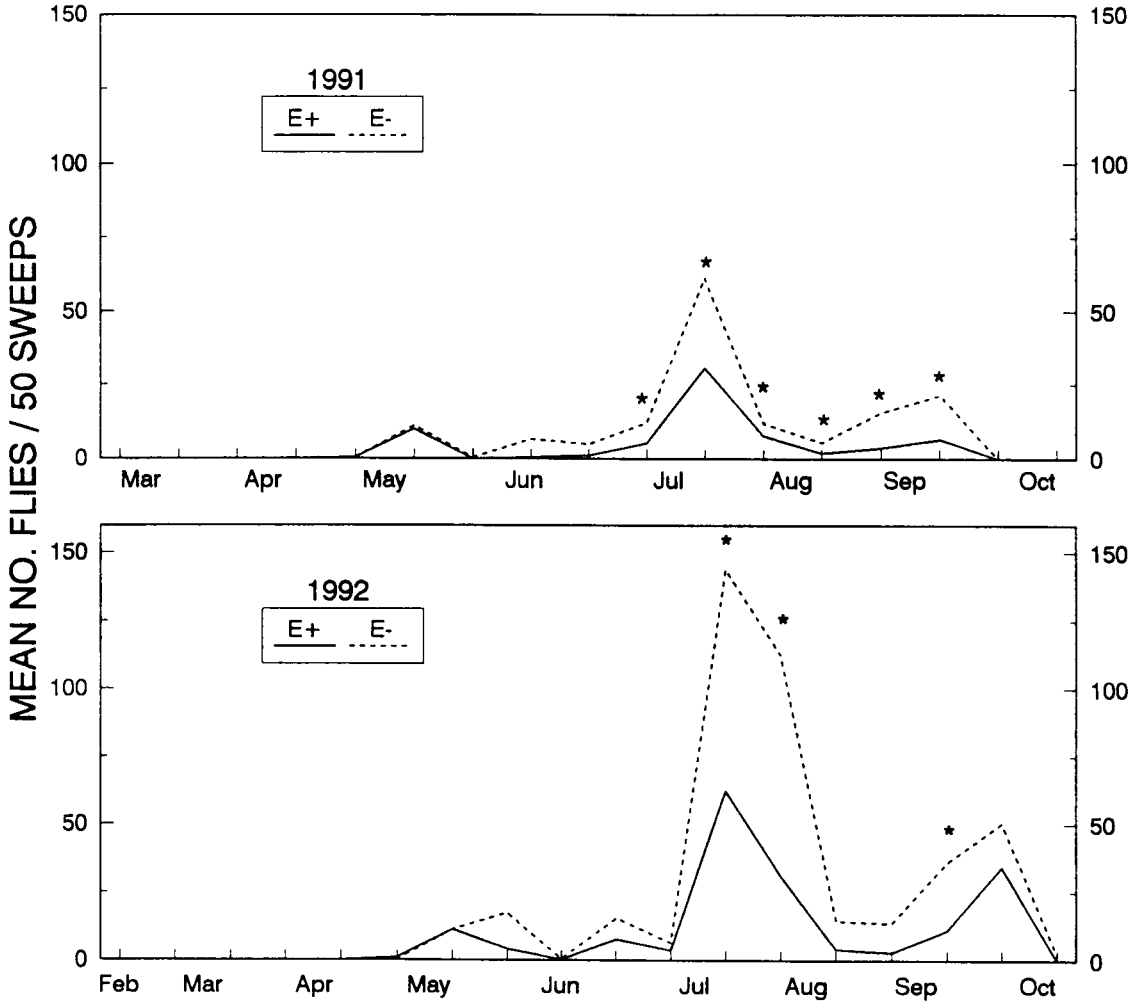


Fig. 7. Seasonal incidence of Monochaetoscinella nigricornis in sweep samples from tall fescue plots in Robertson Co., TN., 1991 and 1992. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

1991, probably because of the lack of rainfall during that period in 1991.

Apallates particeps occurred in significantly greater numbers in E- plots than E+ plots on many sampling dates (Fig. 8). A large peak in population density of this species occurred in early August 1992, but was absent in 1991. Lack of rainfall in summer 1991 appeared to severely limit the number of A. particeps collected in sweep samples.

Apallates neocoxendix did not appear to be severely affected by the presence of A. coenophialum, but generally occurred in greater numbers in E- plots (Fig. 9). This species has been reported from stems of bluegrass exhibiting silver top (Starks & Thurston 1962), a condition of unknown cause in which the seed heads appear pale and seed formation is reduced; however, the percent infestation of chloropids in symptomatic plants was very low. Live fescue plants were not examined for chloropid larvae in my study, but tall fescue has been recorded as a host for A. neocoxendix.

Oscinella frit, the well-known frit fly, was present in the field from May until October (Fig. 10). Larvae of this species are able to infest a variety of grasses, including crops such as wheat and corn. Tall fescue is a preferred host in Germany (Schumann 1988). In the United States O. frit is primarily a pest of turfs, particularly in the northeast. Larvae bore into plants, causing withering of the leaves and death of the growing points. The larvae can

