12-1993

Biological assays to detect seasonal differences in toxicity among four tall fescue clone pairs

John D. Harmuth

To the Graduate Council:

I am submitting herewith a thesis written by John D. Harmuth entitled "Biological assays to detect seasonal differences in toxicity among four tall fescue clone pairs." 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.

Charles D. Pless, Major Professor

We have read this thesis and recommend its acceptance:

Kimberly Gwinn, Reid Gerhardt

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

To the Graduate Council:

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BIOLOGICAL ASSAYS TO DETECT SEASONAL DIFFERENCES IN TOXICITY AMONG FOUR TALL FESCUE CLONE PAIRS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

John D. Harmuth

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ACKNOWLEDGEMENTS

Many individuals contributed toward the completion of this thesis. I would like to thank my major advisor, Dr. Charles Pless, for his guidance and encouragement. My committee members, Dr. Kimberly Gwinn and Dr. Reid Gerhardt, played an integral role in this thesis through their suggestions and constructive editing. I would also like to express my gratitude to Veronica Gibson for maintenance of Drosophila cultures and assistance in the bioassays. I am indebted to Anita Gavin for administering PAS-ELISA tests for the presence of endophyte in tall fescue. Finally, I wish to recognize the Department of Entomology and Plant Pathology in my sincere appreciation for providing a research assistantship and the opportunity to earn the Master of Science degree.

ABSTRACT

Toxicity was compared among four tall fescue clone pairs. Seasonal comparisons were made by measuring development of fruit flies, Drosophila melanogaster Meigen, in diets prepared with endophyte-infected (E+) and endophyte-free (E-) tall fescue leaf powder. Toxicity was also evaluated by observing the behavior of the corn flea beetle, Chaetocnema pulicaria Melsheimer, on E+ and E- tall fescue leaf segments.

Clone pairs developed at The University of Tennessee were designated as 31, 38, 39 and 48. Diets containing 38E+ tall fescue had less fruit flies than other diets during most of the year. Diets containing 39E+ tall fescue had fewer fruit flies than other diets in summer. Diets containing 31E+ had more fruit flies than other E+ diets in spring. Toxic effects of all E+ diets diminished in winter.

In no-choice bioassays, corn flea beetles were found on E- tillers more often than all E+ tillers except 39E+. Amount of feeding was similar on E+ and E- tillers of clone pair 39; whereas, E+ tillers of other clones had less feeding than the respective E- tillers.

Since contrasting levels of toxicity occur in E+ plants, a variety of phenotypes may be expected in a pasture. Results of this study show the importance of evaluating several clone pairs before a particular variety is selected for livestock forage.

TABLE OF CONTENTS

LIST OF FIGURES

1. INTRODUCTION

The fungal endophyte, Acremonium coenophialum Morgan-Jones and Gams, exists symbiotically within tall fescue, Festuca arundinacea Schreber (Schardl et al. 1991). Tall fescue is grown primarily as forage for cattle, but is also used in lawns and for erosion control (Fribourg et al. 1991). The association between endophyte-infected (E+) grass and the fungus confers a competitive advantage over endophyte-free (E-) grasses (Read and Camp 1986). Alkaloid toxins produced by the endophyte have been implicated in resistance to invertebrate and vertebrate herbivores (Siegel et al. 1987). Fescue toxicosis is a disease of livestock which graze on infected fescue. Annual losses to the United States cattle industry have been estimated at over $600 million (Fribourg et al. 1991).

One solution to the fescue toxicosis problem is the development of a vaccine which would alleviate signs of toxicosis. Another solution is the development of a pasture cultivar which is resistant to herbivore pests while reducing clinical signs of mammalian toxicity. Before programs that support E- pasture grass renovation are enacted, host plant resistance studies are needed to determine the benefits of natural insect suppression.

Much of the research on herbivore resistance has regarded all E+ tall fescue as similar in toxicity. Less

literature is available on the importance of plant or endophyte genotypes in the grass/endophyte complex (Breen 1992, Hill 1990). Interactions between specific plants and their accompanying endophyte may have a significant impact on the eventual development of a suitable cultivar. The objective of my research was to determine if significant differences existed among four genotypes of tall fescue in E+/E- clone pairs.

Insects from two orders were used to bioassay differences within and among the genetically identical members of each clone pair. The common laboratory fruit fly, Drosophila melanogaster Meigen (Diptera: Drosophilidae), was utilized because it provided a good model for toxicity studies and is highly amenable to routine culture (Cole et al. 1990). Toxicity among the clone pairs was evaluated as fruit fly developmental time changed depending upon the season. The corn flea beetle, Chaetocnema pulicaria Melsheimer (Coleoptera: Chrysomelidae), was employed as an indicator organism since prior research showed that an inverse correlation existed between beetle abundance and endophyte infestation level (Kirfman et al. 1986).

2. LITERATURE REVIEW

TALL FESCUE

Tall fescue, Festuca arundinacea Schreber, is a perennial grass which was probably introduced into the United States as a contaminant of grass seed from Europe (Fribourg et al. 1991). Evaluation of an ecotype of the grass was initiated in 1931 at the University of Kentucky. Tall fescue received little notoriety until two varieties of the grass were released as 'Alta' and 'Kentucky-31' (K-31) by Oregon and Kentucky, respectively (Cowan 1956). Some of the qualities which made the K-31 cultivar readily welcomed by farmers at the time were its dependability, adaptability, and year-round grazing, which other cool-season grasses could not provide (Buckner et al. 1979).

Other features of the grass that undoubtably helped increase its popularity were its ability to withstand environmental stresses and pest damage as well as provide erosion control. In addition to its agricultural importance as a pasture grass for cattle, tall fescue is grown for its conservational and recreational value (Fribourg et al. 1991). In the United States, tall fescue ranges from Florida to Canada and into certain regions of the Northwest. Although the grass is widespread, it currently predominates the transition zone of the southeastern United States

(Buckner et al. 1979). Today, tall fescue occupies about 12-14 million hectares, and Kentucky-31 is the predominant cultivar.

FESCUE ENDOPHYTE

The common endophyte of tall fescue was originally described as Epichloe typhina (Fr.) Tulasne. A form similar to the asexual state of Epichloe typhina has been reclassified into a new binomial, Acremonium coenophialum Morgan-Jones and Gams. It is placed among a group of clavicipitaceous fungi that belong to the tribe Balansiae. The fungus, an obligate mycosymbiont, lives within tall fescue (Schardl et al. 1991). In one study, endophyte-infected plants were found in 94% of the pastures sampled in the United States. In those infested pastures, an average of 58% of the plants were infected with the endophyte (Shelby and Dalrymple 1987).

Visual detection of an infected plant is not possible without microscopic examination. Hyphae of A. coenophialum are located intercellularly in the mesophyll of the leaf sheath, and the highest concentration occurs at the base of the sheath. The endophyte was not found in tissues of the roots and leaf blades (Hinton and Bacon 1985). In spring, the fungus invades the flower and may then be found between the scutellum and endosperm of the seed. The fungus is

disseminated naturally only via seed from an E+ plant (Hinton and Bacon 1985).

FESCUE\ENDOPHYTE INTERACTIONS

The endophytic fungus which infects tall fescue is an obligate biotroph since it must satisfy its nutritional requirements from the host plant. Regions of high endophyte concentration in the plant act as "sinks" for accumulation of nitrogenous and carbohydrate nutrients (Siegel et al. 1987). Tall fescue also provides the endophyte with a growth substrate as well as its only means of dissemination (Siegel et al. 1987).

Although A. coenophialum derives its nutrition from the host plant, the relationship is considered to be mutualistic rather than parasitic. Host benefits derived from the association include growth stimulation, improved survival, and drought tolerance. Leaf rolling is more common among E+ plants under drought stress than conspecifics (Arachevaleta et al. 1989). Seeds from infected tall fescue have a higher germination rate than those from E- plants (Clay 1987). Infected tall fescue also had more enhanced growth than E- plants (Arachevaleta et al. 1989, Clay 1987). Tall fescue infected with A. coenophialum produces more forage than E- fescue, indicating a competitive advantage for E+ tall fescue in the field (Read and Camp 1986).

ALKALOID TOXINS

Five classes of anti-herbivore alkaloids known to be produced in the grass-endophyte complex include ergot, paxilline indole, pyrrolizidine, azaindolizine, and diazaphenanthrene alkaloids (Bush et al. 1993). Alkaloids are found in both leaf blades and roots where the endophyte does not exist; this suggests that alkaloids are translocated from sites of synthesis in the leaf sheath and stem. Since N-formyl loline is found in the roots, translocation must occur in phloem, but it may also move upwardly in xylem (Bush et al. 1993).

Ergopeptine alkaloids were detected in E+ tall fescue by tandem mass spectrometry (Yates et al. 1985). Ergopeptide alkaloids accounted for 10 to 50 percent of the total ergot alkaloid concentration in fescue tissues, and ergovaline represented more than 80% of the ergopeptides extracted (Lyons et al. 1986). Quantitative seasonal variation in ergovaline levels in Kentucky-31 fescue were detected with high pressure liquid chromatography (Rottinghaus et al. 1991). Ergot alkaloids have been isolated from Acremonium in vitro (Bacon 1988).

Endophyte-infected tall fescue was also found to contain N-formyl and N- acetyl loline (Bush and Burrus 1988). N-formyl and N-acetyl loline are predominant amides of the pyrrolizidine alkaloid and occur in concentrations

100 to 1000 times higher than ergot alkaloids (Siegel et al. 1989). Loline alkaloids are unique to grasses infected with Acremonium coenophialum (Eichenseer et al. 1991). These pyrrolizidine alkaloids are produced by the plant in response to infection since they are absent in fungal cultures (Bacon and Siegel 1988) and their production appears to be dependent upon an infected host (Jones et al. 1983). Loline alkaloid content was found to decrease with increase in leaf age (Hardy et al. 1986). Environmental factors such as water stress and temperature also have an effect on the accumulation of loline alkaloids in tall fescue (Kennedy and Bush 1983). Nitrogen fertilization has not been positively associated with N-acetyl loline or N-formyl loline accumulation.

FESCUE TOXICOSIS

Use of tall fescue rapidly spread with its increase in popularity, but there were soon reports of illness among livestock feeding on it (Pratt and Davis 1954). The condition commonly known as fescue toxicosis is also referred to as "summer slump" due to symptoms occurring most often during summer months. Some of the several signs exhibited by livestock include elevated body temperature, heat intolerance, and rough hair coat (Stuedemann and Hoveland 1988). Cattle grazing on E+ tall fescue spend more

time cooling themselves; whereas, other forages permit grazing during hot times of the day (Stuedemann and Hoveland 1988). Vasoconstriction of blood vessels in cattle has been induced with ergot alkaloids (Solomons et al. 1989) and N-acetyl loline (Oliver et al. 1990).

Steer weight gains were shown to be depressed in steers which grazed on E+ tall fescue (Hoveland et al. 1983). In another study, average daily gains in steers were lower in spring and summer; whereas, the effects of toxicosis were not expressed in the fall (West et al. 1988). Milk production of both lactating beef and dairy cows has been inversely correlated to high endophyte levels (Stuedemann and Hoveland 1988). Endophyte-infected tall fescue causes reproductive abnormalities in gravid mares (Monroe et al. 1988). Agalactia was induced in mares with a synthetic ergot alkaloid (Ireland et al. 1991).

A severe manifestation of toxicosis known as "fescue foot" is a gangrenous condition characterized by eventual sloughing of the extremities (Bush and Buckner 1973). Another extreme disorder of cattle feeding on E+ tall fescue is bovine fat necrosis. This condition occurs when hard fat masses develop in adipose tissue surrounding the intestines (Stuedemann and Hoveland 1988). Total losses due to toxicosis to the United States cattle industry have been estimated at over $600 million annually (Fribourg et al. 1991).

RESISTANCE TO INVERTEBRATES

Endophyte-mediated resistance in E+ tall fescue also occurs on insect herbivores. Although the mode of action remains unclear, plant resistance may arise from antixenotic properties or antibiotic reactions in which insects undergo developmental abnormalities (Clay 1989). Moreover, resistance to insect herbivory by specific alkaloids of E+ tall fescue has been demonstrated. Some ergot alkaloids appeared to function as antibiotics (e.g. ergonovine), feeding deterrents, or both (e.g. ergotamine) against the fall armyworm, Spodoptera frugiperda (J.E. Smith) (Clay and Cheplick 1989). Naturally-occurring loline alkaloid compounds, such as N-formyl and N-acetyl loline, had LC_{50} values against greenbug, Schizaphis graminum Rondani, similar to that of the potent insecticide, nicotine sulfate (Riedell et al. 1991). Two other aphids, the bird-cherry oat aphid, Rhopalosiphum padi L. (Latch et al. 1985), and Russian wheat aphid, Diuraphis noxia (Mordvilko) (Clement et al. 1990), were deterred from feeding on E+ tall fescue. The large milkweed bug, Oncopeltus fasciatus (Dallas), was deterred by the presence of the endophyte in tall fescue (Johnson et al. 1985). In a choice test, significantly more Argentine stem weevils, Listronotus bonariensis (Kuschel), preferred untreated diets to those with endophyte mycelium added to them (Prestidge et al. 1985). Abundance of three

species of leafhoppers and the corn flea beetle, Chaetocnema pulicaria Melsheimer, was negatively correlated to endophyte infestation levels (Kirfman et al. 1986).

Soil-borne organisms are also adversely affected by the presence of the endophyte in tall fescue. Japanese beetle larvae, Popillia japonica Newman, which are common pests of turf grasses, survived differentially on E+ and E- tall fescue (Oliver 1990). Survival and growth of first instar Japanese beetles were lower on E+ than on E- tall fescue (Potter et al. 1992). Infection of tall fescue with the endophyte reduced soil-borne nematode populations in field soils. Population densities of Pratylenchus scribneri (Steiner) and Tylenchorhynchus acutus (Allen) were substantially lower in E+ plots than in E- plots (West et al. 1988). Roots of E+ plants had fewer egg masses and eggs of Meloidogyne marylandi Jepson and Golden than E- plants (Kimmons et al. 1990). Antifungal activity of E+ tall fescue on fungi that would otherwise adversely affect E- plants has been reported in cultures (White and Cole 1985, Siegel and Latch 1991) as well as in greenhouse experiments (Blank et al. 1991).

3. MATERIALS AND METHODS

FESCUE MAINTENANCE

Tall fescue plants were transplanted from a greenhouse into outside cold frames on May 12, 1992. Plants were spaced 51 cm apart from their centers. Fescue plants were placed into one of two rows in a five-block randomized design. Plants received water only through natural precipitation except immediately following transplanting and during extremely hot weather conditions. Each plant was treated with approximately 66 grams of a granular slow-release fertilizer following transplanting; thereafter, plants were not fertilized since nitrogen fertilization was shown to increase ergot alkaloid concentration in E+ plants (Belesky 1988, Rottinghaus 1991). Measurements of pH averaging 7.34 were taken from subsamples of the north, middle, and south portions of the plots. Chlorthal dimethyl was applied around the plots at a rate of 7952 ml per 4042 m^2 to inhibit weed seed germination. Glyphosate was also sprayed around the cold frames at a rate of 473 ml per 4042 m^2 to prevent weed encroachment; thereafter, weeds were removed mechanically.

ENDOPHYTE TESTING

Endophyte-infected and endophyte-free clone pairs were developed at The University of Tennessee by treating one of two genetically identical K-31 plants with the fungicide, propiconizole (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole). Four of these clones pairs were arbitrarily designated numbers 31, 38, 39, or 48. Two tillers from each plant were collected and tested for the presence of the endophyte using Protein A sandwich, enzyme-linked immunosorbent assay (PAS-ELISA) (Reddick and Collins 1988). Endophyte infection was confirmed during March of 1992 and again in October of the same year.

