Winter survival and population dynamics of bean leaf beetle (Coleoptera: Chrysomelidae)

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ABSTRACT

Ecological study of the population dynamics of bean leaf beetle adults, *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae), with special reference to the overwintering populations, was conducted in central Iowa. Ecological habits, spatial distribution, cold tolerance, and winter survival of overwintering beetle adults, were studied for 3-yr (1996 through 1999). Additionally, the population fluctuation of the adult beetles during the growing season was studied for 10-yr, from 1989 through 1998, inclusively. Overwintering beetle adults mostly hibernated in the crop residue of soybean fields (≈20%) and leaf litter of woodlands (≈80%). A few beetles (<1%) overwintered in alfalfa fields, cornfields, and grasslands. The overwintering adults aggregated in the litter of soybean fields and woodlands. Residue in the habitats served as an insulating layer for the overwintering beetles. However, over 50% mortality of the overwintering beetles was observed in both soybean fields and woodlands during the 3-yr study. Laboratory study indicated that most of the overwintering beetles survived more than hundreds of hours when they were kept at -5°C or above, whereas most of the beetles died when they were kept at -10°C or below. This result indicated that cold temperature has a strong effect on the winter survival of the overwintering populations. A model for the estimation of the mortality of overwintering beetle adults, based on the accumulation of the air mean subfreezing winter temperature, was developed. Linear regression and multiple regression stepwise selection procedure analyses were used to relate environmental factors to population fluctuations of bean leaf beetle during the 10-yr study. The analyses showed a strong relationship between the environmental factors, including temperature and precipitation, and beetle population

densities. A predictive model for F_2 adults in the next late season was developed. The major factors affecting the survival of overwintering populations and population fluctuations of the beetles, estimation of winter survival, and the prediction of the population dynamics of bean leaf beetle during seasonal cycles are discussed.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

The modern soybean, *Glycine max* (L.) Merrill, was imported from the East to the United States in mid the 1770s (Wolf and Cowan 1975). In the last few decades, soybean production has increased steadily (Boquet 1994) and has been expanded from a minor to a major crop in the United States (Wilson et al. 1992). Due to their ease of production, long-term storage characteristics, and exceptional protein contents, soybean have been highly regarded as a nutritional food source (Wolf and Cowan 1975, Wilson et al. 1992). Today, soybean is the dominant commodity in both edible oil and feed protein markets. In addition, soybean meal is the protein of choice for livestock and poultry producers (Smith 1994).

In 1992, it was estimated that nearly 19.5 million tons of soybean were exported, netting a total export sales volume of approximately $4.1 billion dollars (Smith 1994). The Midwest is the largest soybean-producing area, accounting for about 65% of the national production (Hammond et al. 1991). In 1998, an agricultural statistics report indicated that in Iowa 1997, over ten million acres were planted with soybean and had a production of more than 400 million bushels. Moreover, Iowa was ranked as the first in production of soybean in the U.S. (Iowa Agricultural Statistic Service 1998).

Over the 60 million acres of soybean are planted annually across 29 states in the U.S., and eight species of insects commonly account for most arthropod damage in soybean. These insects are velvetbean caterpillar, *Anticarsia gemmatalis* Hübner; soybean looper, *Pseudoplusia includens* (Walker); green cloverworm, *Hypena scabra* (F.); Mexican bean beetle, *Epilachna varivestis* Mulsant; bean leaf beetle, *Cerotoma trifurcata* (Förster);

southern green stink bug, *Nezara viridula* (L.); green stink bug, *Acrosternum hilare* (Say); and corn earworm, *Helicoverpa zea* (Boddie) (Way 1994). Among these insects, the bean leaf beetle (Coleoptera: Chrysomelidae), is a species that causes both direct and indirect damage of the crop (Pedigo 1999). Furthermore, bean leaf beetle is an important soybean pest in the southern U.S. (Kogan et al. 1980, Hammond et al. 1991) and has been an occasional pest in the Midwest (Smelser and Pedigo 1991).

In Iowa, the bean leaf beetle develops two generations per year, and the second-generation adults overwinter (Smelser and Pedigo 1991). Each year, the overwintered beetles colonize soybean during the early vegetative stages. Subsequently, the beetles produce two generations in soybean, with the second-generation having the highest population density during the growing season. Possibly the relatively low population density of the overwintered beetles indicated that the winter in Iowa has an effect on insect population fluctuations. Thus, there is a need for the understanding the ecological effects, especially during winter, on the population dynamics of the beetle.

Both the concepts of integrated pest management (Pedigo 1999) and ecologically based pest management system (National Research Council 1996) seek to reduce the status of pests by following principles of ecology and using the latest advancements in technology. To apply the tools of a management program effectively, the approach relies on preventive practices through an understanding of pest biology, ecology, and the appropriate integration of information (Pedigo 1999). Therefore, the main goals of this study were to understand the winter survival and population fluctuations of the bean leaf beetle, so as to improve the preventive management of the pest ecologically.

Objectives

The primary objectives of this study were:

1. To determine, under cold temperatures, the time required for 50% mortality (half lethal temperature, LT_{50}) of the overwintering bean leaf beetles.
2. To understand the ecological habitats and spatial distribution of the overwintering adult beetles in Iowa.
3. To estimate the winter survival of overwintering beetle populations.
4. To understanding the dynamics of the pest populations and to develop a model for the prediction of population fluctuations.

Thesis Organization

This thesis was organized with a general introduction including the thesis objectives, a literature review, four papers for publication in scientific journals, a general conclusion, and references cited. The literature review contains the background information of the biology of the bean leaf beetle, the phenology and population fluctuations of the beetle during the growing season, and the management of the pest. Following the literature review are the four papers for publication. The first paper estimates the cold tolerance of overwintering bean leaf beetle. The second paper determines the ecological habitats and spatial distribution of the overwintering beetles and develops sequential count plans for the beetle in different habitats. The third paper develops a predictive model for the survival of overwintering beetle. The fourth paper describes the beetle density fluctuations and develops a predictive model for the second-generation adult densities in central Iowa. All papers were prepared according to the publications policies and guidelines for manuscript preparation established by the Entomological Society of America (ESA 1992). The references cited section included only those citations in the general introduction, literature review, and general conclusions.