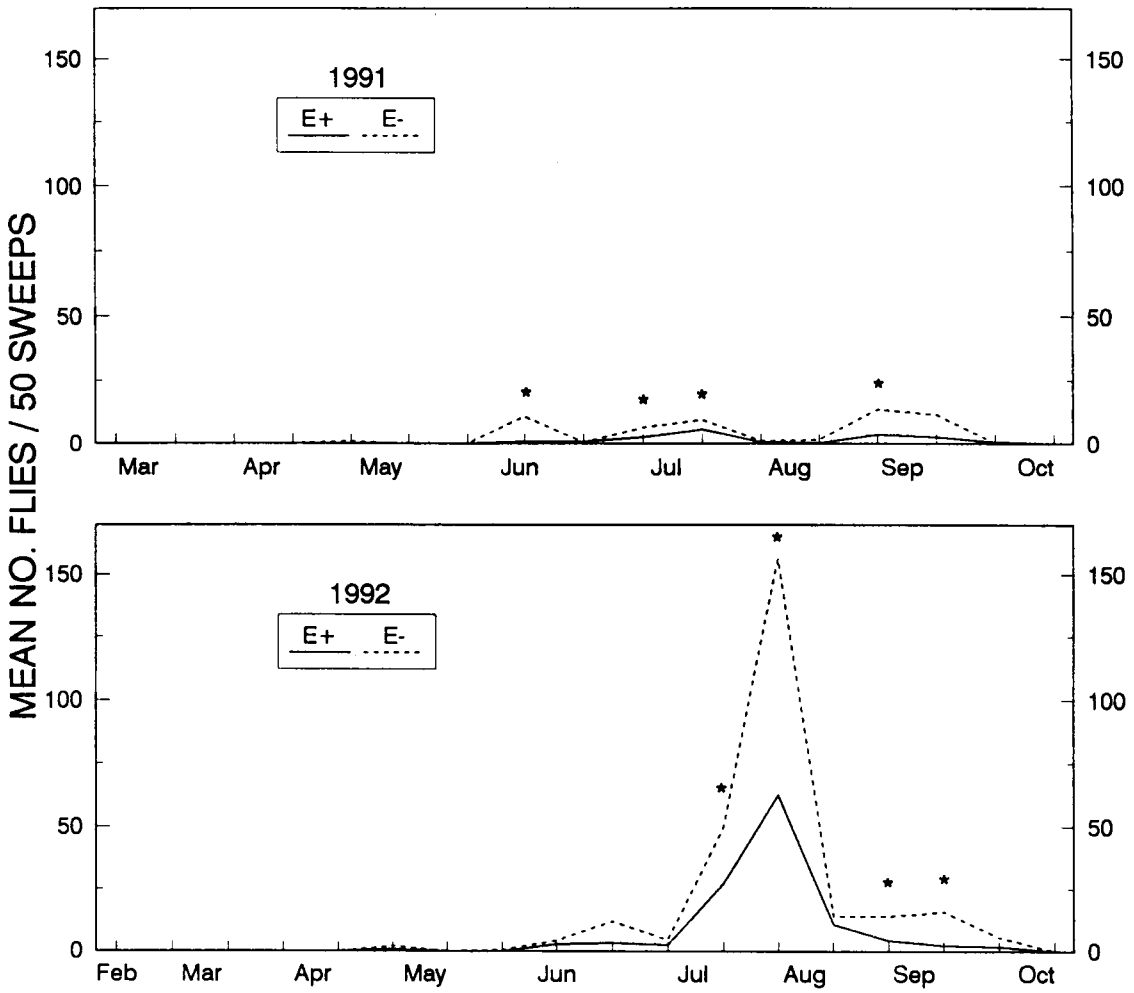


Fig. 8. Seasonal incidence of Apallates particeps in sweep samples from tall fescue plots in Robertson Co., TN., 1991 and 1992. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