MAINTENANCE OF FRUIT FLIES

Stock cultures of wild type fruit flies, Drosophila melanogaster Meigen, and Drosophila Medium® were initially obtained from Ward's Natural Science Establishment Inc., Rochester, NY. Fruit fly cultures were maintained and propagated on medium in 473 ml glass jars in an environmental chamber (25°C, 12L:12D photoperiod). Fruit flies used for bioassay were reared under the same light and temperature regime as flies reared in routine propagation. A piece of moistened Whatman® filter paper (7.5 cm, Grade

362) was placed into each of several 8.8 cm plastic dishes. Approximately 4 grams of Drosophila Medium® were spread evenly onto the filter paper. The medium was saturated with 15 ml of distilled and deionized water (ddw). Approximately 50 adult flies were introduced into each of the dishes. Neonate larvae were picked from the surface of the medium with the aid of a dissecting microscope 4 to 5 days after the introduction of adult flies.

BIOASSAY USING FRUIT FLIES

Bioassays were conducted for the fall, winter, spring, and summer seasons beginning in the fall of 1992. Repeated bioassays, which were contingent upon availability of fescue tissue, were conducted on winter, spring, and summer foliage collections. Samples of foliage were collected on the 25th day of October, January, April, and July. Composite foliage samples from all replicates of each member of each clone pair were harvested separately into plastic bags. Dead leaf material and seed shoots were removed from the foliage. Foliage was stored in a freezer, lyophilized, and ground into powder using a Wiley® mill with a 20-mesh screen. Tall fescue powder was fumigated with propylene oxide to inhibit mold formation and stored in a freezer until needed for bioassay. Tall fescue diet consisted of 4.5 g. of Drosophila Medium® and 1 g. of E+ or E- fescue powder.

These proportions were thoroughly mixed into 120 ml rearing jars to which 20 ml of ddw were added. Ten replicate jars were utilized for each of the eight fescue treatments and one control. Ten neonate fruit flies were added to each jar of diet. Rearing jars were uniformly arranged in a completely randomized design within the environmental chamber (17°C, 18L:6D photoperiod). The number of pupae and adults was monitored daily from the 6th day through the 15th day of each bioassay. Toxicity of each treatment was evaluated by comparisons among diets containing fescue and between the control. An average number of 8 pupae in the control was required by Day 15 of the bioassay in order for the bioassay to be considered valid.

BIOASSAY USING CORN FLEA BEETLES

Approximately 100 corn flea beetles, Chaetocnema pulicaria Melsheimer, were aspirated from Johnsongrass or field corn from the Knoxville Agricultural Experiment Station on each collection day. Each clone pair was bioassayed as follows: Four beetles were placed into each of twenty vials and deprived of food 24 hours prior to the introduction of tall fescue. Corn flea beetles found dead after the deprivation period were replaced with alternate beetles collected and deprived of food during the same period. Two basal leaf segments, each 5 cm long, were

removed from each of the five plants of the treatment. One leaf segment was added to each vial containing corn flea beetles. Mortality and the number of corn flea beetles on fescue tillers was determined 24 and 48 hours after the introduction of tall fescue. Scarification on both sides of the leaf caused by corn flea beetle feeding was measured after 48 hours using a Mini-scale® under the dissecting microscope. Bioassays were performed during July and August, 1992. Clone Pair 39 was tested again in early September. Clone pairs 31 and 38 were tested again in July, 1993.

STATISTICAL ANALYSIS

Data collected from bioassays using fruit flies were analyzed with ANOVA and Duncan's Multiple Range Test ($P < 0.05$) (SAS Institute, 1987). Corn flea beetle mortality and antixenosis data were analyzed using ANOVA and t test. Corn flea beetle feeding data were analyzed using ANOVA and t test as well as Duncan's Multiple Range Test ($P < 0.05$) (SAS Institute, 1987).

4. RESULTS AND DISCUSSION

GENERAL OBSERVATIONS

Endophyte-infected plants had morphological and developmental differences in comparison with their E- counterparts. Endophyte-infected plants produced seedheads up to two weeks earlier than the E- conspecifics (Arachevaleta et al. 1989). More seedheads were produced by clone pair 39 plants; however, there was never any variation in number of seedheads, or any other visible characteristic, between E+ or E- plants within a clone during this research. Every plant from all clone pairs produced seedheads during the spring. More profuse production of seedheads from all clone pair 39 plants during the rest of the year suggested that this was an expression of the plant genotype rather than an influence of the endophyte.

FRUIT FLY BIOASSAY

Fall Bioassay. Fewer larvae developed to the pupal stage on diets containing 38E+ fescue than on all other diets by Day 6 (Fig. 1A). Diets containing 38E+ fescue had no fewer pupae than other E+ diets following Day 6. In all diets, adults began to emerge on Day 8. The control was

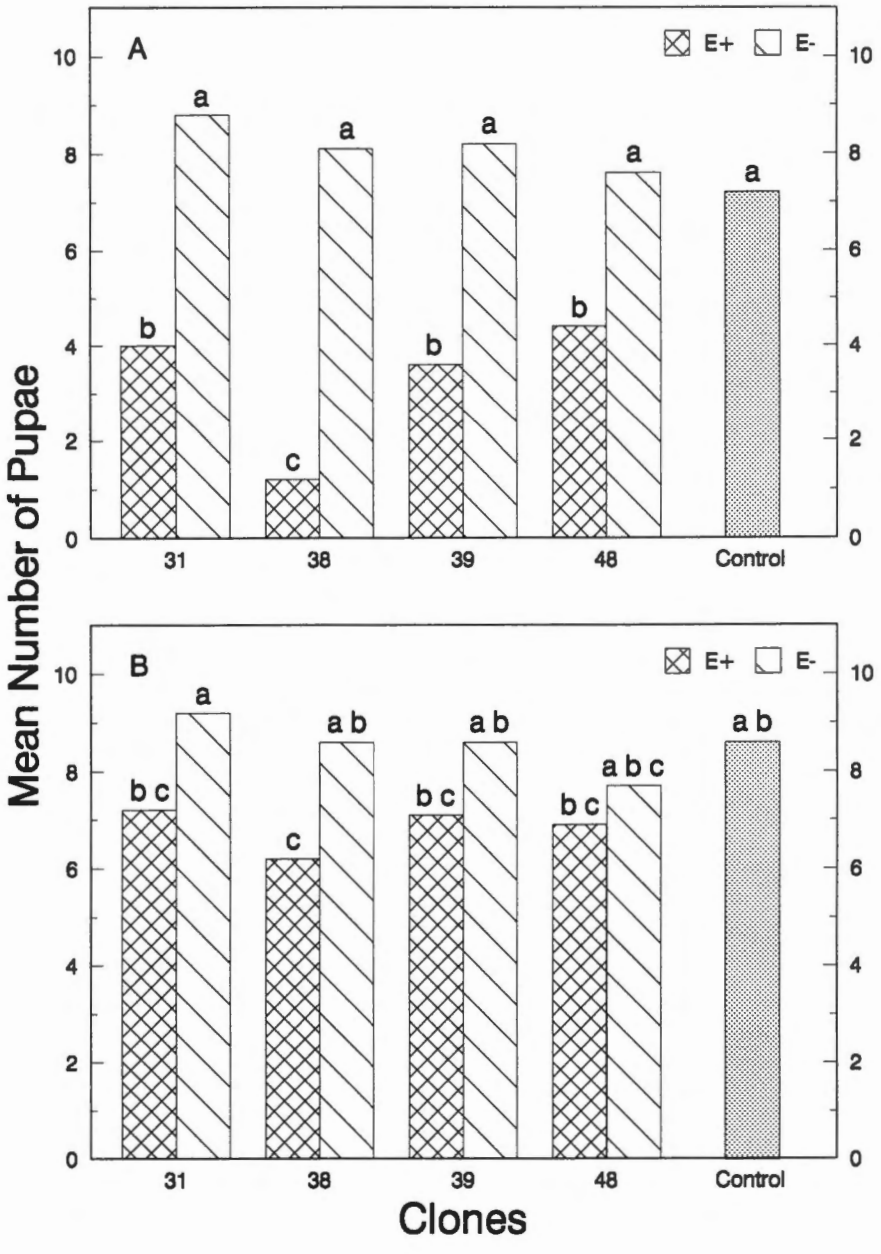

Fig. 1. Number of Drosophila melanogaster which developed from larvae into pupae on control diet or diets containing endophyte-infected (E+) or endophyte-free (E-) tall fescue leaf powder on Day 6 (A) and Day 10 (B) of the fall bioassay. Means with the same letters are not significantly different.

significantly different in terms of diet suitability on the following day (Fig. 2A).

Fewer pupae occurred on E+ diets than the control on Day 6. The number of pupae on diets containing 48E+, 31E+, or 39E+ fescue gradually increased and was not significantly different from the control on Days 7, 8, or 9. Fewer pupae developed into adults on all E+ diets on Days 9 and 10. As in pupal development, the number of adults on E+ diets, except 38E+, eventually increased and became statistically equivalent to the control. Occurrence of fewer pupae and adults on E+ diets than the control during certain days of the bioassay indicated retarded development (Figs. 3A and 4A). Significantly fewer adults on diets containing 38E+ fescue compared to the control by the last day of the bioassay suggests that larval mortality was occurring; however, one larva was observed crawling in a culture near the end of the bioassay.

More adults emerged on diets containing 31E- or 38E- fescue than the control on Day 8. More adults emerged on all E- diets than the control on Day 9; thereafter, control diets did not contain a significantly smaller number of adults than E- diets. More pupae and adults occurring on diets containing E- fescue than the control indicated accelerated development (Figs. 3B and 4B). Growth rate might have been enhanced by some nutritional supplement provided by the fescue.

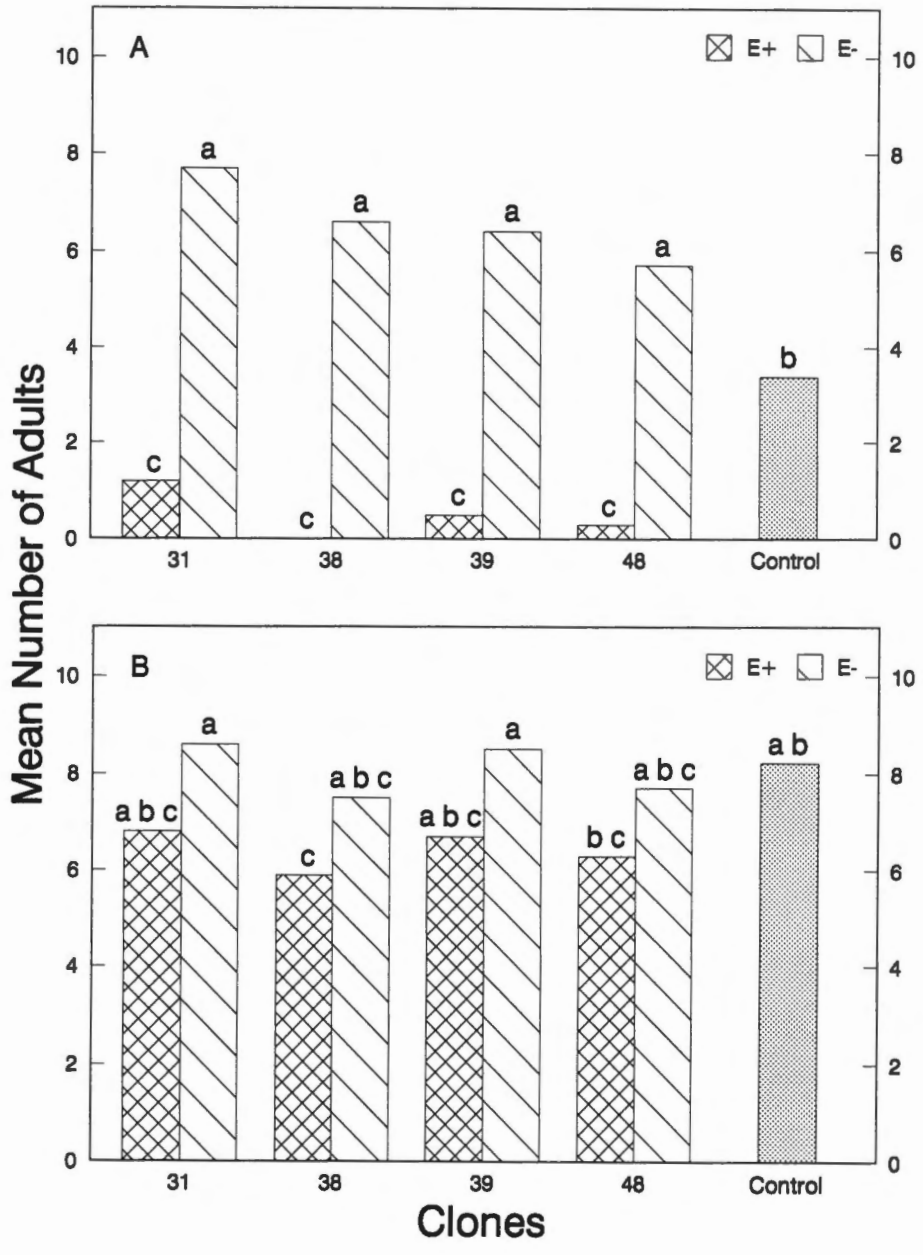

Fig. 2. Number of Drosophila melanogaster which developed from pupae into adults on control diet or diets containing endophyte-infected (E+) or endophyte-free (E-) tall fescue leaf powder on Day 9 (A) and Day 15 (B) of the fall bioassay. Means with the same letters are not significantly different.

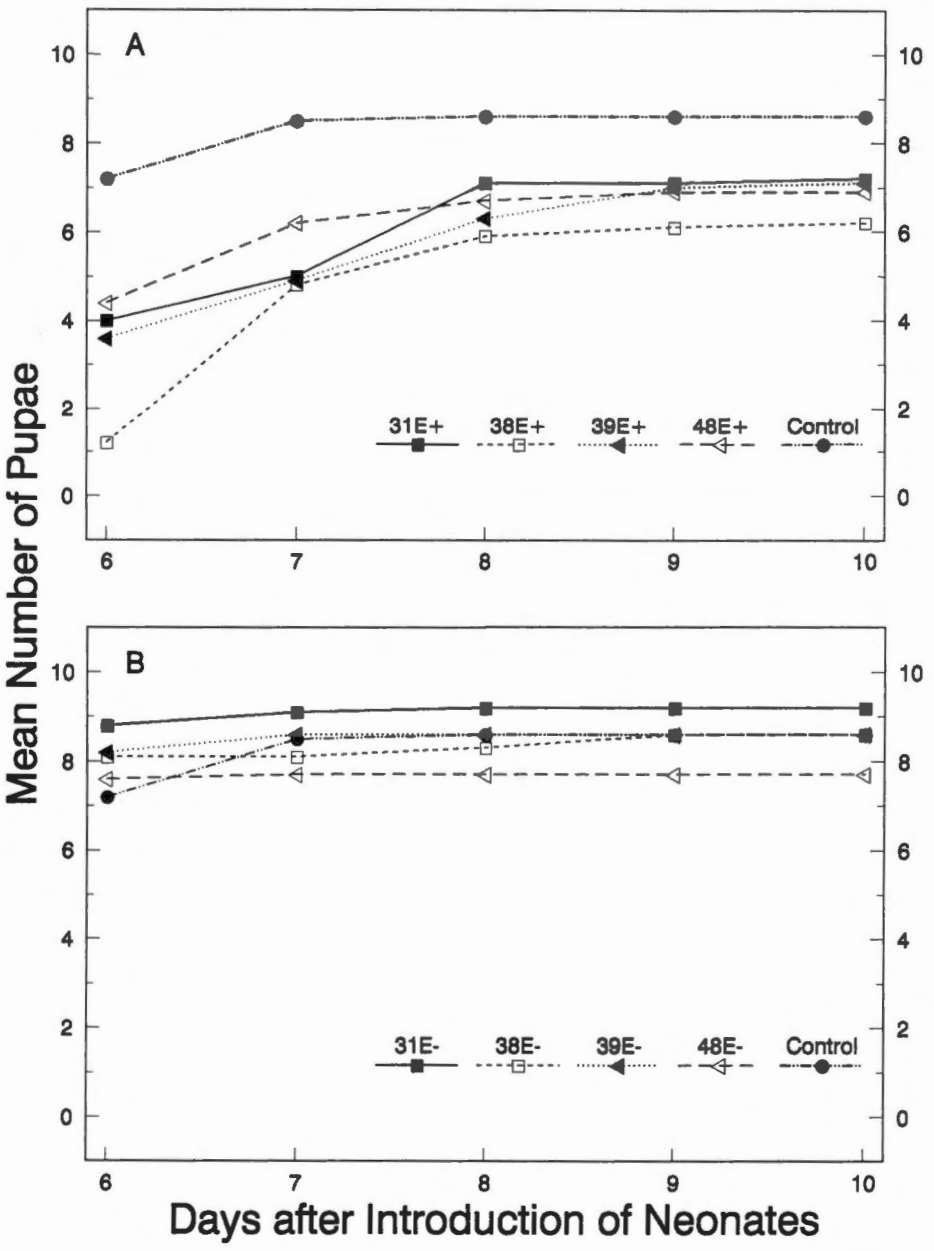

Fig. 3. Number of Drosophila melanogaster which developed from larvae into pupae on diets containing endophyte-infected leaf powder (A) or endophyte-free leaf powder (B) compared to flies on control diet during Day 6 through Day 10 of the fall bioassay.