Literature Review

The Biology of Bean Leaf Beetle

Origin and Distribution. The bean leaf beetle is a native species, widespread in the eastern half of the U.S. (Pedigo 1994). The range of occurrence of the beetle extends from southern Canada to the Gulf States and from the Atlantic coast westward to south Dakota in the north and to Arizona in the south (Kogan et al. 1980).

Description. Bean leaf beetle adults are small (≈5mm long), suboval, and highly variable in coloration. The most common background color of elytra of the beetles is light yellow, likewise, beige, pink, salmon, orange, or crimson may also be found. The elytra are commonly marked with 4 black spots and marginal stripes, however, the elytra may lack spots and stripes. Prominently, a black triangle is always present behind the prothorax (Pedigo 1994). The frons color of female beetle is black, whereas for male beetle is light tan. Eggs, which are spindle-shape (≈0.8mm long) with an orange coarsely reticulated chorion, are laid in the soil of soybean fields. Larvae are whitish, subcylindrical, with three pairs of legs and an anal proleg, whereas pupae are white (Kogan et al. 1980).

Injury to Soybean. The beetle larvae feed on the soybean roots and nodules, thus causing indirect damages in soybean. The adults feed on the leaf tissues and make rounded holes between veins that are typically different from those made by feeding lepidopterous larvae and grasshoppers (McConnell 1915). The second-generation adults also cause direct damage in soybean by feeding on pod external tissues (Pedigo and Zeiss 1996). Bean leaf beetle pod lesions affected the quality and the weight of the seeds directly and may cause the secondary infection of pathogens, such as *Alternaria* spp. of fungi (Shortt et al. 1982, Kunwar et al. 1986, Smelser and Pedigo 1992) and bean pod mottle virus (Patel and Pitre 1976).

Life History. The beetle develops two generations a year in Iowa (Smelser and Pedigo 1991). During spring, the overwintered adults emerge from the overwintering sites, move to wild hosts (Helm et al. 1983) and alfalfa, until soybean emerges, then colonize the soybean during early vegetative stages (Smelser and Pedigo 1991). The beetles lay their eggs in the soil of soybean fields. The female beetles lay ≈350 eggs within a month (Eddy and Nettles 1930, Isely 1930).

Eggs hatch after 5-7 d at 26°C (Herzog et al.1974). Larvae live in the soil and feed on the roots, root hairs, and nodules of soybean (Pedigo 1994). The larvae molt 3 times and pupate in earthen cells. The mature larvae are ≈10 mm in length. The pupal stage requires ≈7 d at 26°C for adult emergence. The complete life cycle from eggs to adult emergence takes ≈30 - 45 days depending on soil temperatures (Kogan et al. 1980).

In Iowa, the first-generation adults emerge at the end of July to early August, whereas the second-generation adults emerge at late August to early September (Lam and Pedigo 1998). The second-generation adults move to overwintering sites at late September but some move to alfalfa, stay for a short time, then move to overwintering sites. Most of the overwintering adults hibernate in soybean fields and woodlands.

Integrated Management of the Bean Leaf Beetle

An integrated pest management program involves both preventive and therapeutic practices. Preventive management is the ultimate form of integrated pest management and also is the first line of defense against a pest population. However, therapeutic practice is a back up program when preventive tactics fail (Pedigo 1999).

The recommended preventive strategy for bean leaf beetle is to plant soybean as late as possible in the recommended planting period. Additionally, soybean are planted adjacent to nonhost crop, such as corn, and as far from alfalfa as possible. Planting late reduces the food sources of the beetles. Thus, many overwintered beetles may die or have to find other habitats before the soybean emerge. This tactic reduces the colonization of beetles in soybean and disrupts the synchronization of the second-generation adults and soybean pod development. Therefore, pod injury by the beetles is lowered in the late planting soybean (Pedigo and Zeiss 1996).

A therapeutic strategy is recommended using insecticide applications when the beetle population reaches the economic threshold. Economic thresholds are based on the treatment costs per acre, which provided appropriate guidelines for a given soybean market value, and specific management costs. During the R4 to R7 soybean developmental stages the fields are scouted to estimate the beetle population density. Control is justified if the number of beetles per foot of row equals or exceeds the economic threshold (Rice and Pedigo 1994).

Integrated pest management emphasizes that appropriate preventive measures should be initiated before pest populations reach damaging levels. Such an approach requires phenological prediction of the seasonal events and the spreading and establishment of the species (Tauber et al. 1986). The forecasting of seasonal events in insect populations and the prediction of adaptation by colonizing species require detailed knowledge of seasonal responses and the genetic variability underlying the populations. Moreover, all these require the understanding of pest population ecology in both natural environments and those modified by humans (Price 1997).

Seasonality and Pest Management

Predicting Seasonal Events. The fundamental unit of agricultural pest management is the crop ecosystem (Huffaker et al. 1984, Price 1984). Effective manipulation of an agroecosystem requires phenological prediction of both recurring seasonal events and the spreading and establishment of colonizing species during the cycles (Tauber et al. 1986). Hence, knowledge of the seasonal adaptations and the seasonal variability of insects in the system provide a basis for predicting population fluctuations of the pests in the following season.