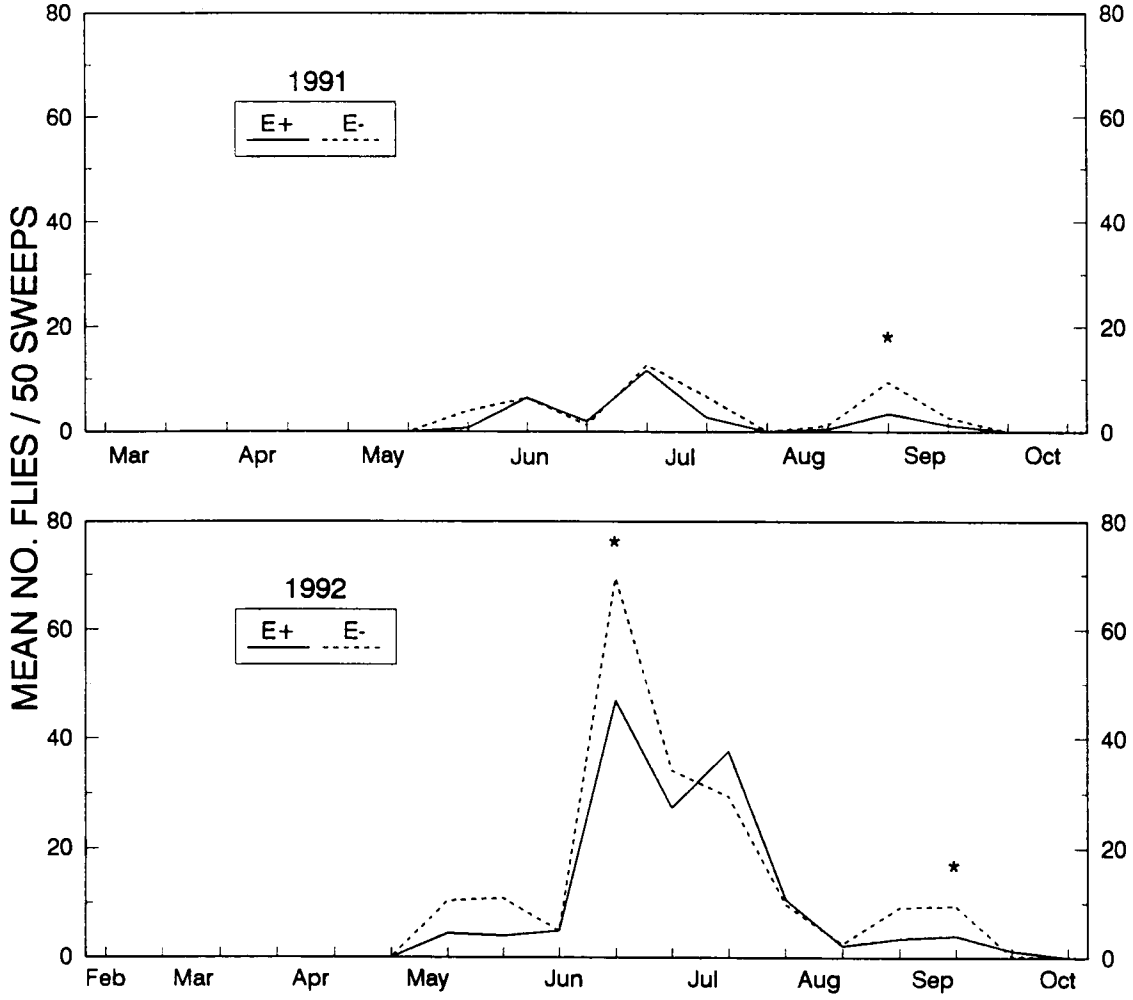


Fig. 9. Seasonal incidence of Apallates neocoxendix in sweep samples from tall fescue plots in Robertson Co., TN., 1991 and 1992. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

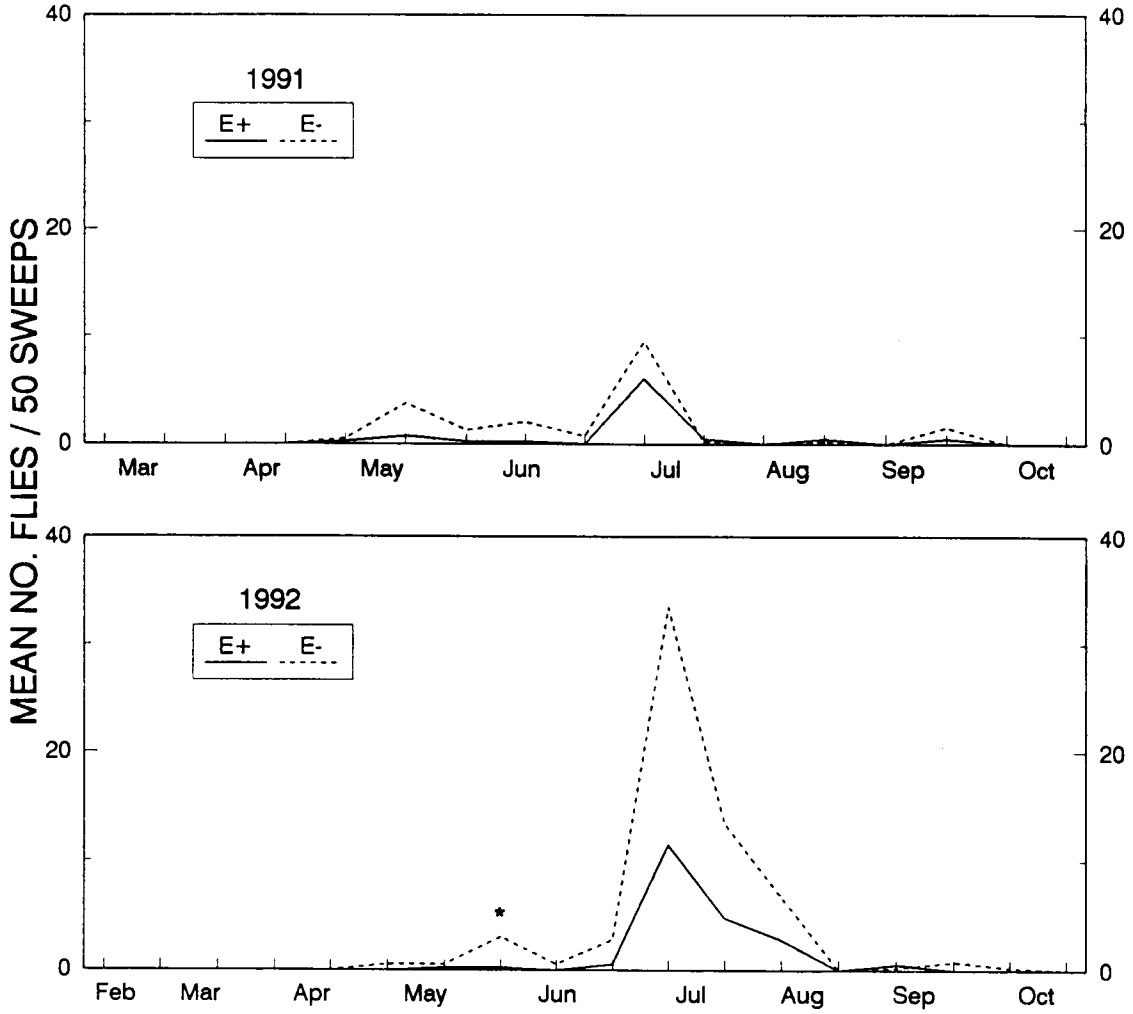


Fig. 10. Seasonal incidence of Oscinella frit in sweep samples from tall fescue plots in Robertson Co., TN., 1991 and 1992. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

migrate from previous grass crops into newly sown swards, causing significant damage (Bentley & Clements 1989). Significantly more frit fly adults were collected in E- plots than E+ plots on only one sampling date; however, numbers caught varied considerably between replications. The trend toward greater infestation in E- plots should be investigated further to determine the possibility of this insect becoming a problem in renovated (E-) pastures.