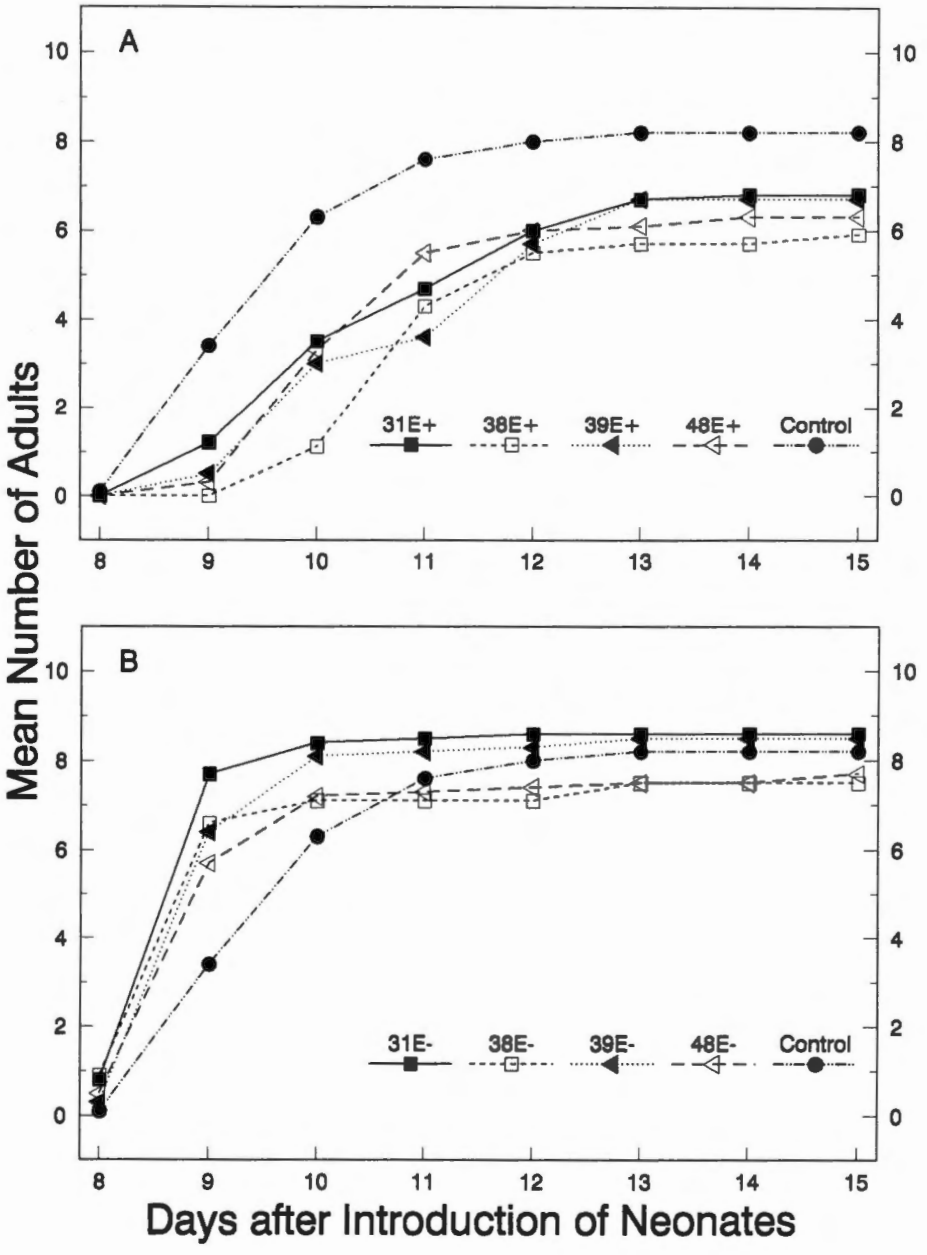

Fig. 4. Number of Drosophila melanogaster which developed from pupae into adults on diets containing endophyte-infected leaf powder (A) or endophyte-free leaf powder (B) compared to flies on control diet during Day 8 through Day 15 of the fall bioassay.

Winter Bioassay. Number of pupae was the same on diets containing 38E+ or 39E+ fescue compared to the respective E- diets during the entire bioassay. The number of pupae was not statistically different among any of the diets on Day 10 (Fig. 5B). Number of adults was significantly different among treatments on Days 11 and 12. After Day 12, number of flies on all diets was not significantly different. Statistically same number of adults on all diets occurred for most of the winter bioassay as exemplified on Day 9 and Day 15 (Fig. 6A and B).

On Day 6, more pupae occurred on diets containing 38E+ or 48E+ fescue than the control. More pupae occurred on diets containing 38E+ fescue than the control on Day 7. More adults emerged on diets containing 38E+ or 48E+ fescue than the control by Day 11. These data indicated a faster rate of development for flies on these diets during winter (Figs. 7A and 8A). Nutritional quality of diets containing fescue may have overrode the toxic effects of E+ fescue during winter.

More pupae occurred on E- diets than the control on Day 6. More pupae occurred on diets containing 31E- or 48E- fescue than the control on Day 7. More pupae occurred on diets containing 31E- fescue on Day 8 as well. More adults emerged on all diets containing E- fescue than the control on at least on day. More adults emerged on diets containing

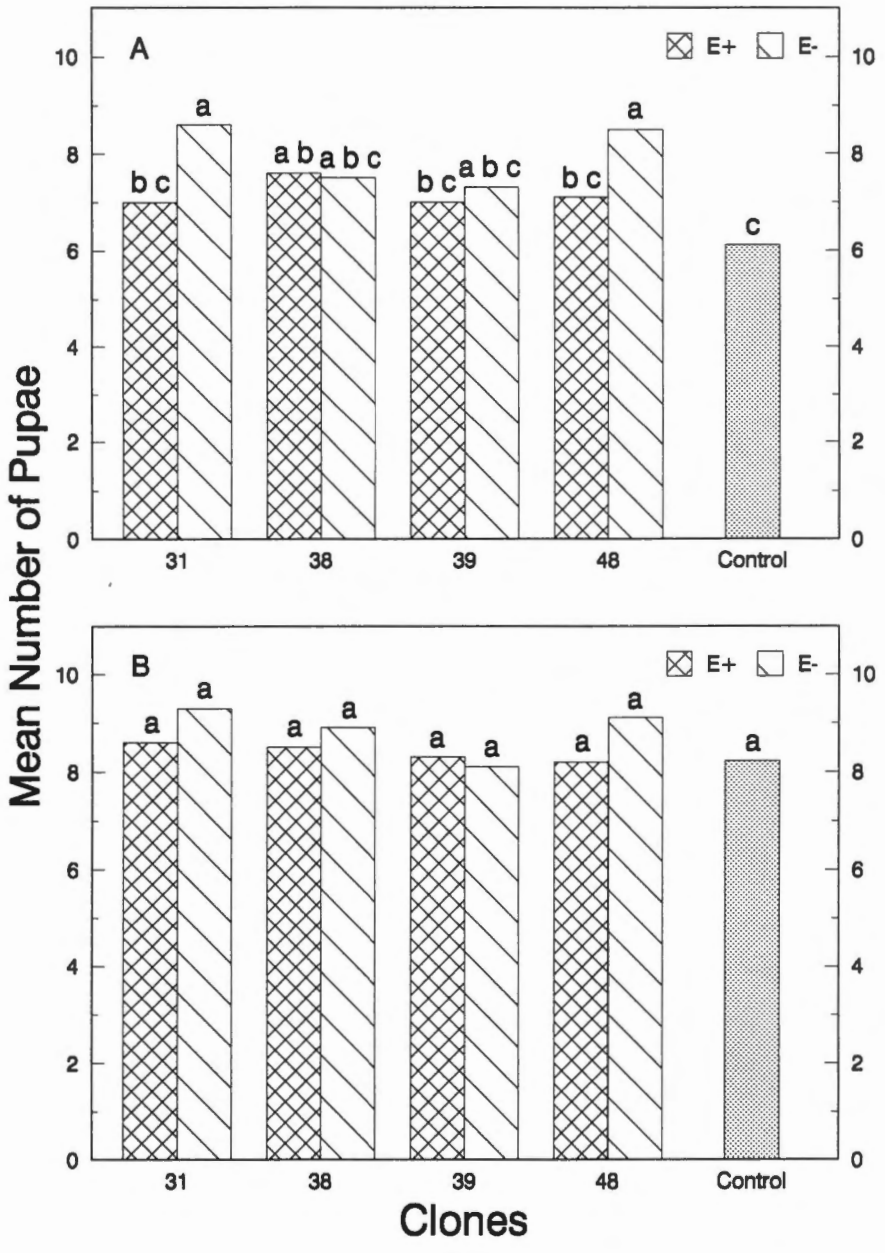

Fig. 5. Number of Drosophila melanogaster which developed from larvae into pupae on control diet or diets containing endophyte-infected (E+) or endophyte-free (E-) tall fescue leaf powder on Day 7 (A) and Day 10 (B) of the winter bioassay. Means with the same letters are not significantly different.

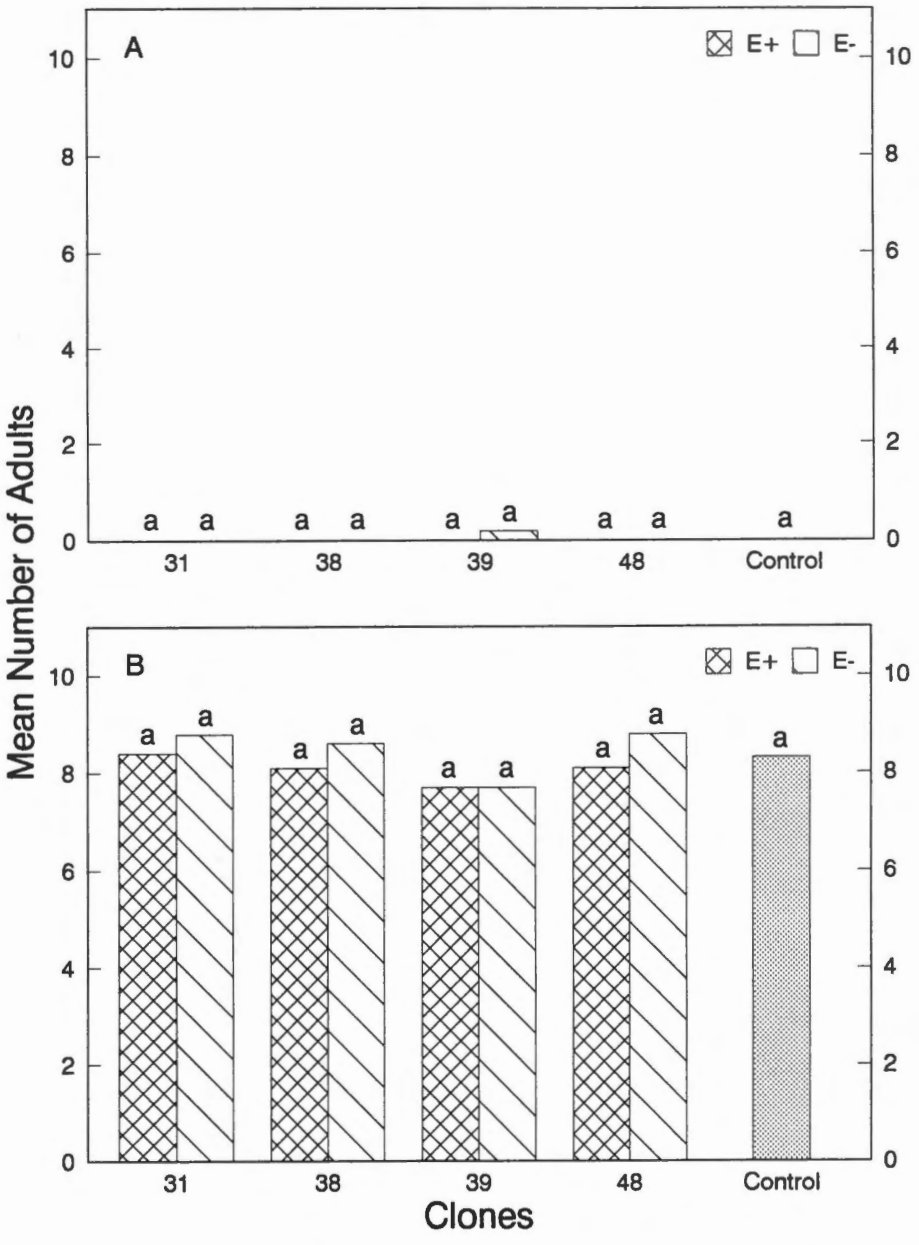

Fig. 6. Number of Drosophila melanogaster which developed from pupae into adults on control diet or diets containing endophyte-infected (E+) or endophyte-free (E-) tall fescue leaf powder on Day 9 (A) and Day 15 (B) of the winter bioassay. Means with the same letters are not significantly different.