Prediction of seasonal events entails the modeling of two major components: (1) the onset and termination of diapause and (2) the continuous non-diapause development that occurs during the growing season (Bradshaw 1974). However, much of the research on insect pests has concentrated on the insect-host plant relationship during the summer. Attention focused on the growing season is understandable because the pest population density usually reaches a peak, and plant protection is required then. In fact, the numbers of insects on crops during the early growing stages is determined mainly by the density of the colonizing population, which is closely related to the survival of the overwintering stages. It is increasingly apparent that the role of the winter season in the population dynamics of insect pests has been overlooked (Bale 1991).

Cold Tolerance. Cold tolerance or cold hardiness refers to the capacity of an organism to survive exposure to low temperature (Denlinger 1991, Lee 1991). Practically, cold tolerance is usually measured as the length of survival periods at a certain low temperature at which half the test population is killed (half lethal temperature, LT_{50}) (Hori and Kimura 1998). Thus, estimating the cold tolerance of overwintering adult bean leaf beetles and understanding the survival of the overwintered populations under cold temperatures during Midwest winters is desirable in managing the pest in the following season.

Winter Survival. For most insects, overwintering mortality is correlated primarily with low temperature exposure. Generally, winter mortality increases with time, along a sigmoid curve, and the slope of the curve increasing at lower constant temperatures (Salt 1966, Casagrande and Haynes 1976). However, to assess the effects of cold winters on insects, there are three main requirements for the insect populations and sampling: (1) a sufficient population density to reveal changes in numbers as the winter proceeds, (2) an accurate sampling method to assess changes in density, and (3) a frequent sampling estimate so that shifts in abundance can be related to distinct climatic events (Bale 1991).

As expected, pest damage during the growing season is mainly a function of postwinter abundance. If a consistent relationship can be found between levels of mortality observed in the laboratory and the decline in natural populations in winter in relation to known climatic events, then the combined data can be analyzed to provide models to forecast the likely abundance or outbreak of the pests.

Winter Habitats. Natural populations are controlled by mortality factors that vary among species and among times and places in the same species (Clark et al. 1967).

Estimation of field mortality from standard meteorological records is more complicated than has often been supposed and usually cannot be made based on the laboratory study of cold tolerance alone. In fact, winter survival of insects depends on cold tolerance and the choice of winter microhabitats (Danks 1978).

Variations in winter survival of insects depend on the insulating power of sites and the climatic fluctuations in years. For example, winter survival of the boll weevil, *Anthonomus grandis*, varied from 14.4 to 100% in Texas (Davis et al. 1975), whereas the survival of the same species varied from 0 to 100% in Mississippi (Pfrimmer and Merkl 1981). In most insect species, ecological or behavioral means of moving to suitable overwintering sites and avoiding low winter temperatures are important to withstand the winter season (Danks 1991).

Ecological Adaptations. Most insect species move from summer feeding areas to different overwintering sites. The majority of species move to more sheltered, protected, and warmer microhabitats for winter (Danks 1991). However, some species, such as the monarch butterflies, migrate to different regions far to the south of their summer range in the United States and Canada to winter in California (western populations) and Mexico (eastern populations) (Calvert and Brower 1986, Hardman 1998). Many insects, such as the cocinellid (Lee 1980, Sotherton 1985) and chrysomelid beetles (Boiteau et al. 1980), move on a smaller-scale from fields to woodlands or groves for hibernation. Additionally, most insects enter microhabitats and are protected by plant material and snow, or they burrow in soil, or overwinter in the plant parts and bark.

Ecological factors related to insect winter survival seem to correlate chiefly with habitat and the insect life history. Winter mortality can vary widely among species, populations, and sites. Furthermore, mortality in a single species potentially varies greatly in space and time accordingly to a wide range of site features. Therefore, research on winter survival and ecological adaptations of insects to cold temperatures should consider simultaneously several aspects, including physiological, biochemical, and ecological studies.

Population Dynamics and Pest Management

Population dynamics and regulation are the centerpiece of ecology (Murdoch 1994). The basis for understanding the population dynamics of any organism is the identification of the limiting factors on populations (Allee and Parks 1939). These limiting factors, which are likely to act as controlling factors, are responsible for setting limits on the density of a species that is common in the area studied (Solomon 1949). In addition, Morris (1957 and 1959) has used the term key factor for an influence that causes a degree of mortality that is closely related to changes in population density, from generation to generation, and which has predictive value. However, the definition of the term key factor has differed, leading to differences in analysis and sometimes misunderstandings (Podoler and Rogers 1975, Price 1997). To avoid confusion and misunderstanding in this thesis, the term limiting factor is used to imply the major factor that acts as a limiting (Allee and Park 1939) or controlling (Solomon 1949) influence on population dynamics.

Many factors and combination of factors can have a regulatory role in population fluctuations. Regulation is defined as the return of a population to equilibrium density (Murdoch 1970). However, a regulated population would fluctuate about some trend but would not drift unboundedly away from it (Royama 1992). Therefore, the ultimate goal of studying population dynamics in applied entomology is to develop models based on the ecological understanding of systems involved, resulting in predictive value (Goodenough and McKinion 1992, Metcalf and Luckmann 1994).

Improvement for the Preventive Management of Bean Leaf Beetle

If winter survival and population dynamics of the bean leaf beetle populations are related to environmental factors are known, then models for the prediction of population fluctuations can be developed. Forecasting the relative population density of the pests in the following season allows an intelligent decision for insect control, thus improving the preventive management of the beetle. Therefore, the main objectives of this study are to understand the winter survival of the overwintering populations and the population dynamics of damaging populations during the growing season.