Meromyza americana, the wheat stem maggot, is a minor pest of small grains in the U.S.; its larvae bore into the stems of plants. Like frit fly, infestation in my research plots varied considerably between replications, but significantly fewer M. americana were collected in E+ plots than E- plots on some sampling dates (Fig. 11). Trends toward higher population densities in E- plots were evident. This species might contribute to the overall poor performance of E- pasture, and effects of the fescue endophyte on M. americana should be investigated further.

Thaumatomyia glabra larvae are predaceous on root aphids and are an important natural enemy of at least one aphid species, Pemphigus populivenae Fitch, the sugarbeet root aphid (Summers & Newton 1989). Root aphids were not sampled in this study, but the trend toward more T. glabra in E- plots, and one significant difference in May 1992 (Fig. 12), might be related to the fly's larval food source, if a suitable aphid species occurred in my plots.

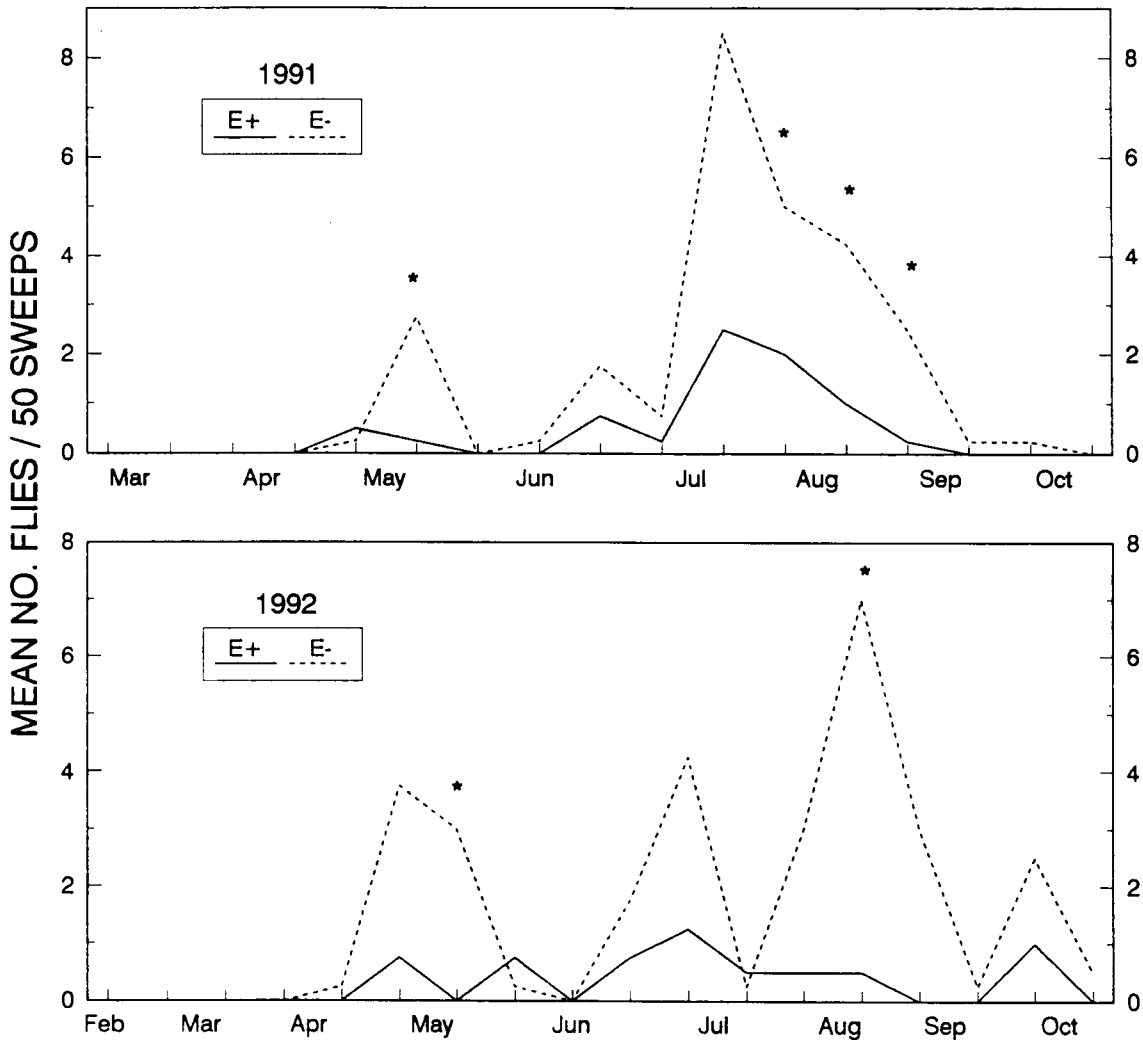


Fig. 11. Seasonal incidence of Meromyza americana in sweep samples from tall fescue plots in Robertson Co., TN., 1991 and 1992. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