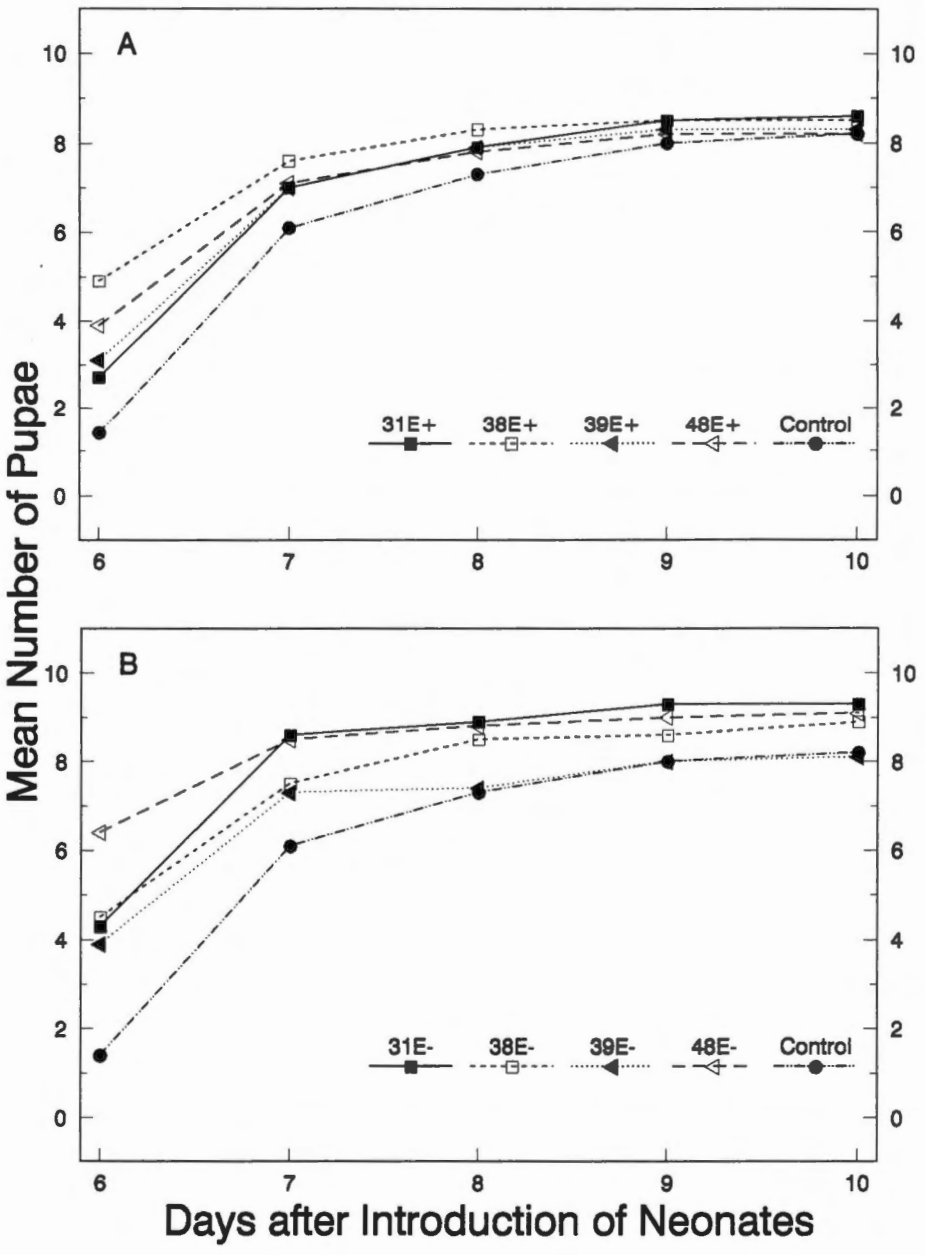

Fig. 7. Number of Drosophila melanogaster which developed from larvae into pupae on diets containing endophyte-infected leaf powder (A) or endophyte-free leaf powder (B) compared to flies on control diet during Day 6 through Day 10 of the winter bioassay.

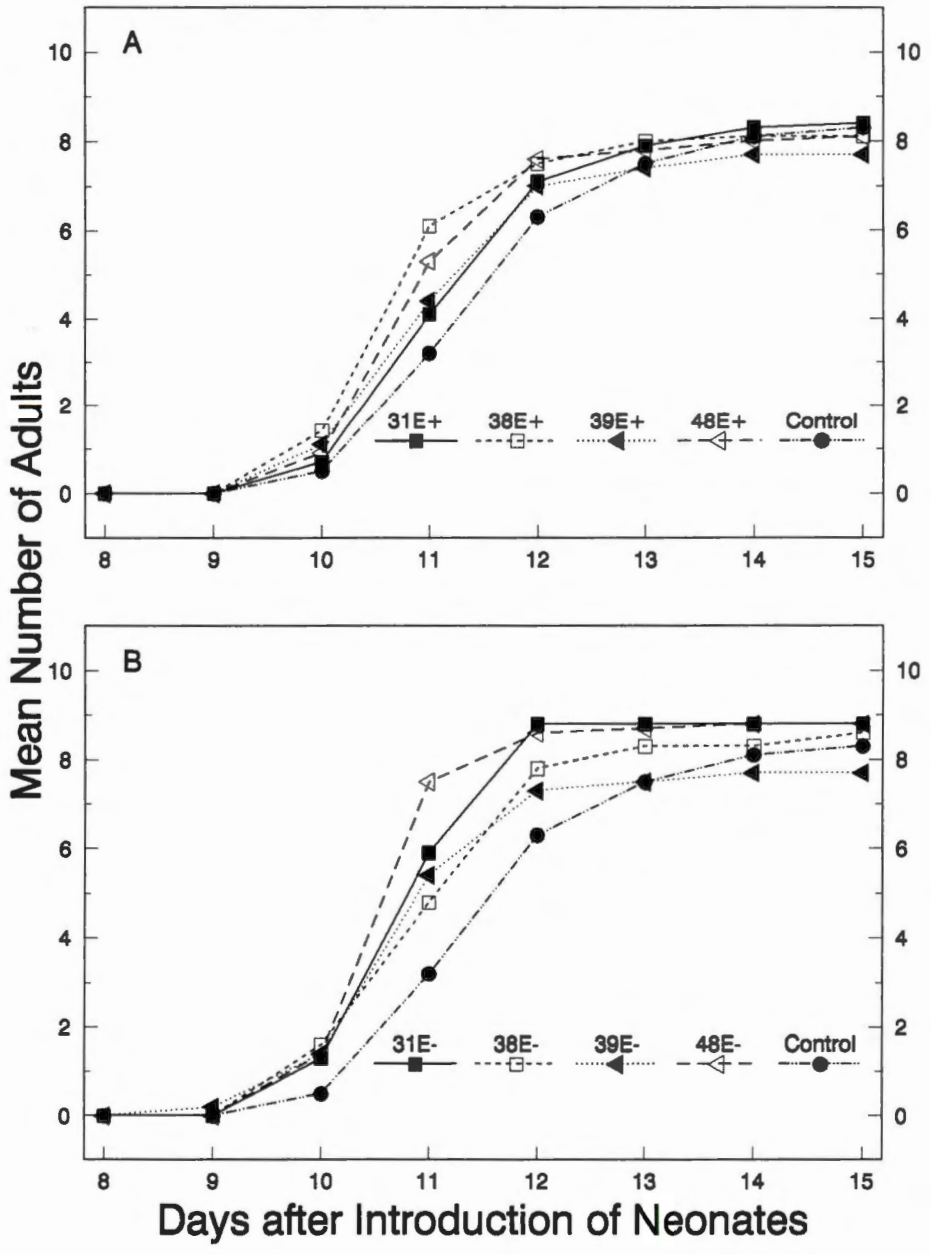

Fig. 8. Number of Drosophila melanogaster which developed from pupae into adults on diets containing endophyte-infected leaf powder (A) or endophyte-free leaf powder (B) compared to flies on control diet during Day 8 through Day 15 of the winter bioassay.

31E- or 48E- fescue than control on Days 11 and 12. Number of adults on all diets was not significantly different after Day 13. Diets containing 31E- or 48E- fescue were most conducive to rapid fly development. Other E- diets were less conducive to accelerated fly development (7B and 8B).

Spring Bioassay I. Number of pupae and adults on the control was statistically different from E+ and E- treatments during spring on Days 7 and 13 (Figs. 9A and 10A). More pupae occurred on diets containing 31E+ fescue than other E+ diets from Day 8 to the end of the bioassay (Fig. 9B). More adults also occurred on 31E+ diet than other E+ diets during and after Day 13 (Fig. 10A and B). In contrast, fewer pupae occurred on diets containing 38E+ fescue compared to other E+ diets (Fig. 9B). Fewer adults emerged on diets containing 38E+ fescue than on other E+ diets on Day 15 (Fig. 10B).

The effect of E+ diets on larval development became apparent on Day 7. By Day 9, the number of pupae on diets containing 31E+ fescue was the only number of flies on E+ diets which was not statistically different from the control. All E+ diets, except 31E+, also had fewer adults than control during and after Day 13. Diets containing 31E+ fescue were the only E+ diets which had as many adults as the control by the end of bioassay. Fewer flies on all

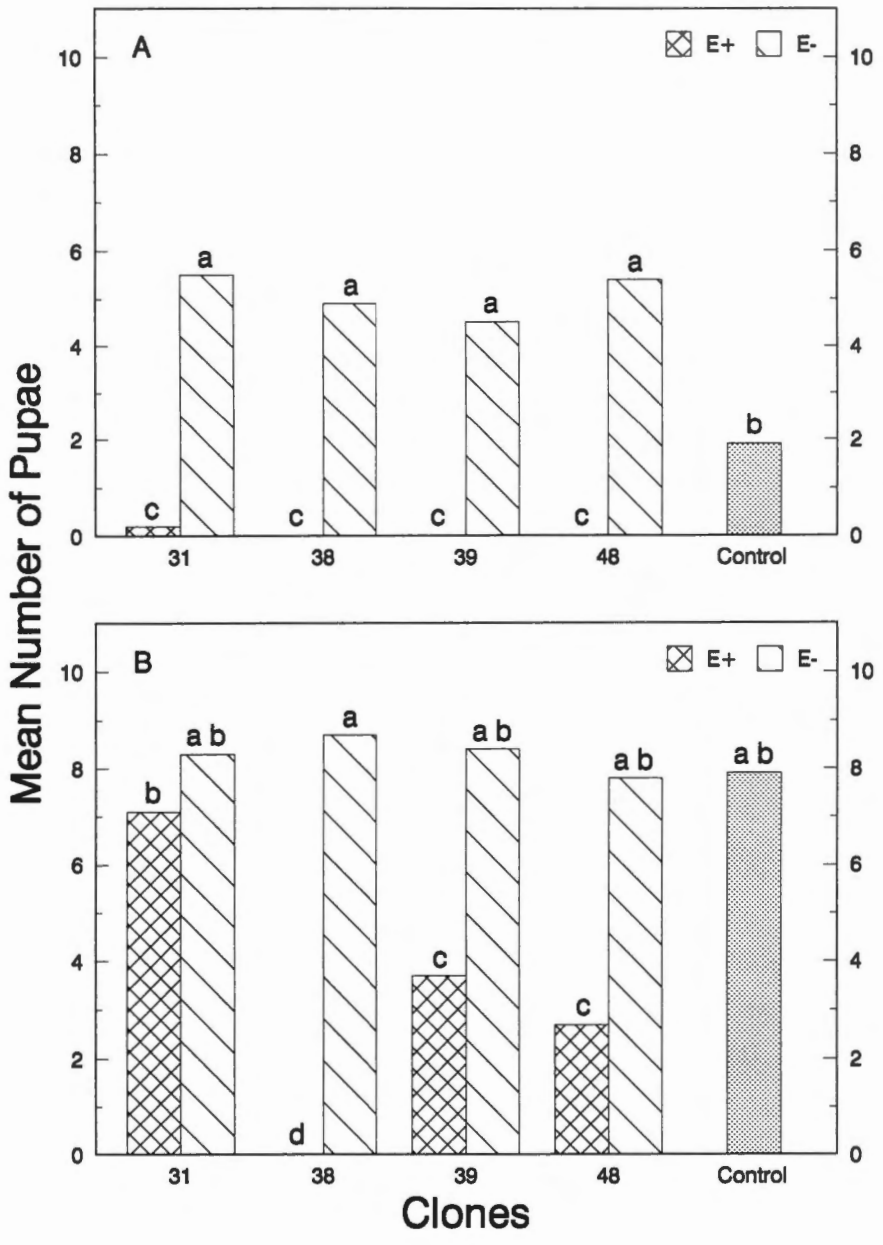

Fig. 9. Number of Drosophila melanogaster which developed from larvae into pupae on control diet or diets containing endophyte-infected (E+) or endophyte-free (E-) tall fescue leaf powder on Day 7 (A) and Day 10 (B) of spring bioassay I. Means with the same letters are not significantly different.

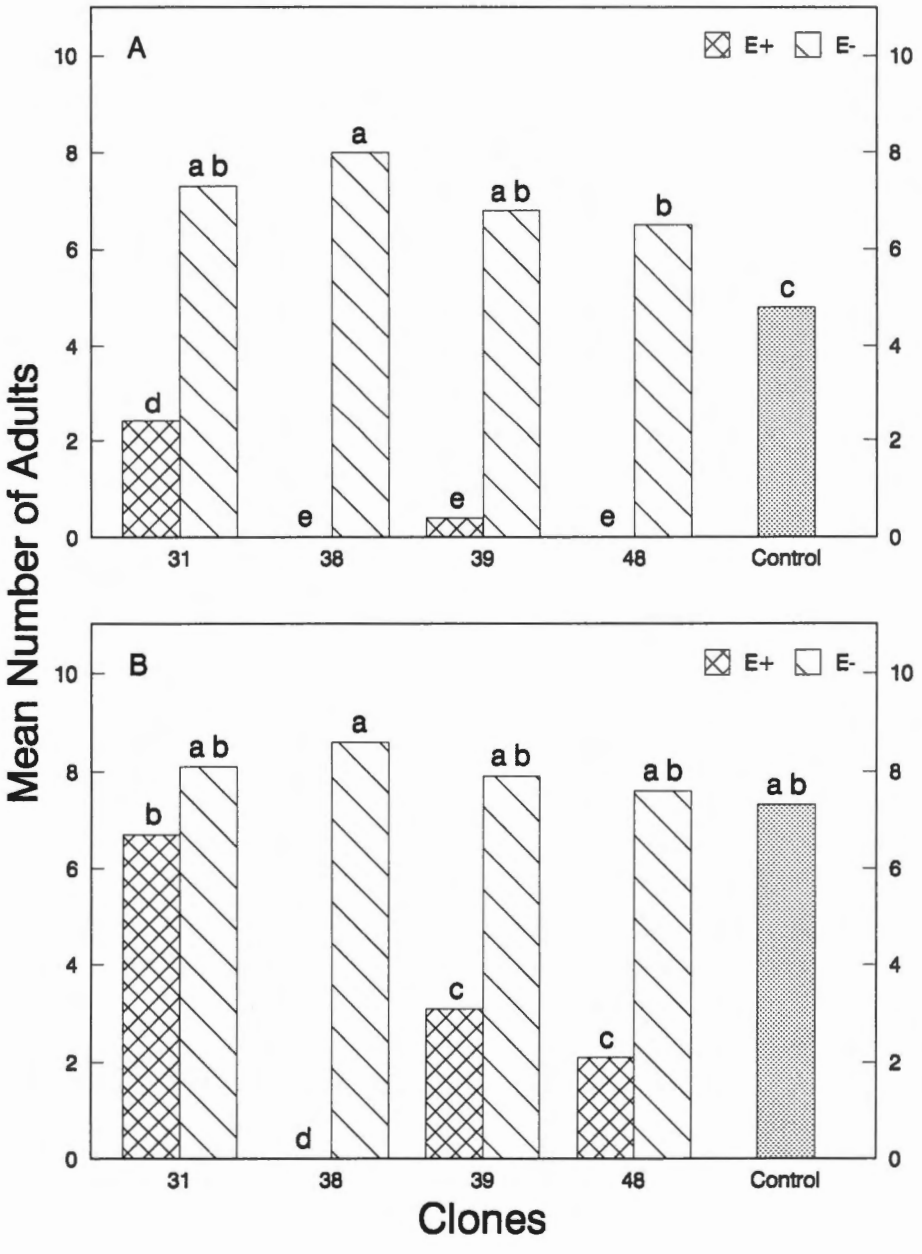

Fig. 10. Number of Drosophila melanogaster which developed from pupae into adults on control diet or diets containing endophyte-infected (E+) or endophyte-free (E-) tall fescue leaf powder on Day 13 (A) and Day 15 (B) of spring bioassay I. Means with the same letters are not significantly different.

other E+ diets by the end of the bioassay attested to the lethal effect of the endophyte (Figs. 11A and 12A).

No developmental lag occurred in flies on E- diets. More pupae occurred on all E- diets than the control on at least one occasion during Days 6, 7, and 8; thereafter, the control and all E- treatments were not significantly different. More adults emerged on all E- diets than the control on at least two consecutive days. More adults emerged on diets containing 38E- fescue than the control during days 12, 13 and 14. Flies on E- diets, therefore, had a faster rate of development than flies on the control (Figs. 11B and 12B).