CHAPTER 2. COLD TOLERANCE OF OVERWINTERING BEAN LEAF BEETLES (COLEOPTERA: CHRYSOMELIDAE)

A paper to be submitted the Journal of Environmental Entomology

Wai-Ki F. Lam and Larry P. Pedigo

Abstract

Laboratory studies were conducted during the winters of 1996 and 1997 to determine the cold tolerance of overwintering bean leaf beetle adults, *Cerotoma trifurcata* (Coleoptera: Chrysomelidae). Second generation adults were collected during early September from soybean fields near Ames, Iowa. The collected beetles were reared at 24°C with a photoperiod of 10:14 [L:D] h in the laboratory for 3 wk. The beetles then were transferred to a chamber with high relative humidity at 5°C and photoperiod of 9:15 [L:D] h for evacuation of gut contents preparing for the cold tolerance study. After 2 wk at 5°C, the beetles were randomly selected and subjected to cold baths maintained at temperatures -15, -10, -5, 0, and 5°C (5, 14, 23, 32, and 41°F). At each temperature studied, 30 test tubes containing 5-pr adults (5♂: 5♀) in small vials with blotting-paper discs and 30 test tubes containing 5-pr adults in small vials without blotting-paper discs were prepared. At certain time intervals, 6 test tubes containing beetles, 3 test tubes with and 3 test tubes without blotting-paper discs, were retrieved from the cold baths at different temperatures for the observation of time-mortality. More than 50% of the adult beetles survived over hundreds of hours at -5, 0, and 5°C, whereas most of the beetles died after 15 minutes at -10 and -15°C. In addition, the blotting paper covering enhanced the survival of overwintering beetles under cold temperatures. The survivorship of the overwintering adults under cold temperatures and the

correlation of the air temperature and the leaf litter temperature in beetle hibernating woodland habitats are discussed.

Introduction

The bean leaf beetle, *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae), is a native species widespread in the eastern half of United States (Pedigo 1994). The beetle is a serious pest affecting soybean production in all soybean growing regions of U.S. (Hammond et al. 1991) and develops 2 generations per year in Iowa (Smelser and Pedigo 1991, Lam and Pedigo 1998). Although phenology of the beetle on soybean and alfalfa during the growing season was thoroughly studied by Waldbauer and Kogan (1976), Boiteau et al. (1979a), and Smelser and Pedigo (1991), not much is known about the survivorship of the overwintering adult populations in Iowa.

The overwintering adult beetle displays a typical reproductive diapause (Boiteau et al. 1979b). Studies in North Carolina found that dormant females were characteristically unmated, had small and immature ovaries, and possessed an increased fat content (Boiteau et al. 1979b). However, when compared with nondiapausing beetles, the respiration and feeding rates of diapausing beetles were relatively insensitive to temperatures (Schumm et al. 1983). In Iowa, most of the F_2 adults overwinter inside the withered leaves in the forest-floor litter and the crop residues of soybean fields (unpublished data).

Cold tolerance or cold hardiness refers to the capacity of an organism to survive exposure to low temperature (Denlinger 1991, Lee 1991). Practically, cold tolerance is usually measured as the length of survival periods at a certain constant low temperature at which half of a population is killed (half lethal temperature, LT_{50}) (Hori and Kimura 1998).

For many insects, survival of overwintering stages plays the major role in determining the pest status of the species in the following year (Bale 1989). The numbers of insects on crops during the early growth stages is determined mainly by the density of the colonizing insect, which is closely related to the survival of the overwintering population (Bale 1991). Moreover, Salt (1936) indicated that preparations for the control of pest species could be made more intelligently if information was available on the ability of the insect to resist winter temperatures. Hence, understanding the survival of overwintering bean leaf beetle populations under cold temperatures during Midwest winters is desirable in managing the pest the following year.

Both the concepts of integrated pest management (Pedigo 1999) and ecologically based pest management (National Research Council 1996) seek to reduce pest status by following principles of ecology and using advanced technology. Ecological or behavioral means of avoiding low winter temperatures in insect species are essential in surviving cold winter temperatures. Furthermore, the protection of the overwintering insects from low temperatures depends primarily on the insulating characteristics of the substrate (Danks 1991). The objective of this study was to estimate the cold tolerance of overwintering adult beetles, under simulated ecological substrate, measured by half lethal temperature.

Materials and Methods

Water Baths. Treatment temperatures, including -15, -10, -5, 0, and 5°C (5, 14, 23, 32, and 41°F), were maintained in 5 cold baths, containing 1:1 (v:v), water and antifreeze (propylene-glycol). The cold baths containing 2 sets of 30 test tubes were prepared in 5 different walk-in freezers in September 1996 and 1997. The temperatures inside the cold baths were measured with a thermocouple thermometer at 24-h intervals. The maximum temperature fluctuation within the baths was maintained at ±1°C.

Bean Leaf Beetle Treatment. In early September 1996 and 1997, ≈5000 F_2 adults were collected from soybean fields near Ames, Iowa. In the laboratory, beetles were fed on soybean leaves collected from the late planting field and reared at 24°C (75.2°F) with a photoperiod of 10:14 [L:D] h. After 3 wk, the beetles were transferred to containers in a walk-in freezer held at 5°C (41°F) with a photoperiod of 9:15 [L:D] h. Inside the containers, rolled and curved blotting paper was provided for the beetles as simulating the withered soybean leaves for hibernation. In addition, 2 jars of deionized water covered with muslin cloth were provided for maintaining a high relative humidity. The beetles were kept in the containers for 2 wk to allow for the evacuation of the gut contents (SØmme and Block 1982).

The experiment was conducted at 5 different temperatures, including -15, -10, -5, 0, and 5°C. When treatment temperature was -5°C or below, the beetles were cooled at a rate of 5°C per 12 h until the temperature was reached. At each temperature studied, 30 test tubes (20 x 150 mm) containing 5-pr adults (5♂: 5♀) in a vial (15 x 45 mm) covered with blotting-paper discs and 30 test tubes containing 5-pr adults (5♂: 5♀) in a vial without blotting-paper discs were prepared. The prepared vials with blotting-paper discs attempted to simulate the ecological habitat of withered rolled leaves enclosing the hibernating beetles. In addition, 2 ml deionized water was placed in each test tube for maintaining a high relative humidity. Likewise, a small screen was placed inside the test tube to serve as a support for the vial. All test tubes were capped and randomly located in racks inside the baths.