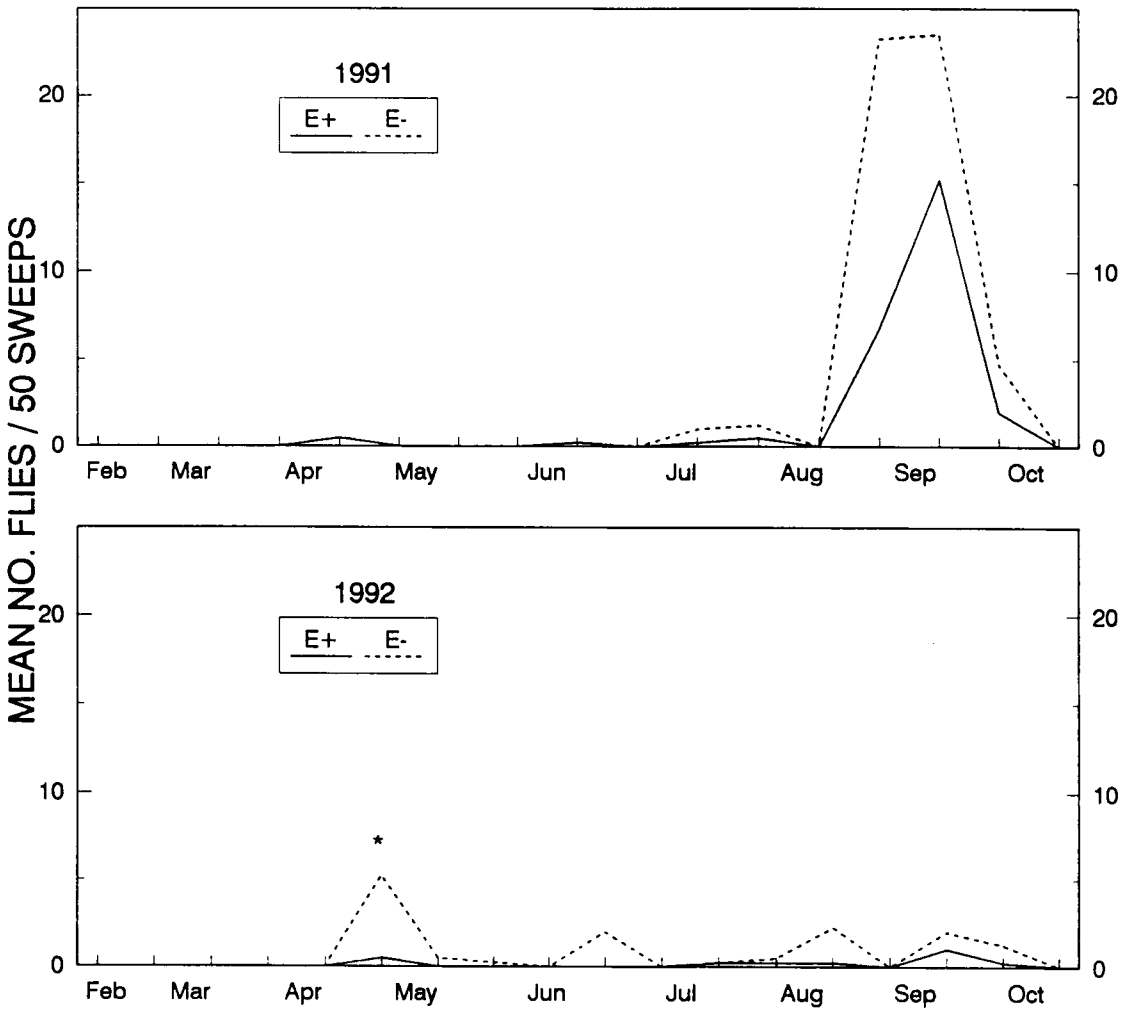


Fig. 12. Seasonal incidence of Thaumatomyia glabra in sweep samples from tall fescue plots in Robertson Co., TN., 1991 and 1992. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

Significantly fewer Thaumatomyia pulla var. punctum were collected from E+ plots than E- plots on one date in May 1992. No statistically significant differences were found in numbers of any chloropids, other than those already discussed, collected from E+ or E- plots on any sampling dates. Specimens of Conioscinella ? poss. n. sp. and Oscinella frit var. or n. sp. have been retained by C. W. Sabrosky for taxonomic study.

Emergence traps

None of the chloropids captured in emergence traps occurred in significantly greater numbers in E+ or E- plots on any sampling date, although trends toward greater emergence in E- plots than E+ plots were evident. The number of chloropids taken in emergence traps was small compared to the number collected in sweep samples, and there was a great deal of variation between replications, probably due to the small surface area sampled by traps. Data from 1991 are not given. Total chloropids caught in emergence traps in 1992 are presented in Table 2. Trap data confirmed that several chloropid species, including Rhopalopterum carbonarium, Apallates dissidens, Monochaetoscinella nigricornis, R. umbrosum, A. neocoxendix, Incertella minor, Elachiptera erythropleura, Oscinella frit, and Conioscinella? n. sp., were reproducing in my research plots. The other species emerging into traps were collected in such small numbers that no conclusion can be made as to

Table 2. Chloropids collected in emergence traps placed in tall fescue plots, Robertson Co., TN., 1992.

SPECIES	TOTAL NO. TRAPPED		EMERGENCE PERIOD
	E+	E-	
Rhopalopterum carbonarium	69	100	Apr-Oct
Apallates dissidens	13	35	Apr, Jun-Oct
Monochaetoscinella nigricornis	27	130	Jun-Oct
Rhopalopterum umbrosum	23	46	May-Oct
Apallates neocoxendix	13	31	Jun-Sep
Incertella minor	4	9	May-Aug
Elachiptera erythropleura	2	11	May-Jun
Oscinella frit	16	8	Aug
Conioscinella? poss. n. sp.	6	3	May-Aug
Meromyza americana	0	1	May
Olcella trigramma	2	0	Jun
Liohippelates pallipes	0	1	Jun
TOTAL	173	373	

their possible reproduction in the plots. The grass beneath the traps often became chlorotic between sampling dates, and the environment beneath the traps was damper than the surrounding area. This altered environment could have affected the growth or survival of chloropid larvae. Interestingly, no Apallates particeps were caught in emergence traps, yet they were relatively abundant in sweep samples. A. particeps may not reproduce in tall fescue, or the altered environment beneath emergence traps may not have been suitable for larval development.