Spring Bioassay II. A bioassay which was repeated on leaf material collected during spring provided results similar to those obtained from the initial spring bioassay. Although treatments had a similar pattern of toxicity relative to each other, all diets had more pupae and adults during the second spring bioassay than during the initial spring bioassay. Once again, diets containing 38E+ or 31E+ fescue had greater or lesser toxicity, respectively. Fewer pupae occurred on diets containing 38E+ fescue than all other diets from Days 7 through 10 (Fig. 13A and B). Treatment 38E+ was significantly different from all other treatments from Day 12 until the final day of adult counts (Fig. 14A and B). More pupae and adults occurred on diets

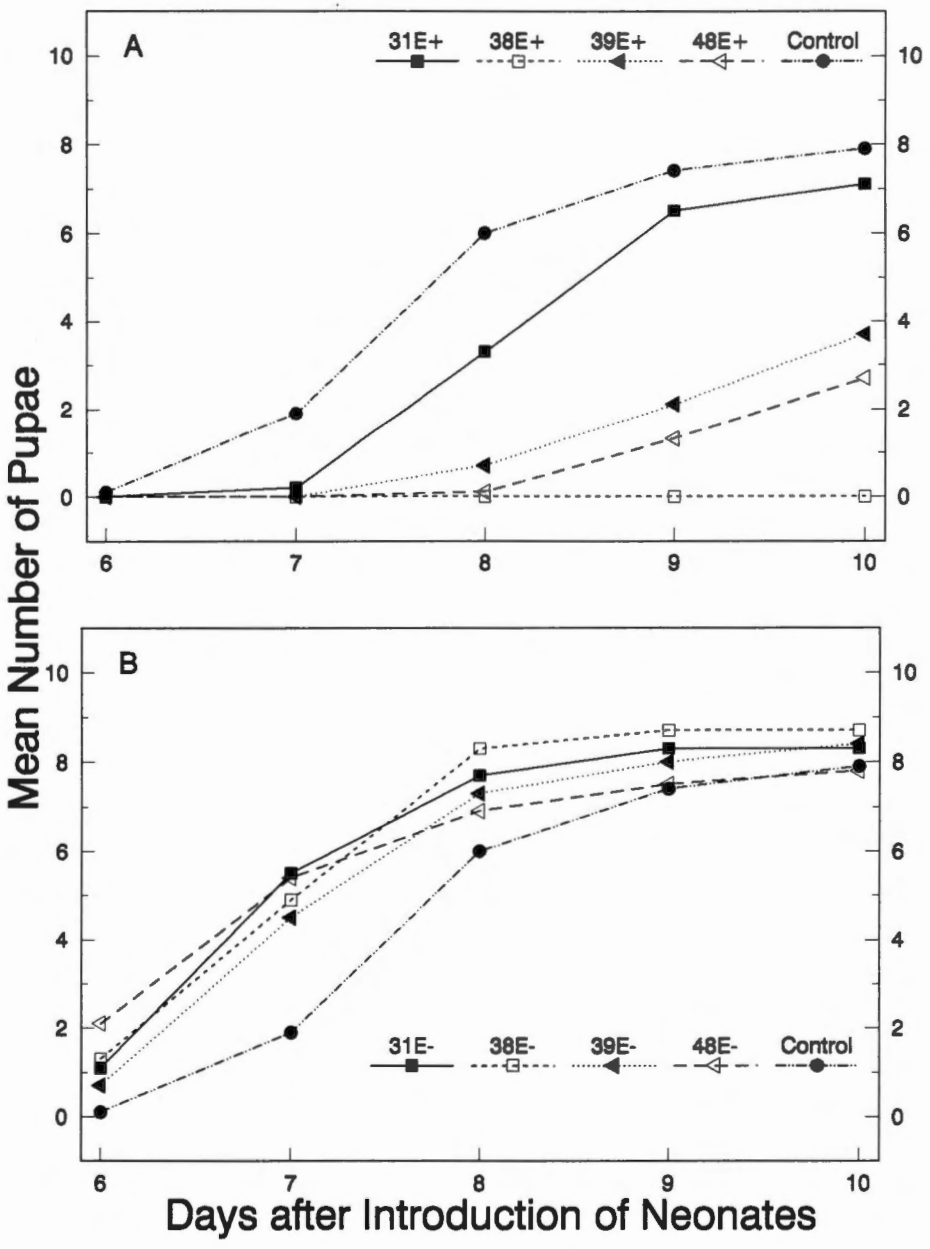

Fig. 11. Number of Drosophila melanogaster which developed from larvae into pupae on diets containing endophyte-infected leaf powder (A) or endophyte-free leaf powder (B) compared to flies on control diet during Day 6 through Day 10 of spring bioassay I.

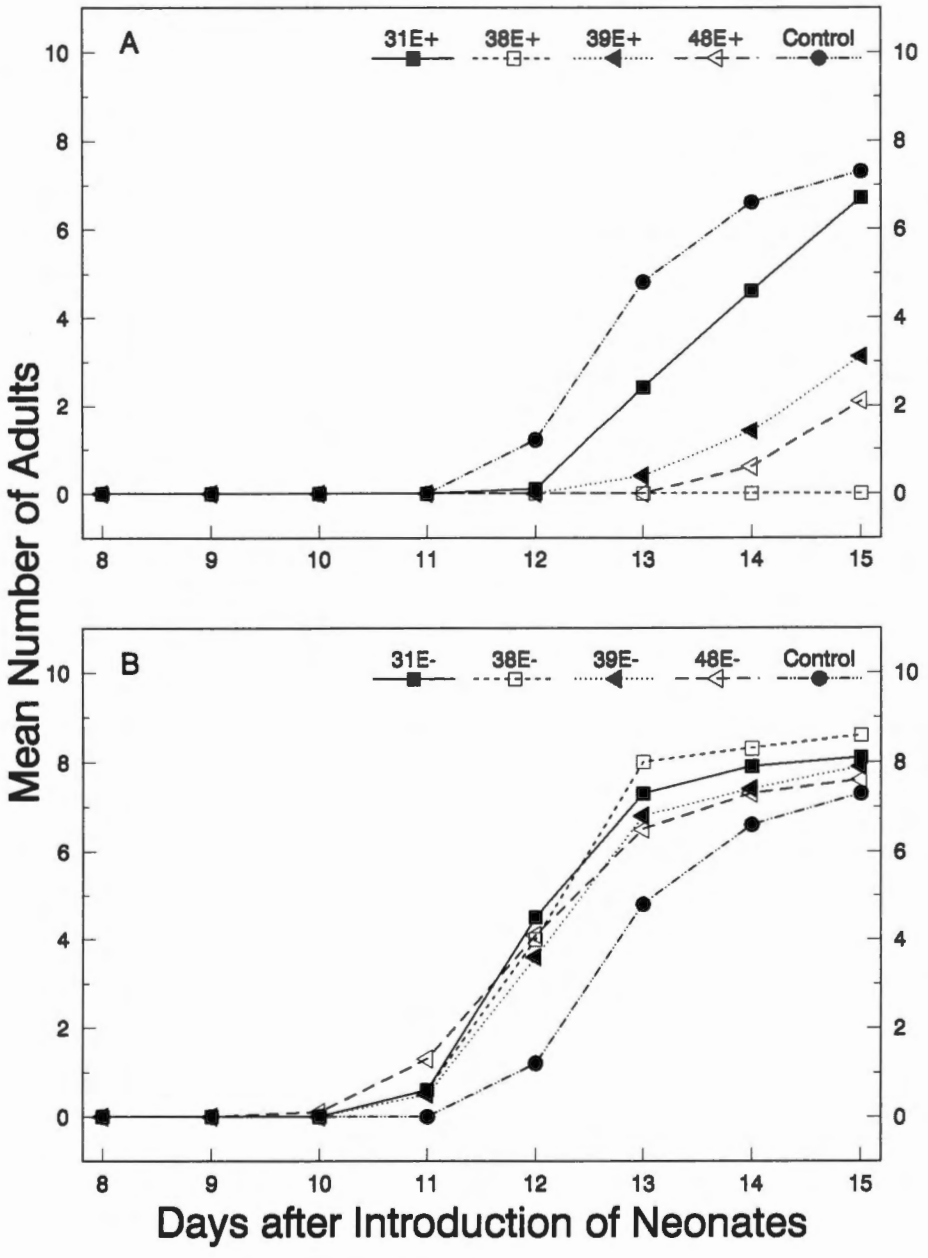

Fig. 12. Number of Drosophila melanogaster which developed from pupae into adults on diets containing endophyte-infected leaf powder (A) or endophyte-free leaf powder (B) compared to flies on control diet during Day 8 through Day 15 of spring bioassay I.

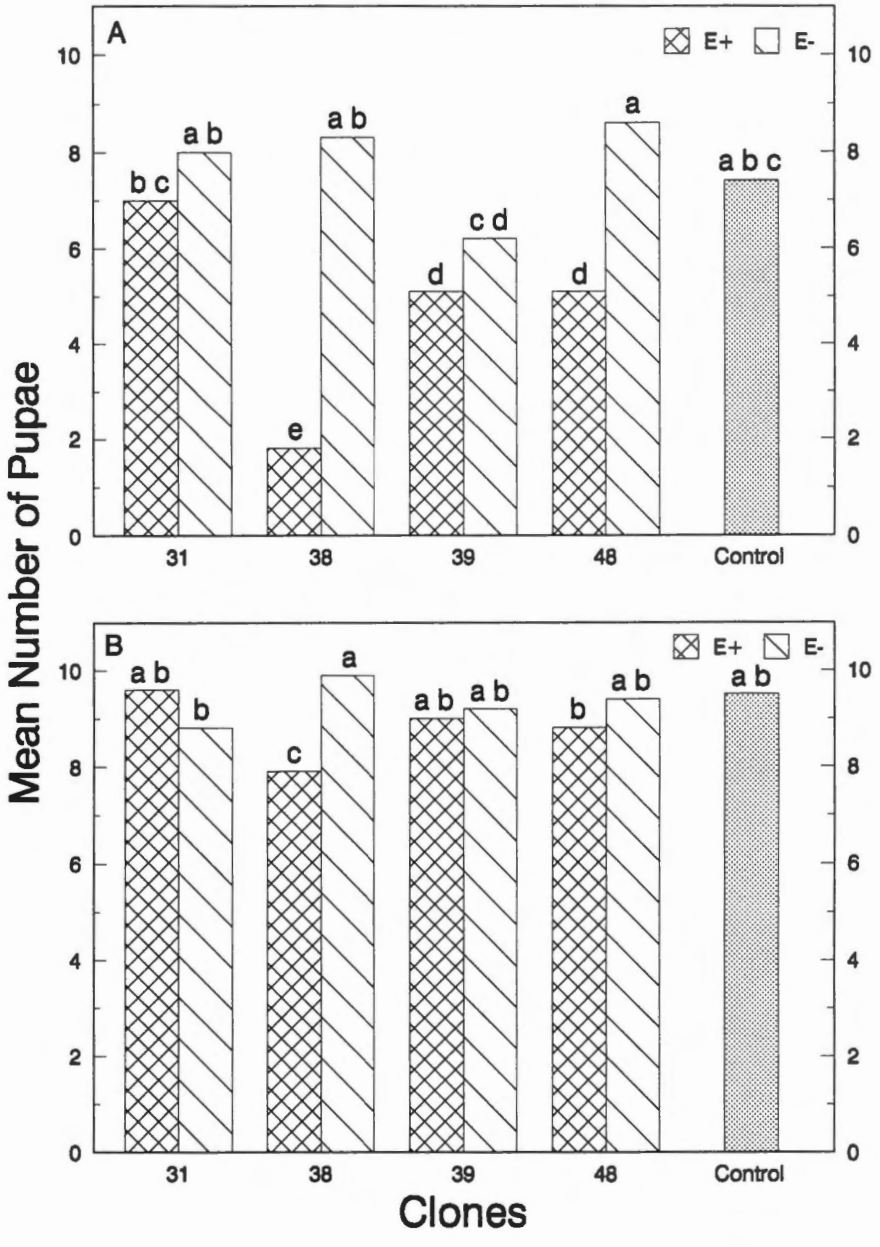

Fig. 13. Number of Drosophila melanogaster which developed from larvae into pupae on control diet or diets containing endophyte-infected (E+) or endophyte-free (E-) tall fescue leaf powder on Day 7 (A) and Day 10 (B) of spring bioassay II. Means with the same letters are not significantly different.

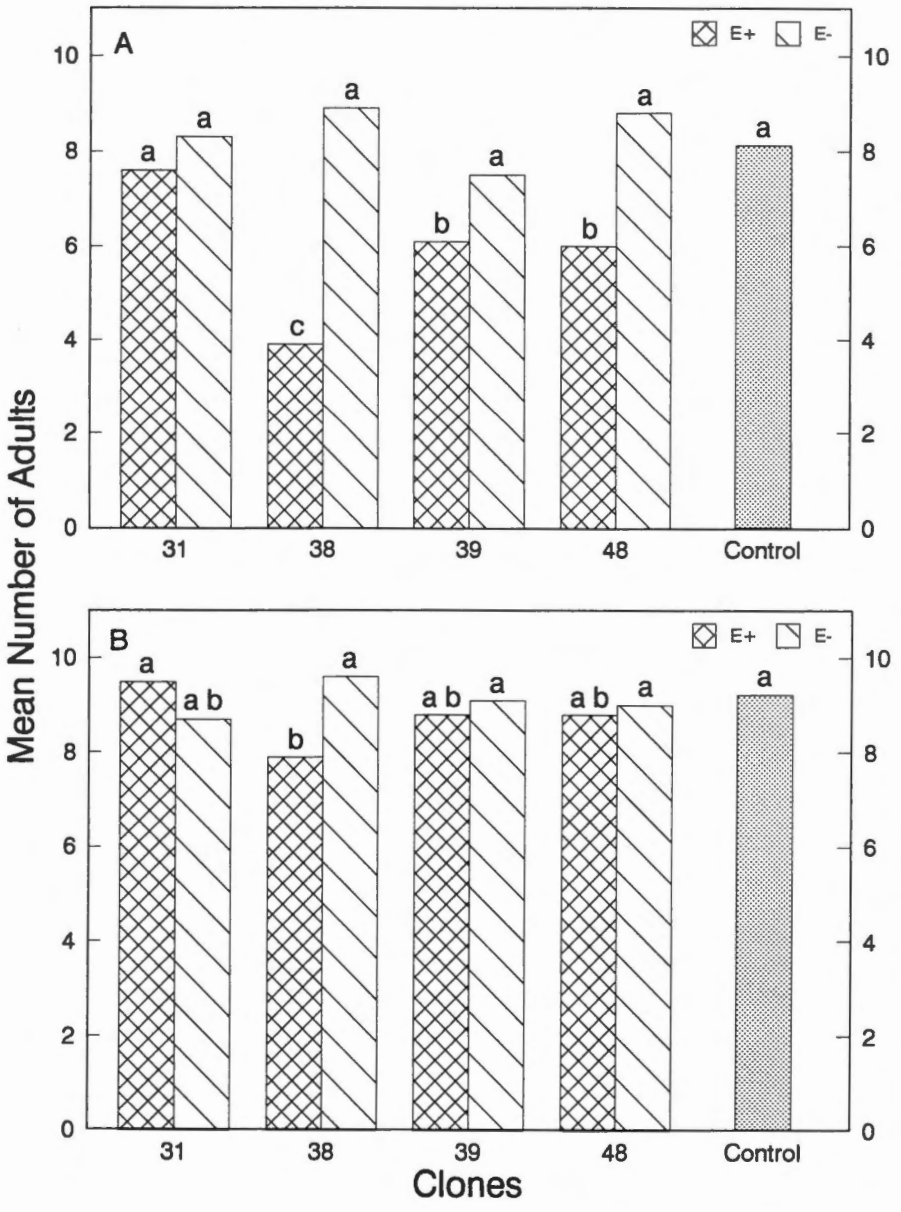

Fig. 14. Number of Drosophila melanogaster which developed from pupae into adults on control diet or diets containing endophyte-infected (E+) or endophyte-free (E-) tall fescue leaf powder on Day 12 (A) and Day 15 (B) of spring bioassay II. Means with the same letters are not significantly different.

containing 31E+ fescue than all other E+ diets on Days 7 and 12 of the bioassay, respectively (Fig. 13A and 14A). Compared to other E- diets, diets containing 39E- fescue had fewer pupae on Day 7 (Fig. 13A) and fewer adults on Day 10. Low number of pupae in 39E+ diets may, in part, have resulted from poor fescue nutritional value rather than solely alkaloid toxins.