Periodically 6 test tubes, 3 with and 3 without blotting-paper discs, were retrieved from the cold bath. The beetles from each vial were separated and kept at 5°C for 24 h.

Then the beetles were transferred to 24°C (75.2°F) for another 100 h. Subsequently, beetle activities were examined. After the treatment, if beetles were able to stand and walk, they were considered alive, otherwise, they were counted as dead (Boiteau et al. 1980, Slosser et al. 1996).

Environmental Temperatures. A data set recorder (Model LI-1200S Minimum Data Set Recorder, LiCor, Lincoln, NE) was used to collect the daily meteorological data from mid November (1996-97) or mid October (1997-98) to mid April of the following year in a woodland habitat near soybean fields. The data collected were analyzed for the correlation between the air temperature and the temperature beneath the leaf litter.

Data Analysis. Insect mortality data were analyzed for temperature treatments and time of 50% mortality by using analysis of variance (ANOVA) procedures (SAS Institute 1989). In addition, regression analysis was used to observe the mortality rates at different temperatures and the correlation between air and leaf-litter temperatures.

Results

Bean Leaf Beetle Treatment. At all the 5 temperatures studied, the scattergram of beetle time-mortality seemed to fit a sigmoid shape (Fig. 1). Figure 1 gives the pooled data in the 2-yr study (1996 and 1997) at the 5 constant temperatures studied. However, the maximum mortality rate of each temperature was determined by the regression analysis on the steepest slope of the mortality curve.

Table 1 presents the regression models and the times for 50% time-mortality of the beetles at different temperatures. Most of the beetles in the vials with blotting-paper discs had a lower mortality rate when compared with those without discs, except at 5°C. In addition, comparing the time-mortality between the beetles with and without blotting-paper treatments, only those maintained at -15°C ($F = 11.39$; df = 1, 5; $P = 0.006$) and -10°C ($F = 68.15$; df = 1, 5; $P = 0.0001$) were highly significant. The results indicated that more than 50% of the beetles were killed within 12 minutes when maintained at extreme temperatures, i.e., -10 and -15°C. However, when kept at -5°C or above, hundreds of hours were required to kill 50% of the beetles.

Environmental Temperatures. The air mean temperatures and leaf-litter mean temperatures were measured in 1996-97 (mid November to mid April) and 1997-98 winters (mid October to mid April) at a typical woodland habitat near Ames, Iowa. The leaf-litter mean temperatures in 1996-97 winter had 87 d (Table 2) below zero, with an average of -1.968°C. Whereas, in 1997-98 winter, the leaf-litter mean temperature below 0°C occurred only 15 d, with an average of -1.562°C. These cold temperatures were recorded in either December or January of the years studied. Figure 2 shows the correlation between the leaf-litter mean temperature and air mean temperature each day in the 2-winter study. The results showed that when the air temperature was -10°C or above, there was a strong relationship ($P > 0.75$) between the litter and air mean temperatures. However, when the air temperature was below –10°C, the litter temperature had a low correlation ($P < 0.25$) with the air temperature (Table 3).

Discussion

Bean Leaf Beetle Treatment. The environmental cues that regulate diapause are token stimuli, mainly including photoperiod, temperature, moisture, and biotic factors (Tauber et al. 1986). In most insects, short photoperiod, low air temperature, and change in the food nutrients are the most important environmental factors for inducing diapause (Lee 1991). Additionally, evacuating gut contents maximizes winter supercooling in insects (Bale 1989). SØmme and Block (1982) studying the cold hardiness of Antarctic Collembola at Signy Island found that supercooling point was greatly influenced by feeding status. They found that the supercooling points of the Collembola studied fell into high (above -10°C) and low (below –20°C) groups. A field-collected Collembola, *Cryptopygus antarcticus*, mainly had high supercooling points, but starvation at 5°C for 6 d greatly increased the number with low supercooling points. Moreover, further starvation at 5°C at 12 and 18 d did not increase the proportion of specimens with low supercooling points.

The F_2 adult beetles collected in the field for this study were reared at 24°C with a photoperiod of 10:14 [L:D] h for 3 wk, then the beetles were transferred to containers with high relative humidity at 5°C with a photoperiod of 9:15 [L:D] h and starved for 2 wk. It was observed that the beetles showed some movements even when maintained at 5°C for days. Numerous pellets of excreta were found on the blotting papers inside the containers after the beetles had been held for 2 wk. Within the 2-wk starvation period, it was believed that most of the beetles evacuated their gut as preparing for diapause, thus lowering their supercooling point.

Simulation of the Overwintering Habitats. In Iowa, most of the F_2 adults hibernate in the leaf litter of woodlands and the crop residues of soybean fields through the winter. In soybean fields, the beetles overwinter inside the withered rolled soybean leaves and pods. Whereas in woodlands, the beetles overwinter either inside the withered rolled dicotyledous leaves or hibernate inside the dry-hollowed acorn seed (unpublished data). Danks (1991) had indicated that the insulating power of the substrate of winter habitats and ecological adaptations of migrates to overwintering sites are important for winter survival of hibernating insects. The behavior of the overwintering beetles moving to the litter of forest-floor probably helped the insects to locate a microhabitat avoiding extreme cold temperatures. In addition, the leaf litter served as an insulating layer to enhance the survival of the hibernating beetles.

In this study, at extreme cold temperatures (-10 and -15°C), the highly significant lower mortality rate of those beetles covered with blotting-paper discs demonstrated the importance of an insulating layer in the overwintering habitat. However, compared with the natural situation of leaf litter in a typical woodland, the vials and test tubes used in the study were very small. Under extreme temperatures (-15 to -10°C) at a short period of time (minutes), the discs inside the vials served as an insulating layer; it took a longer time for the beetles in the vials reached 50% mortality. Therefore, those beetles with discs inside the vials, studied at -15 and -10°C, showed significantly lower time-mortality than those without discs. Conversely, at higher temperatures (-5°C or above) and at a long period of study (hours), the paper discs seemed unimportant.