Grass clipping experiments

Liohippelates pallipes was by far the most abundant species collected in grass clippings from HRES (Fig. 13). This species is one of a group of chloropids commonly called eye gnats, referring to the habits of the adult females which feed on secretions of animals and humans, often around the eyes. The fact that significantly fewer L. pallipes were collected from E+ clippings than E- clippings in one collection from emergence cups, and the general trend toward more flies reared from E- clippings, indicates that this species merits additional study.

Hummadi & DeFoliart (1967) reported finding larvae of L. pallipes 5.1 to 6.7 cm below the surface of moist, disturbed soil. Considering the small number of L. pallipes collected in emergence traps in this study, and the very large numbers collected from piles of grass clippings, the

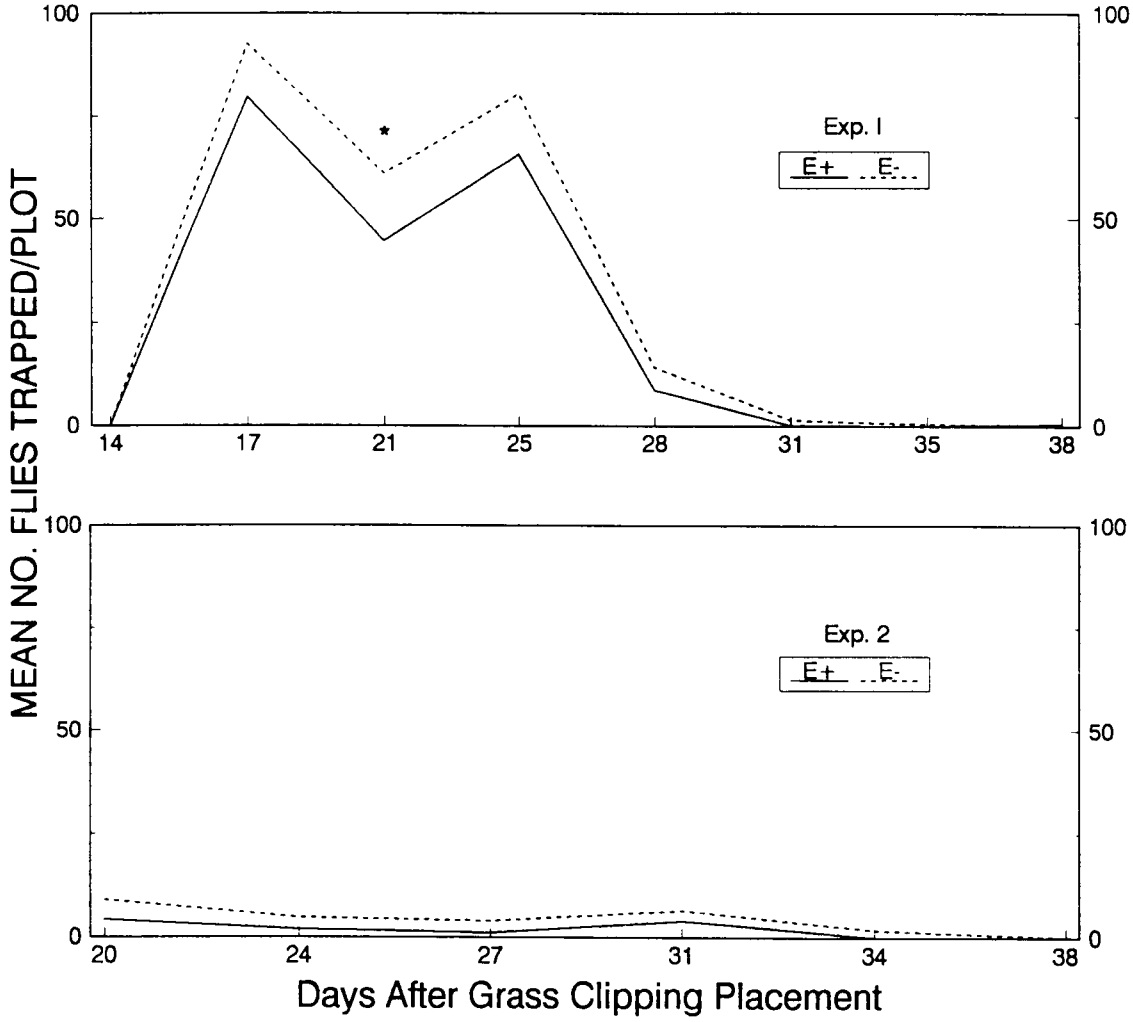


Fig. 13. Emergence of Liohippelates pallipes from tall fescue clippings, Robertson Co., TN. An asterisk above data points indicates that they are significantly different ($P < 0.05$).

densely packed clippings above the soil surface provided a better medium for larval growth than did soil in the plots. The lack of adult flies in sweep samples is probably due to the females seeking vertebrate hosts upon emerging. Males are often found on flowers (Sabrosky 1935). Watering the piles in grass clipping experiment I might have contributed to the higher number of L. pallipes collected in experiment I compared to experiment II, in which clippings were not watered.

Rhopalopterum carbonarium and Apallates dissidens emerged in greater numbers from E- clippings than E+ clippings; however, no significant differences occurred (Figs. 14 and 15, respectively).

Laboratory Studies

Six days after placing oviposition dishes into cages containing adult R. carbonarium, larvae were observed in the fescue leaf treatment. Pupae were present after 14 days; adults emerged after 18 days. No larvae were detected in the live fescue tillers or ball clover roots, and no adult flies emerged from either. The adults (ca. 20) which emerged from the fescue leaves were identified as R. carbonarium.