Toxic qualities which made diets containing 38E+, 39E+, or 48E+ fescue lethal during spring bioassay I may have been marginal since there were as many adult flies on E+ diets as the control by Day 15 of spring bioassay II. Fewer pupae and adults occurred on 48E+ diets than the control during three consecutive days of each life stage. Fewer pupae and adults occurred on 39E+ diets than the control during two consecutive days of each life stage. Fewer pupae and adults occurred on 38E+ diets for much of the experiment, but had as many adults as the control by Day 15. Diets containing 31E+ fescue never retarded development in either larvae or pupae during the second spring bioassay (Figs. 15A and 16A).

All E- diets, except 39E-, had more pupae than the control on Day 6; thereafter, all E- treatments were not statistically different from one another. All E- diets, except 39E-, also had more adults than the control during at least one day during the second bioassay. Diets containing 39E- fescue were the only E- diets in which flies did not undergo developmental acceleration (Figs. 15B and 16B).

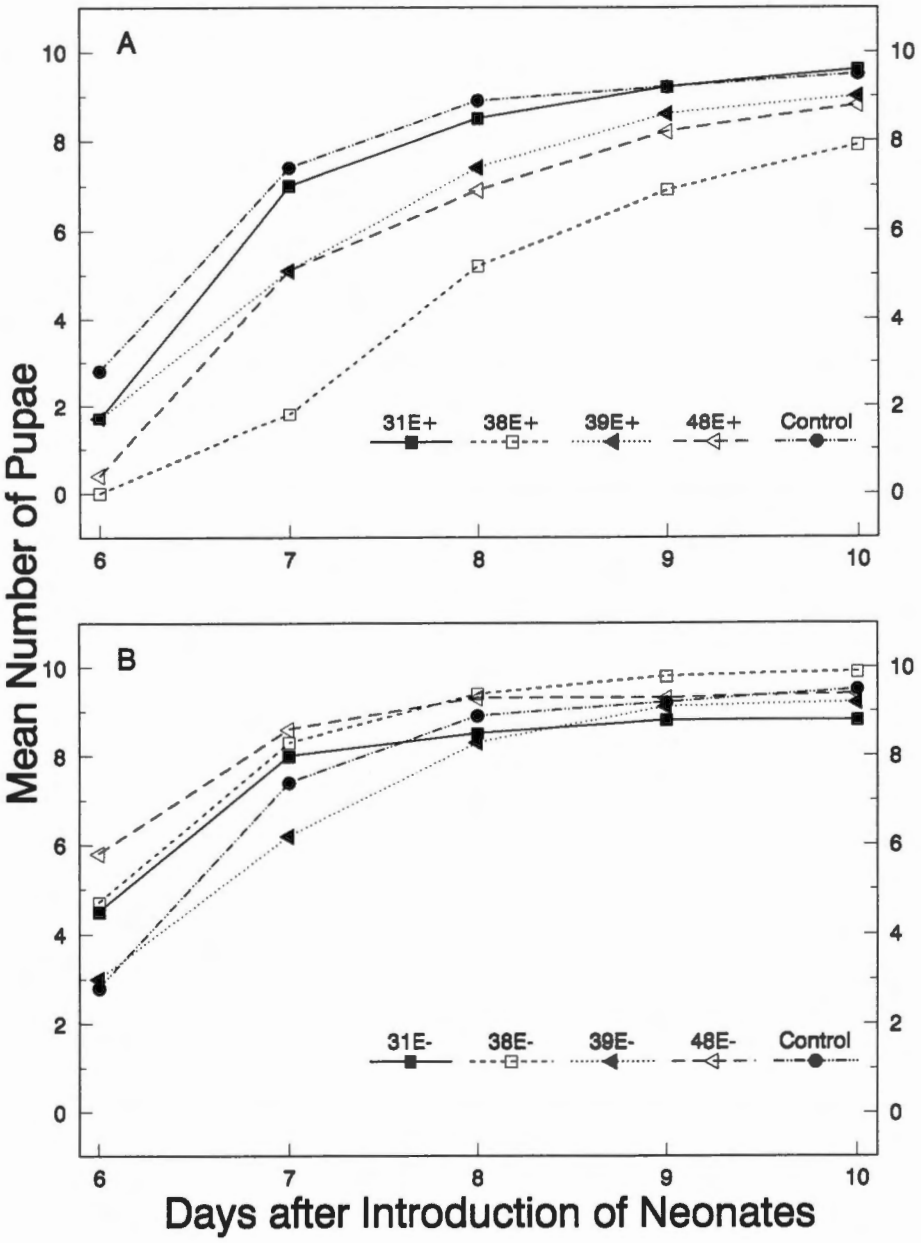

Fig. 15. Number of Drosophila melanogaster which developed from larvae into pupae on diets containing endophyte-infected leaf powder (A) or endophyte-free leaf powder (B) compared to flies on control diet during Day 6 through Day 10 of spring bioassay II.

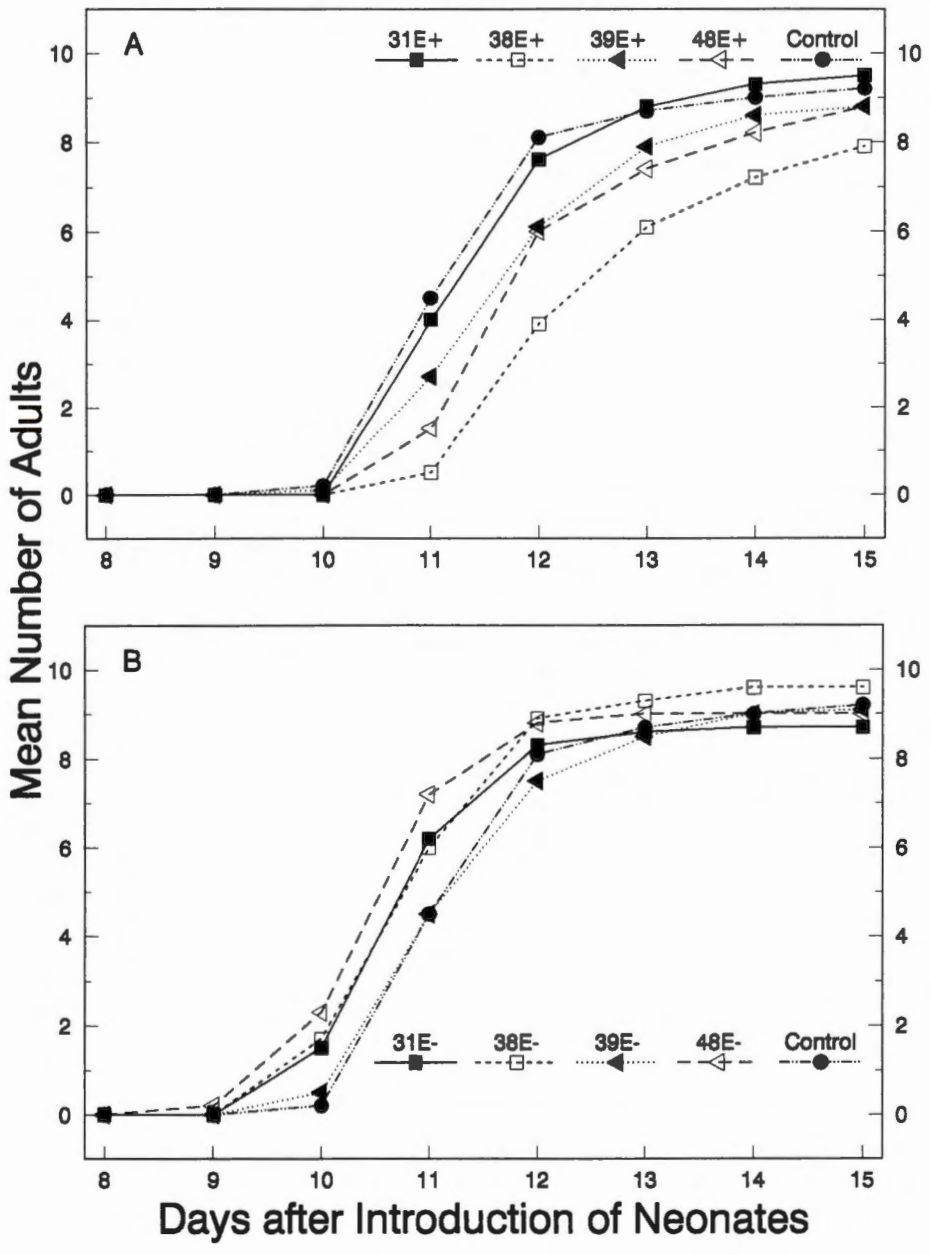

Fig. 16. Number of Drosophila melanogaster which developed from pupae into adults on diets containing endophyte-infected leaf powder (A) or endophyte-free leaf powder (B) compared to flies on control diet during Day 8 through Day 15 of spring bioassay II.

Summer Bioassay. More pupae occurred on diets containing 31E- fescue than all other diets on Day 6 (Fig. 17A). More adults emerged on diets containing 31E- fescue than other diets by Day 10. Fewer pupae occurred on diets containing 39E+ fescue than all other diets on and after Day 9. Fewer adults emerged on diets containing 39E+ fescue than all other diets on and after Day 13 (Fig. 18A and B). Number of adults on diets containing 38E+ fescue was between that of diets containing 39E+ fescue and other E+ diets on Day 13 (Fig. 18A).

Fewer pupae occurred on E+ diets during all but the first day of monitoring. There were also fewer adults on E+ diets from Day 11 to Day 15. This again demonstrated a concentration of toxins lethal to fruit flies (Figs. 19A and 20A).

More pupae occurred on diets containing 38E- fescue than the control on Day 7. More pupae and adults occurred on 31E- diets than the control during two and three consecutive days of bioassay, respectively. Flies on diets containing 31E- fescue may have undergone developmental acceleration (Figs. 19B and 20B).

Seasonal Trends. Diets containing 38E+ fescue were more toxic than other E+ diets during spring. High levels of toxicity in 38E+ diets caused mortality in fruit flies during fall, was completely absent in winter, reappeared

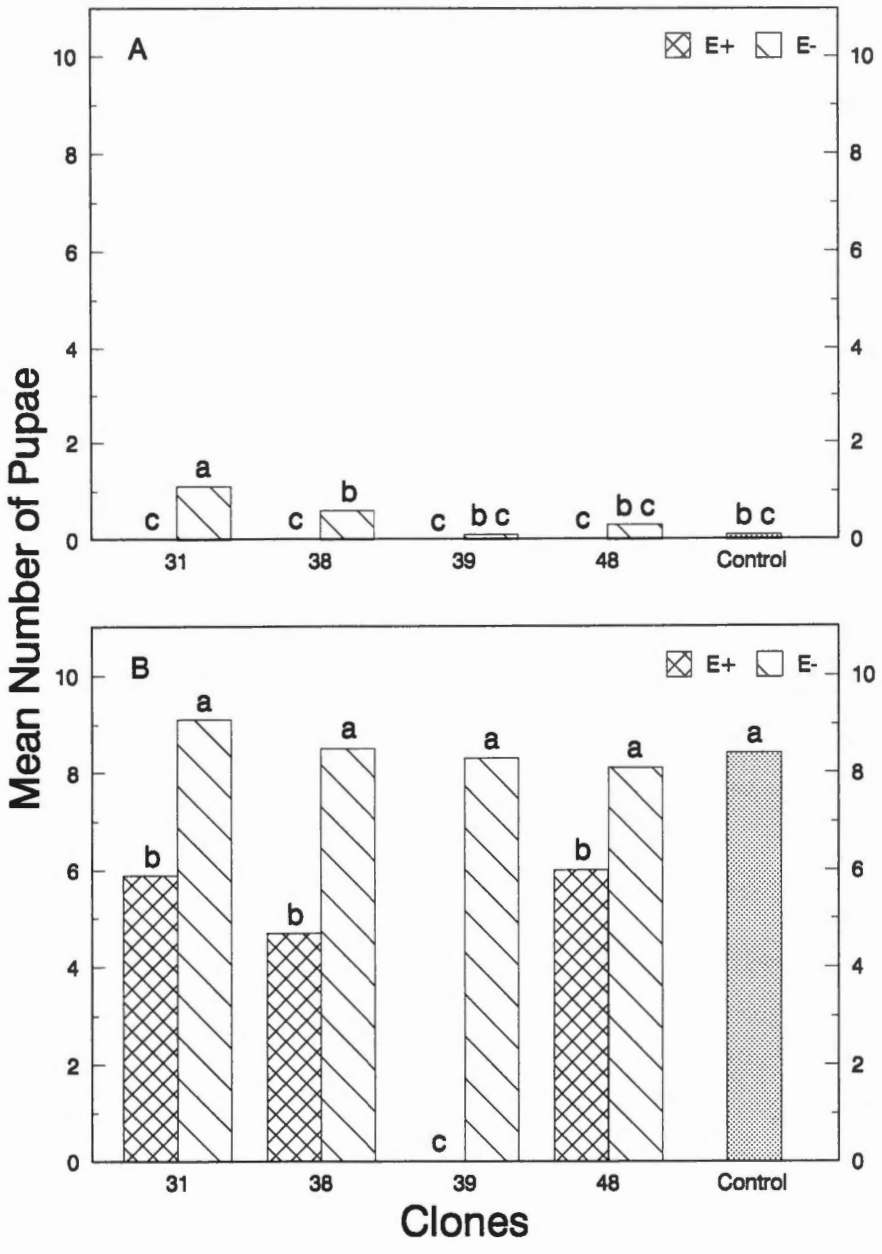

Fig. 17. Number of Drosophila melanogaster which developed from larvae into pupae on control diet or diets containing endophyte-infected (E+) or endophyte-free (E-) tall fescue leaf powder on Day 6 (A) and Day 10 (B) of the summer bioassay. Means with the same letters are not significantly different.