Correlation Between Air Mean and Leaf-litter Mean Temperatures. As expected, the leaf-litter mean temperature was always lower than the air mean temperature in the 2-yr study. However, the results indicated that there was a high correlation between these temperatures when the air temperatures were at -10°C or above. Below -10°C, the leaf-litter mean temperature had a low correlation with the air mean temperature and maintained an average of about -2°C. Within the 2-yr study, leaf litter temperature dropped below -5°C for 3 d only (Table 2). These results suggest that the overwintering beetles seldom experienced temperatures below -10°C when hibernating inside the rolled leaves of the woodland litter.

Cold Tolerance of Overwintering Beetles. In this 2-yr study, most overwintering adults could not survive longer than 30 minutes at temperature -10°C or below. However, at -5°C or above, hundreds of hours were required to kill 50% of the overwintering beetles. This suggests that the critical temperature causing significant mortality of the beetle would be below and close to -5°C. These results agree strongly with those of Boiteau et al. (1980), who found that there was a large difference in survival times of beetles between -5.5 and -8°C. Furthermore, they suggested that the highest cold temperature causing significant mortality for their southern beetles were near -5.5°C. It is noteworthy that the critical temperature for significant mortality is similar for both southern and northern bean leaf beetles.

Winter survival of insects depends on the choice of winter microhabitat and on cold-hardiness (Danks 1978). Millers and Hart (1987) studied the overwintering survivorship of mimosa webworm, *Homadaula anisocentra* Meyrick (Lepidoptera: Plutellidae) and found that the overwintering pupae aggregated behind shutters, under eaves, and other areas associated with heated structures could survive extended cold periods during winter. Parajulee et al. (1997) studied the overwintering habitats of boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae) and found that differences in the isolation provided by overwintering habitats can influence boll weevil survival and subsequent emergence from diapause. In addition, Slosser et al. (1996) indicated that boll weevils did not survive exposure $\geq$ 40 h duration at -10.0°C. In this study, most of the bean leaf beetles maintained inside the vials with blotting-paper discs took a longer time to reach 50% mortality, although only at -10°C or below were there significant differences (Table 1). The blotting-paper discs simulating withered rolled leaves in the overwintering habitats allowed the beetles to avoid extreme temperature fluctuations. This demonstrated that an insulating cover is important for the survival of the overwintering beetles. Hibernating in the leaf litter of the forest-floor allows the persistence of the bean leaf beetle at the latitude in Iowa.

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Table 1. Regression models for 50% mortality of overwintering adult beetles at 5 different temperatures (-15, -10, -5, 0 , and 5°C) with and without blotting-paper discs in the 2-winter study (96-97 and 97-98)

Temperatures (°C)	Blotting Paper[a]	Regression Model	R^2	Time for 50% Mortality
-15	-	$y = 11.038x - 10.496$	0.9789	5.48 min
	+	$y = 8.5911x - 10.954$	0.9724	7.1 min[b]**
-10	-	$y = 6.4002x - 6.5437$	0.8822	8.83 min
	+	$y = 5.0043x - 8.3671$	0.7699	11.66 min[c]**
-5	-	$y = 0.0777x + 18.1$	0.8807	410.55 h
	+	$y = 0.0813x + 17.468$	0.945	400.15 h
0	-	$y = 0.0398x + 15.66$	0.9585	862.81 h
	+	$y = 0.0364x + 16.629$	0.94	916.79 h
5	-	$y = 0.0481x - 2.1178$	0.9467	1083.53 h
	+	$y = 0.0444x - 1.6994$	0.9455	1164.4 h

[a] - : without blotting-paper discs; + : with blotting-paper discs

[b] $F = 11.39$; df = 1, 5; $P = 0.006$

[c] $F = 68.15$; df = 1, 5; $P = 0.0001$

** highly significant when compared with beetles without blotting-paper discs at the same temperature

Table 2. Number of days with litter temperatures of woodland below 0°C in the 2-winter study (96-97 and 97-98)

Leaf litter mean temperature (°C)	No. of days below 0°C[a] 96-97	97-98
-0.01 to -1.0	32	6
-1.01 to -2.0	16	4
-2.01 to -3.0	13	4
-3.01 to -4.0	11	0
-4.01 to -5.0	9	1
-5.01 to -6.0	2	0
-6.01 to -7.0	1	0
Total	87	15

[a] temperatures were measured from mid November (96-97) or mid October (97-98) to mid April of the following year.

Table 3. Regression analyses of air mean and leaf-litter mean temperatures in woodland during the 2-winter study (96-98)

	Temperature range (°C)			
Year [a]	Air mean	Leaf-litter mean	Regression analysis [b]	R^2
96-97	14.18 to -10.0	10.73 to -4.24	$y = 0.0196x^2 + 0.3608x + 0.1391$	0.767
	-10.01 to -22.76	-1.05 to -6.67	$y = 0.0104x^2 + 0.4674x + 1.0948$	0.1795
			$y = 0.1247x - 1.5509$	0.1685
97-98	16.49 to -10.0	13.86 to -2.63	$y = 0.0191x^2 + 0.4889x + 2.0463$	0.8543
	-10.01 to -16.24	0.76 to -4.03	$y = 0.2263x^2 - 6.2349x - 42.936$	0.2398
			$y = 0.218x - 3.9855$	0.0726

[a] 96-97: from mid November to mid April; 97-98: from mid October to mid April.

[b] polynomial and linear regression analyses were shown for air mean <-10°C in each year.