Other observations were made while keeping a colony of R. carbonarium in 1991, but replicated experiments proved difficult. Female flies oviposited erratically onto

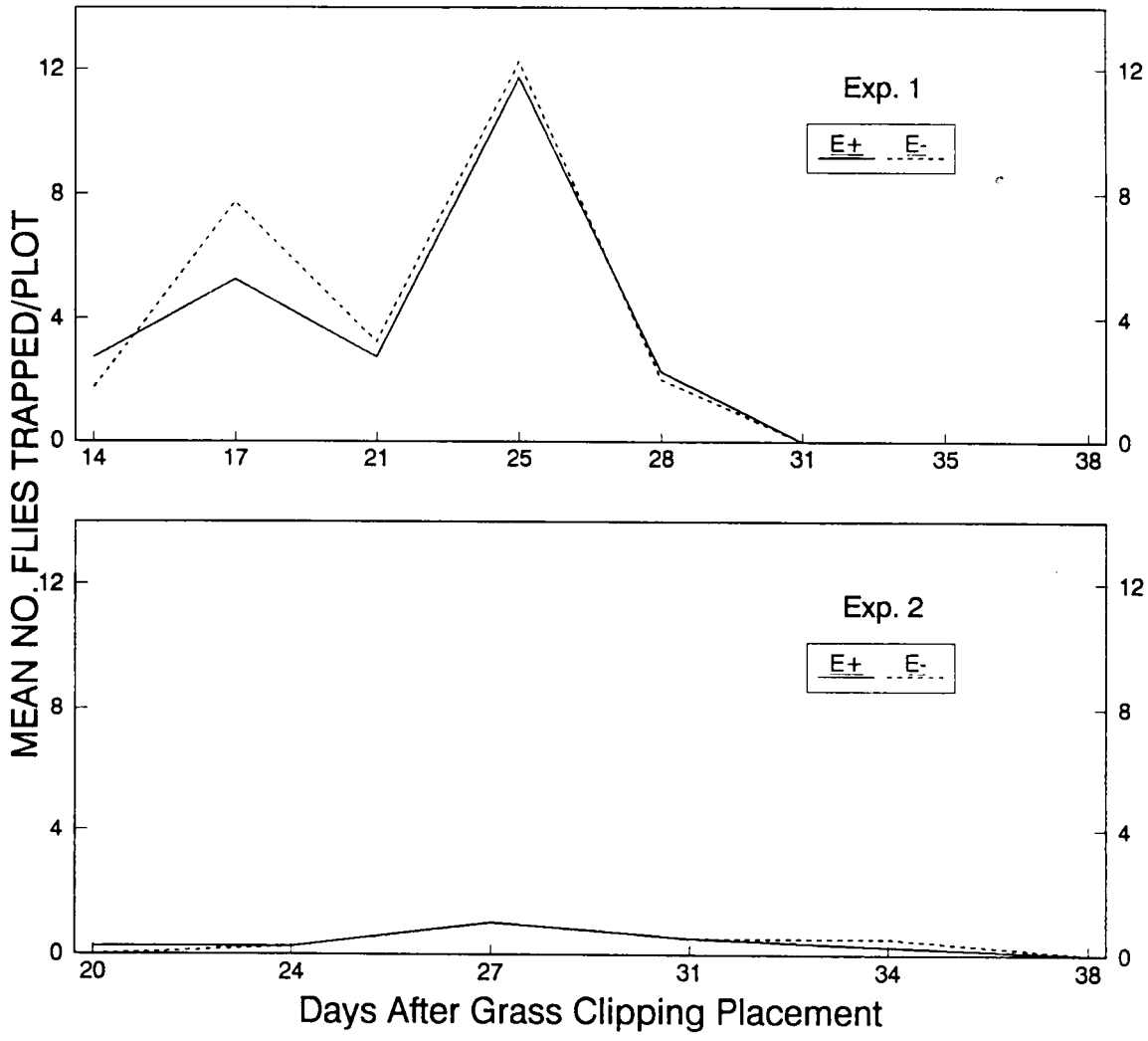


Fig. 14. Emergence of Rhopalopterum carbonarium from tall fescue clippings, Robertson Co., TN.