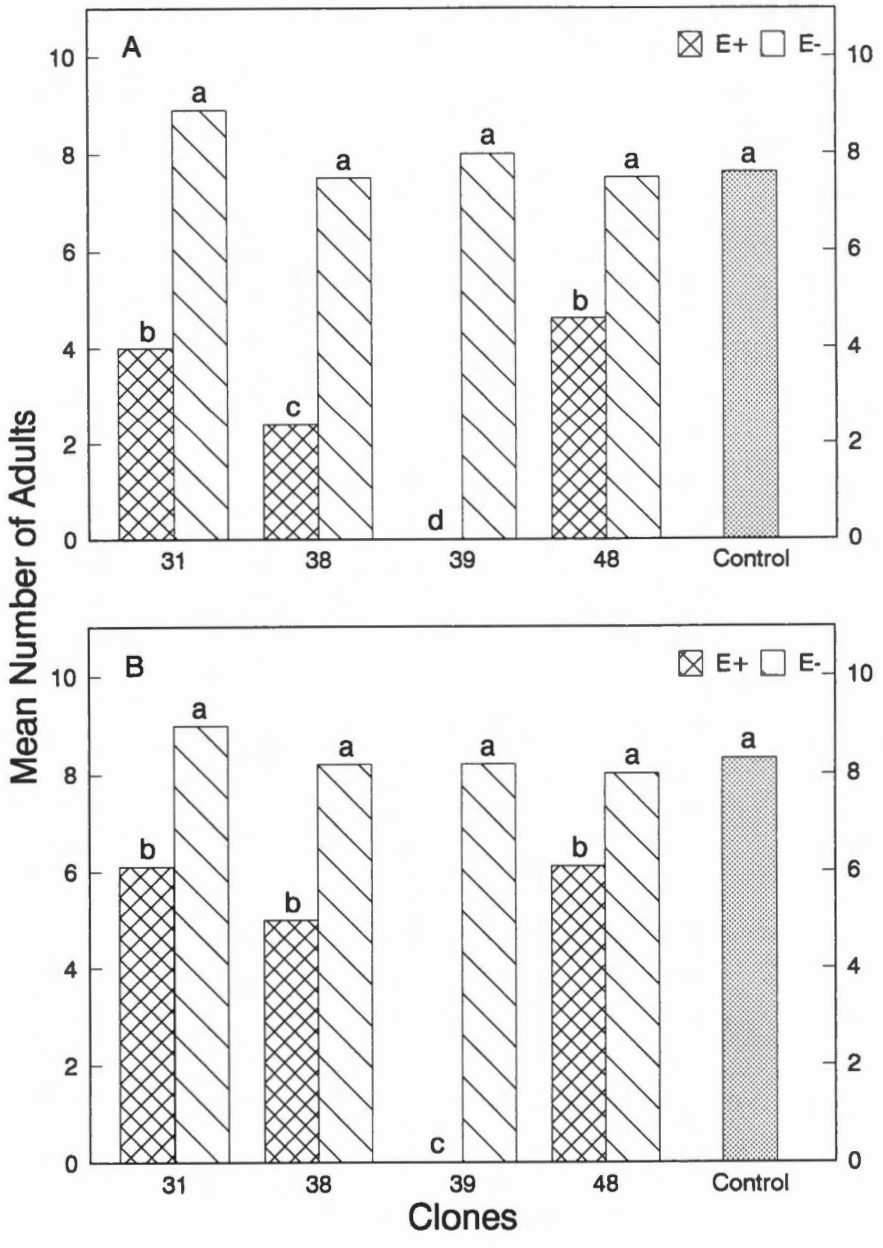

Fig. 18. Number of Drosophila melanogaster which developed from pupae into adults on control diet or diets containing endophyte-infected (E+) or endophyte-free (E-) tall fescue leaf powder on Day 13 (A) and Day 15 (B) of the summer bioassay. Means with the same letters are not significantly different.

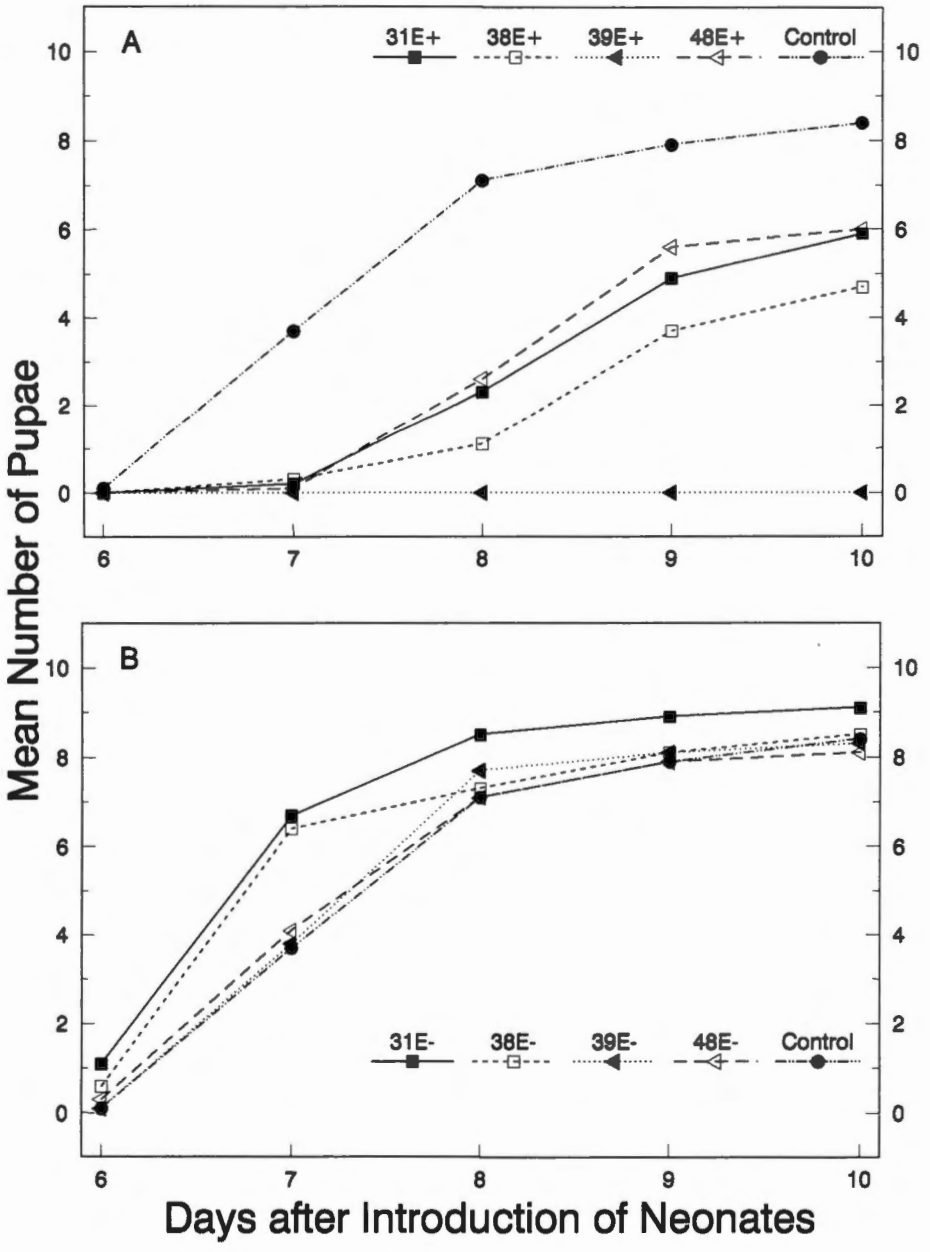

Fig. 19. Number of Drosophila melanogaster which developed from larvae into pupae on diets containing endophyte-infected leaf powder (A) or endophyte-free leaf powder (B) compared to flies on control diet during Day 6 through Day 10 of the summer bioassay.

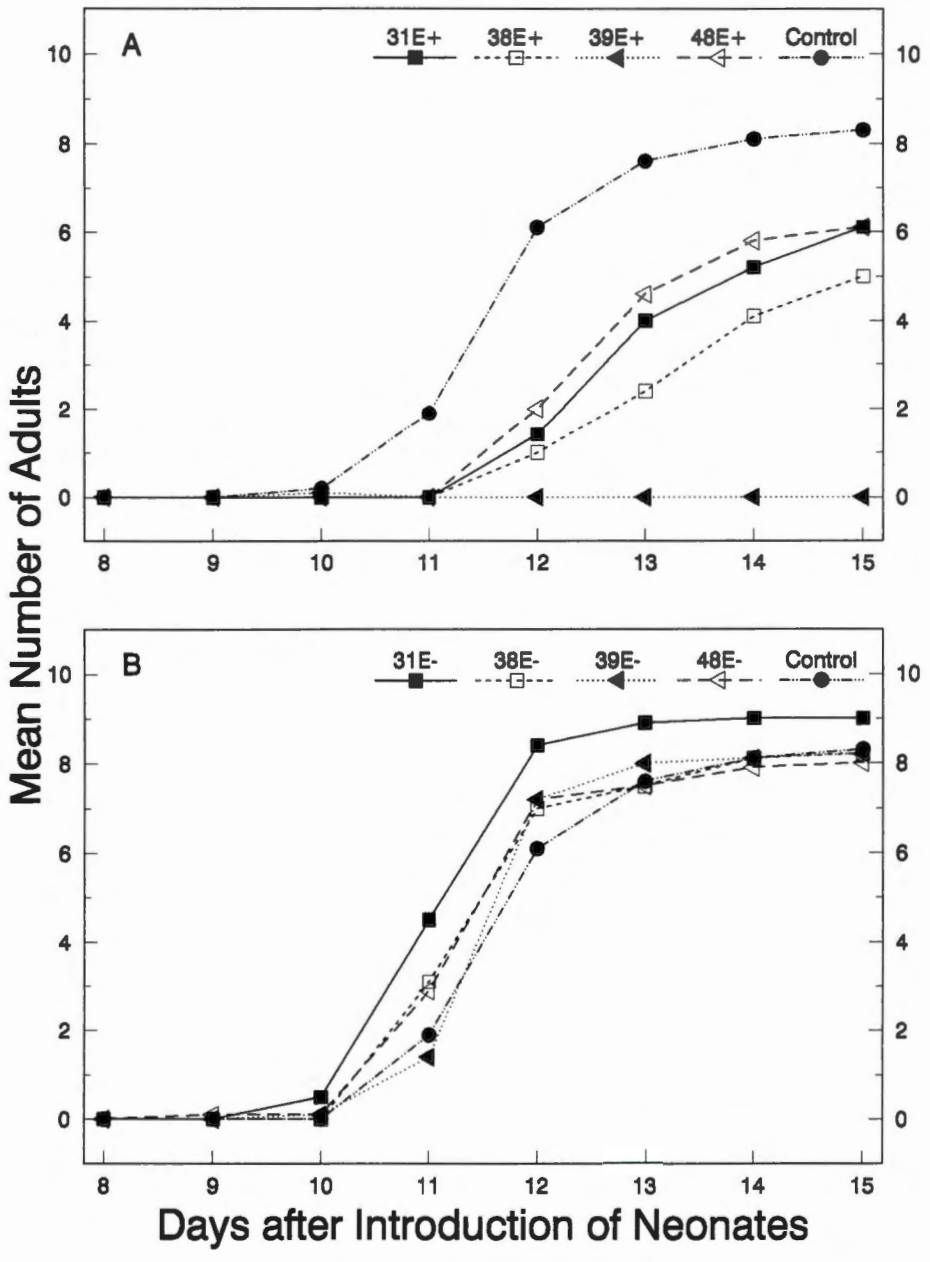

Fig. 20. Number of Drosophila melanogaster which developed from pupae into adults on diets containing endophyte-infected leaf powder (A) or endophyte-free leaf powder (B) compared to flies on control diet during Day 8 through Day 15 of the summer bioassay.

during spring, and carried into the summer months. This coincides to some extent with the seasonal incidence of ergovaline peaks detected by tandem mass spectrometry (Belesky et al. 1988) and high-performance liquid chromatography (Rottinghaus et al. 1991).

In contrast to diets containing 38E+ fescue, 31E+ diets had lesser toxic effects. Rate of fly development was not inhibited by diets containing 31E+ fescue during spring when all other E+ diets delayed fly development. This may be partially explained by an increase in alkaloid concentration by some infected plants at different times of the year. In a study using cloned individuals, low ergovaline production remained constant in one genotype, but increased in another (Hill et al. 1990). The nutritive value of the grass, in combination with low alkaloid levels, may help explain why 31E+ diets were generally more favorable for fly development. Diets containing 31E- fescue appeared to be the most beneficial diet for fly success during a brief period in the summer.

The control generally ranked between E+ and E- diets, becoming most obvious in fall and spring bioassays. Toxicity increased in all E+ treatments, except 31E+, during both spring and summer. All E+ treatments were comparable to or less toxic than the control during winter; therefore, toxicity due to the presence of the endophyte seemed to diminish during winter. Fescue qualities amenable to fly

development may have overridden any toxic effects enhanced by the presence of the endophyte during other times of the year. All E- diets generally accelerated fly development compared to the control throughout the year.

CORN FLEA BEETLE BIOASSAY

Mortality of corn flea beetles was not different on E+ and E- tillers of a clone pair except on one occassion during the bioassay. More beetles were found dead on treatment 48E+ than on 48E- after 48 hours. Since presence of the endophyte did not cause mortality for the most part, beetles were able to feed on both E+ and E- tillers for an equal duration of the bioassay. Although E+ fescue tillers did not have any antibiotic effects on the beetles, they exhibited antixenotic properties during the summer of 1992 (Fig. 21). More beetles were counted on treatments 31E- and 48E- compared to the respective E+ tillers after 24 hours. More beetles were counted on E- tillers of clone pair 31 and 38 compared to the respective E+ tillers after 48 hours. Clone pair 39 was the only treatment which had an equivalent number of corn flea beetles on both E+ and E- tillers after both 24 and 48 hours. A second bioassay conducted on clone pair 39 provided similar results. During the summer of 1993, clone pairs 31 and 38 had the same number of beetles

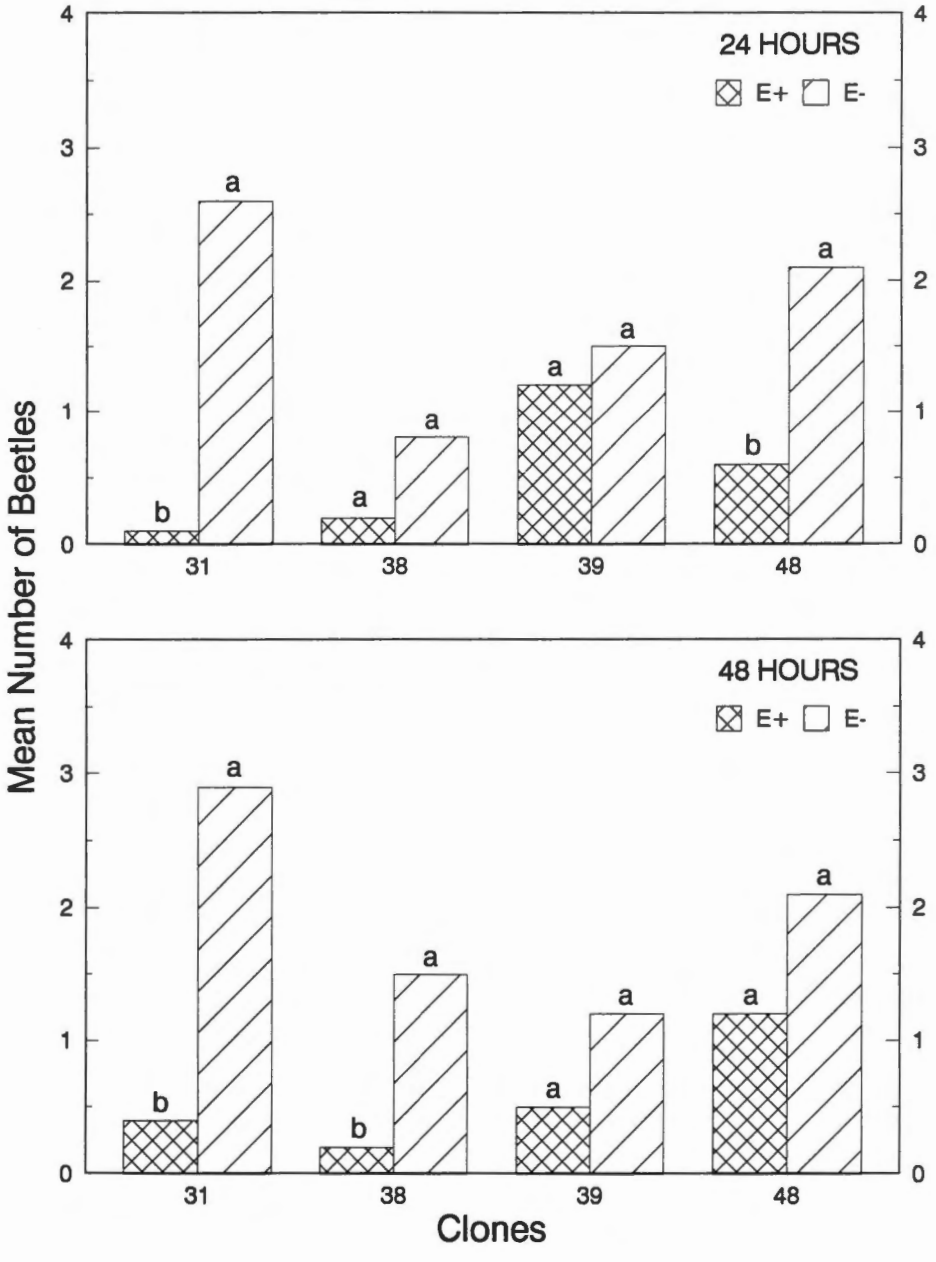

Fig. 21. Number of Chaetocnema pulicaria on endophyte-infected (E+) or endophyte-free (E-) tillers of tall fescue clone pairs 24 hours (A) and 48 hours (B) after introduction of beetles during the summer, 1992. Means with the same letters are not significantly different.

on E+ tillers as the respective E- tillers after both 24 and 48 hours (Fig. 22).