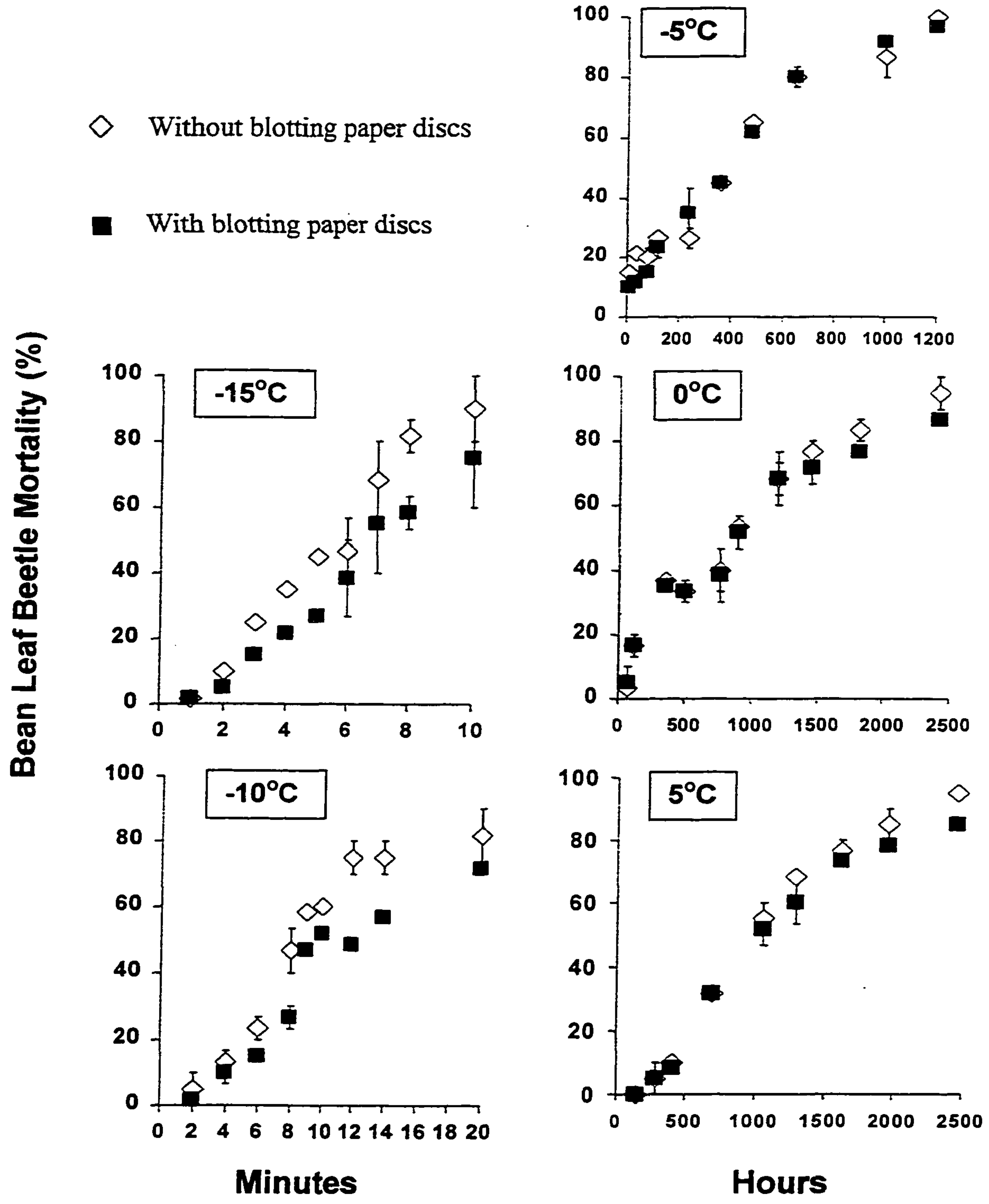

Fig. 1 Time-mortality (%) of overwintering adult beetles with and without blotting paper discs in the vials when exposed to 5 different temperatures (-15, -10, -5, 0, and 5°C) in the 2-winter study (96-97 and 97-98).