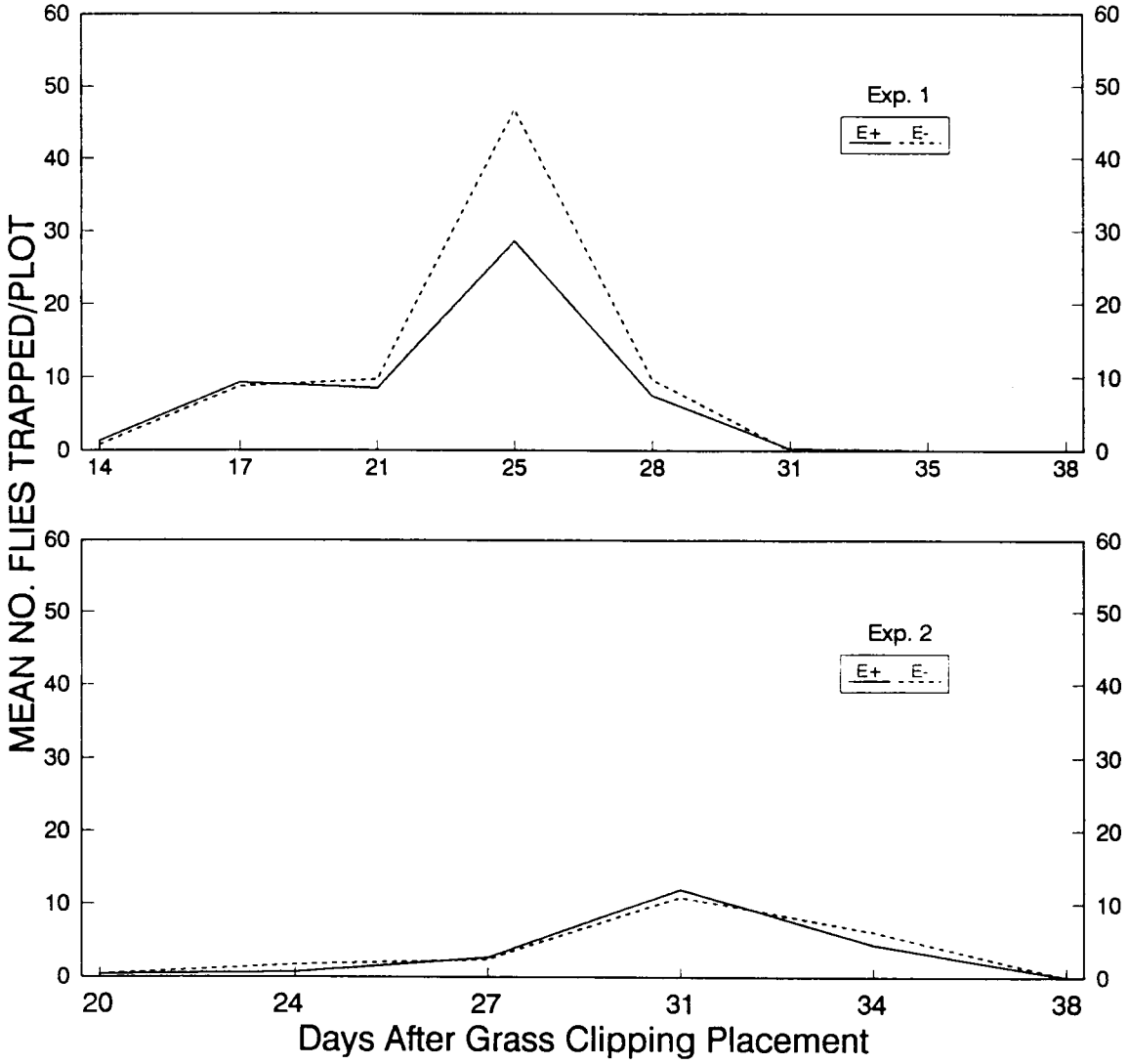


Fig. 15. Emergence of Apallates dissidens from tall fescue clippings, Robertson Co., TN.

treatment diets; thus, wide variations existed in the number of adults emerging from replications of the same treatment. Eggs of R. carbonarium were collected once from the surface of grass tissue, after allowing adults collected from the field to oviposit onto fescue leaves, by washing the leaf tissue onto a fine mesh screen with tepid water. Eggs are ca. 0.5 mm in length, oval, white, with numerous striations.

Larvae of R. carbonarium were observed moving on and within dead fescue tissue. Larvae did ingest plant material, since green material was observed within the guts of active larvae. Pupation occurred either on the substrate, or, more commonly, between the epidermal layers of fescue leaves, usually with one end of the pupa exposed. Pupae were commonly found clustered at tiller bases with one end exposed through the cut base when whole tillers were used for rearing. Developmental time from egg to adult varied from 13-18 days, averaging 15 days. R. carbonarium developed successfully on crabgrass and on E+ and E- fescue collected from HRES in August 1991; quantitative data are not available. In general, containers that yielded large numbers of adult R. carbonarium had little mold growth compared to those that yielded few adults. The colony began to decline in October 1991 and few flies developed before rearing was stopped in November 1991. Difficulties were encountered in starting a new colony in 1992, and further

data on life cycle and mass rearing of R. carbonarium was not collected.

Prior to this research the larval feeding habits of R. carbonarium were unknown, although Sabrosky (personal communication) suspected that it fed on decaying leaf sheaths. R. carbonarium is a scavenger, and aids in the breakdown of organic matter at the soil level. R. carbonarium might prove useful in assays of tall fescue, since little preparation of the test material would be required.

5. SUMMARY AND CONCLUSION

Several insects which feed on live fescue plants have been shown to be negatively affected by the presence of Acremonium coenophialum. Endophyte-mediated resistance to scavenging and plant-feeding dipterans has been ignored in field studies of insects in tall fescue. My research has demonstrated that several members of the family Chloropidae, which inhabited tall fescue in Robertson Co., TN., occured in lower numbers in E+ tall fescue than in E- tall fescue. Chloropids which feed on dead plant material as well as live plant material were negatively affected by the fescue endophyte.

Fewer specimens of the three known pest species collected in my study (Oscinella frit, Meromyza americana, and Liohippelates pallipes) were collected in samples from E+ plots than from E- plots. Additional studies are needed to determine what effect planting E- tall fescue might have on the pest status of these species. The two live plant feeders, O. frit and M. americana, may contribute to the poor performance of E- pasture and to the difficulties often encountered in establishing E- stands.

It is difficult to say with certainty what effect lowered population densities of beneficial (scavenging) chloropids have on E+ stands over time. In my study, scavenging chloropids collected in E+ plots might have emerged from weed tissues present in the research plots, or

adult flies may have flown from one plot to another. Emergence trap data are indicative of less chloropid reproduction in E+ plots than E- plots.

Laboratory studies of Rhopalopterum carbonarium confirmed that this common species feeds on dead and decaying plant material. The short life cycle of this species, and the fact that it is not host-specific, suggest that it might be a useful laboratory insect for studies of endophyte-mediated herbivore resistance. Additional study is needed for several of the species of chloropids collected in my study to determine their life cycles and biologies. By investigating the interactions of chloropid species with tall fescue, further insight might be gained into the mechanisms of herbivore resistance exhibited by Acremonium-infected grasses.

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