More scarification occurred on treatments 31E-, 38E-, and 48E- than the respective E+ tillers during the summer of 1992 (Fig. 23). The same amount of scarification occurred on E+ and E- tillers of clone pair 39 when initially tested; however, more feeding occurred on 39E- tillers than 39E+ tillers when the bioassay was repeated. Treatments 31E- and 38E- had more scarification than the respective E+ tillers during the summer of 1993 (Fig. 23). Presence of the endophyte deterred feeding of corn flea beetles on all E+ treatments except 39E+.

Leaves from treatment 31E- plants had more scarification than all other treatments during the summer of 1992 (Fig. 24). Treatments 31E- and 38E- exhibited equal amounts of feeding during the following summer. Treatment 39E- had less scarification than other E- tillers during the summer of 1992. Although treatment 39E+ was not significantly different than other E+ tillers, tillers of treatment 39E+ did have minimal scarification; whereas, other E+ tillers had no scarification in 1992. Feeding upon 39E+ tillers may have occurred if not all tillers were infected. Noninfected tillers could result from a failure of the fungus to grow along an infected tiller bud (Hinton and Bacon 1985). During the summer of 1993, more

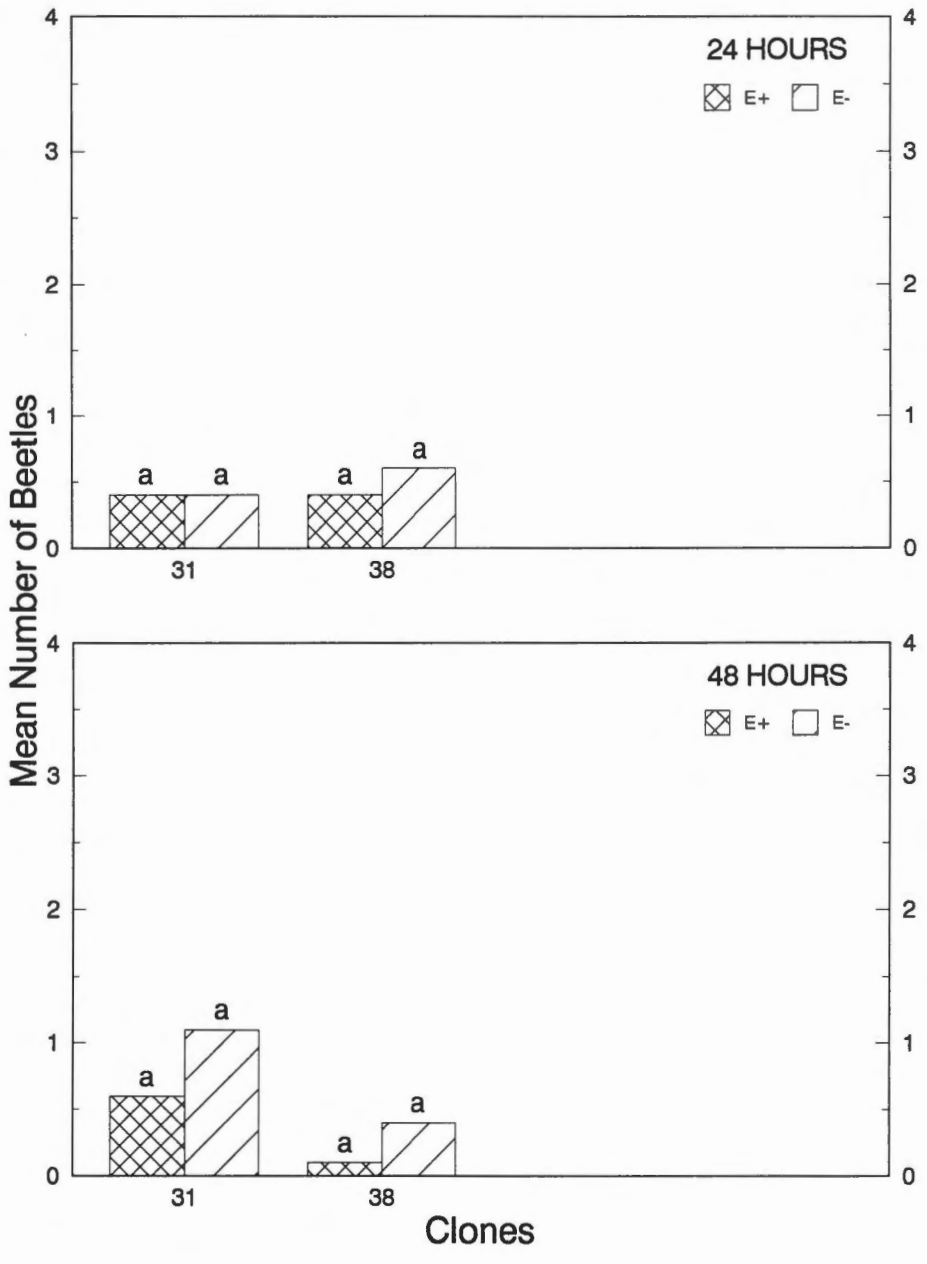

Fig. 22. Number of Chaetocnema pulicaria on endophyte-infected (E+) or endophyte-free (E-) tillers of tall fescue clone pairs 24 hours (A) and 48 hours (B) after introduction of beetles during the summer, 1993. Means with the same letters are not significantly different.

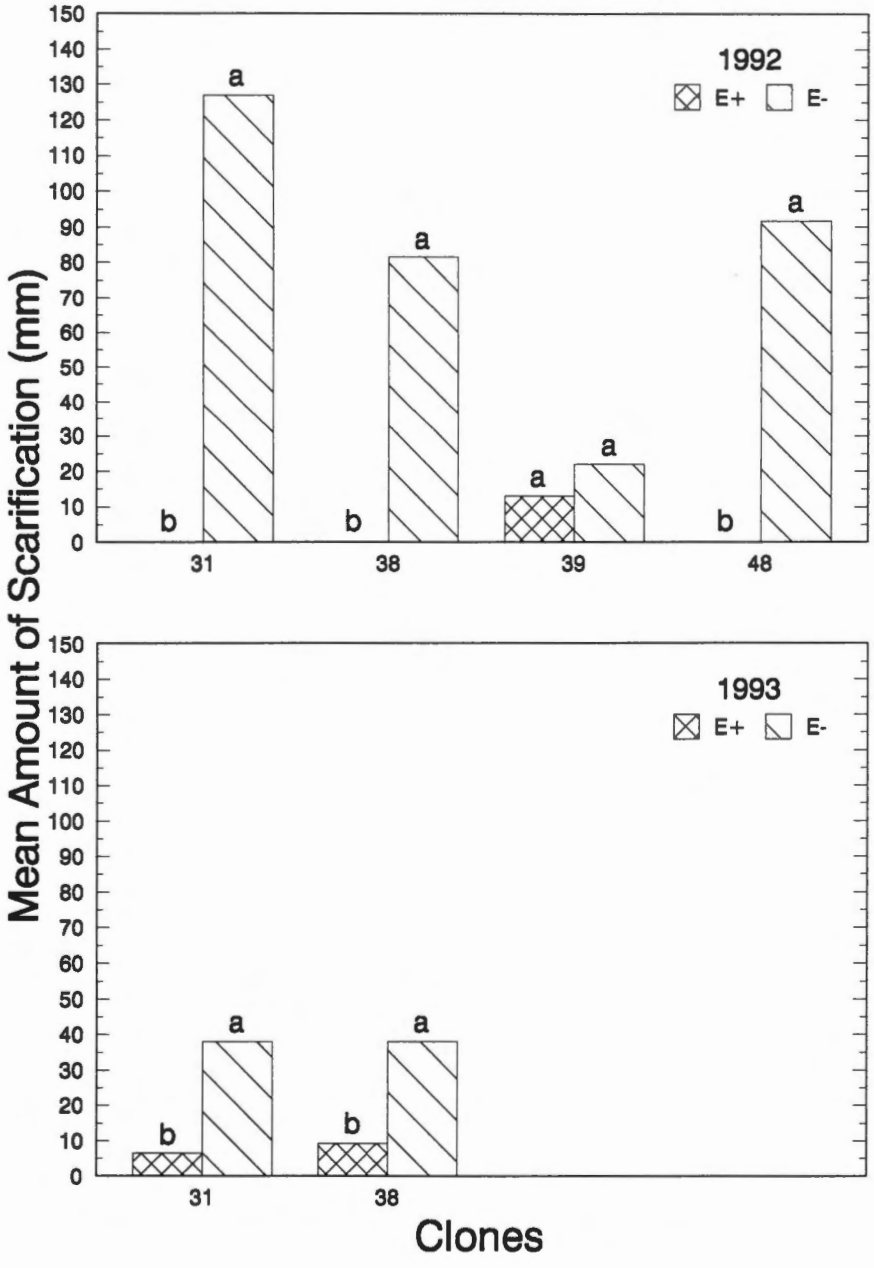

Fig. 23. Amount of feeding by Chaetocnema pulicaria on endophyte-infected (E+) or endophyte-free (E-) tall fescue tillers compared within clone pairs during the summer, 1992 and 1993 (t test). Means with the same letters are not significantly different.

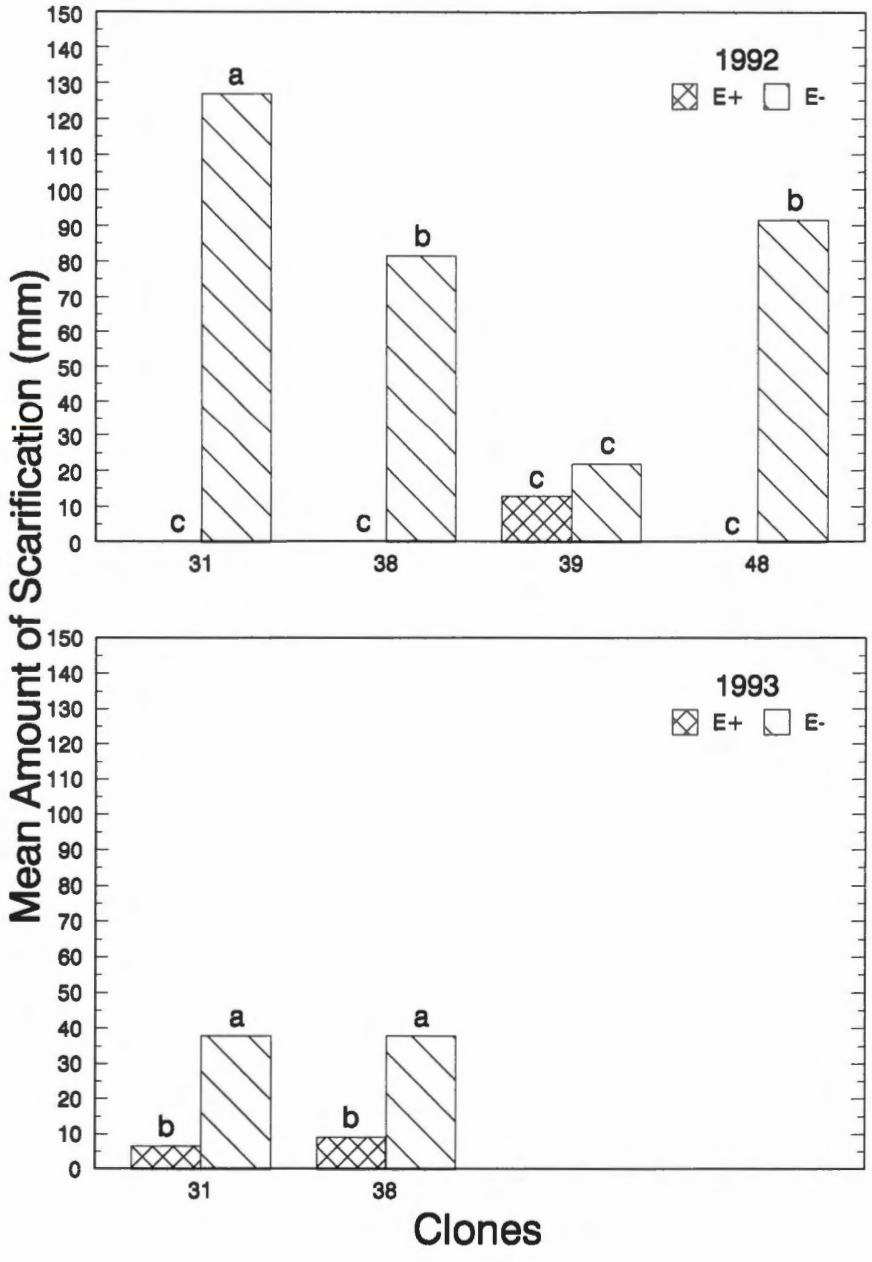

Fig. 24. Amount of feeding by Chaetocnema pulicaria on endophyte-infected (E+) or endophyte-free (E-) tall fescue tillers compared among all treatments during the summer, 1992 and 1993 (Duncan's Multiple Range Test). Means with the same letters are not significantly different.

scarification occurred on E- tillers than on E+ tillers, although some feeding did occur on 31E+ and 38E+ leaves.

5. SUMMARY AND CONCLUSION

Research on the relationship between Acremonium coenophialum and its tall fescue host indicated that E+ plants exhibit greater resistance to insect pests compared to their E- conspecifics. Varieties of E+ tall fescue can be developed to produce grass/endophyte complexes with a unique combination of traits. High levels of endophyte are associated with reductions in animal performance. Endophyte influence at intermediate levels is of particular importance to pasture renovation (Stuedemann and Hoveland 1988). Low alkaloid levels, perhaps such as in treatment 31E+, suggest that variability in E+ treatments may enable development of a fescue/endophyte complex desirable for foraging purposes.

Level of toxicity of treatment 38E+ was clearly higher than other treatments tested during most of the year, and the adverse effects of diets containing 38E+ fescue on fruit fly development greatly increased during spring. On the other hand, the innocuous effects diets containing 31E+ fescue had on fruit flies compared to other E+ diets became particularly apparent in spring as well. The E- member of clone pair 31 showed a higher affinity to both fruit flies and corn flea beetles. Developmental time was generally accelerated with flies on E- diets and retarded with flies on E+ diets. The results obtained from fruit fly and corn

flea beetle bioassays on clone pair 39 warrant its further investigation.

With the contrasting levels of toxicity in E+ treatments, a great diversity of phenotypes may be expected in a pasture situation. Given the large range of genotypes in K-31 individuals, other clone pairs may not provide results shown in this study. Results of this study demonstrate the importance of testing several clone pairs before suitability of a particular variety is determined.

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VITA

John David Harmuth was born in Lebanon, PA, on March 31, 1965. He graduated from Lebanon Catholic High School in 1983. He enlisted in the U.S. Army for three years as an armor specialist. John returned to school and later received a Bachelor of Science degree in marine biology from The University of West Florida in August, 1991. He entered the Department of Entomology and Plant Pathology at The University of Tennessee, Knoxville, in January of 1992 as a Graduate Research Assistant. He obtained a Master of Science degree in Entomology and Plant Pathology in December, 1993. Mr. Harmuth is a member of the Entomological Society of America and plans to pursue a career with the U.S. Navy as a medical entomologist.