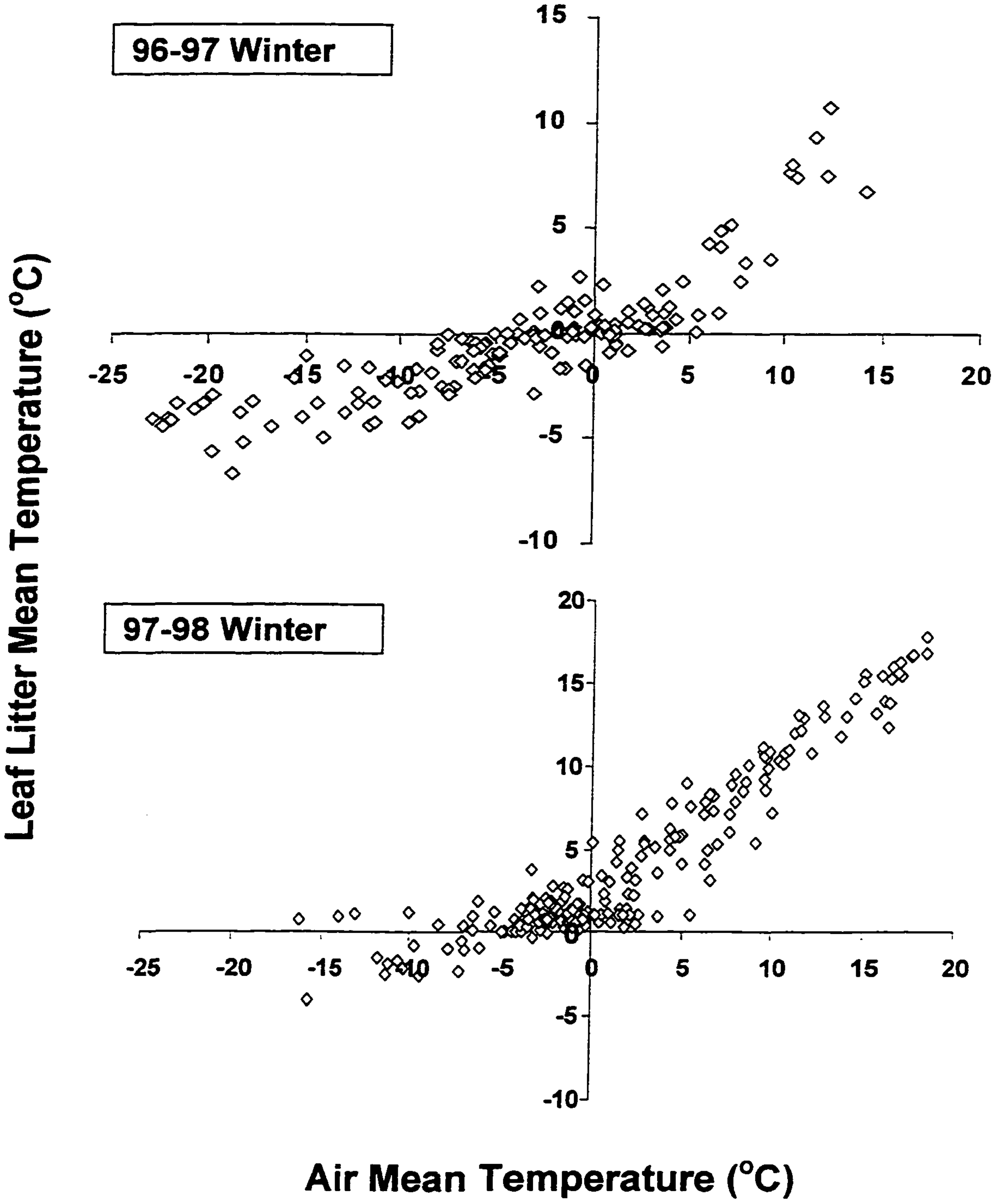

Fig. 2. Relationship between air mean temperature and leaf litter mean temperature in a typical woodland habitat near Ames, Iowa from mid-November (96-97) or mid-October (97-98) to mid-April of the following year.

CHAPTER 3. SPATIAL DISTRIBUTION AND SEQUENTIAL COUNT PLANS FOR OVERWINTERING BEAN LEAF BEETLES (COLEOPTERA: CHRYSOMELIDAE)

A paper to be submitted the Journal of Environmental Entomology

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Abstract

Overwintering bean leaf beetle adults (*Cerotoma trifurcata* (Förster)) were sampled in early winter to estimate spatial distribution of populations and to develop sequential count plans in different habitats. Crop residue or leaf litter in alfalfa fields, cornfields, grasslands, soybean fields, and woodlands, were collected for absolute estimates of hibernating adults during 3 winters, 1996-99. More than 99% of the diapausing adults overwintered in soybean fields and woodlands, whereas a few beetles (<1%) hibernated in alfalfa fields, cornfields, and grasslands. The dispersion of the overwintering population in soybean fields and woodlands was tested by different mathematical analyses, including Poisson distribution, negative binomial distribution, index of clumping, Green's index, Taylor's power law, and Iwao's regression. The results of these analyses were used to develop sequential count plans by using Kuno's formula for the diapausing populations. The overwintering-population distribution and the sequential count plans in both soybean fields and woodlands are discussed.

Introduction

Spatial distribution, which is one of the fundamental properties of species, is an important characteristic of ecological communities (Taylor 1984, Ludwig and Reynolds 1988). Knowledge of insect dispersion patterns is essential for the development of realistic population models and efficient sampling strategies (Boiteau et al. 1979), as well as ecologically sound pest management programs (Pedigo and Zeiss 1996). Economic-injury levels and models to predict insect population densities are useful and important, only if strategies of assessing insect density or population indices are available and accurate (Smelser and Pedigo 1992).

The bean leaf beetle, *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae), is an occasional soybean pest in the Midwest. Although spatial distribution of the bean leaf beetle populations during the growing season is known (Boiteau et al. 1979, Smelser and Pedigo 1992), not much is understood about dispersion and sampling of overwintering adults. Survival of overwintering stages, which is closely related to the initial colonizing population, plays the primary role in determining the status of the pest in the following year (Bale 1991). Hence, a sampling program of the overwintering population is essential to estimate the colonizing population for the next season.

Developing a sampling program requires understanding spatial distribution in order to determine a sequential count plan for insect populations. A number of mathematical attempts have been conducted to better understand insect dispersion by using individual counts per sampling unit and developing sequential count plans for insect pests during the growing season (Bechinski et al. 1983, Davis and Pedigo 1989, Smelser and Pedigo 1992). This study was designed to determine the dispersion of diapausing bean leaf beetle adults in overwintering sites using different mathematical analyses. From this information, a sequential count plan can be developed for overwintering populations.

Materials and Methods

Sampling Plan. A preliminary study, conducted during the winter of 1995-96 showed that hibernating bean leaf beetle adults overwintered mostly in crop residue of soybean fields and leaf litter of woodlands in Iowa (unpublished data). In the 3 consecutive winters of 1996 through 1998, hibernating adults were studied in 5 different habitats: alfalfa, corn, grassland, soybean, and woodland. In the 1996-97 winter, a study was conducted at 5 locations near Ames, Iowa, but in the 1997-98 and 1998-99 winters, study was conducted at 4 locations (Table 1).

In the 1996-97 winter, three sites of three 10x10 m of each habitat type were reserved randomly to sample overwintering beetles at each location. Woodland areas were reserved randomly in a 10m border from the edge of an adjacent soybean field, whereas grasslands, alfalfa, corn, and soybean fields were reserved randomly throughout the field. Leaf litter samples were collected monthly from November through January. On each sampling date, a $1m^2$ sampling unit of crop residue or leaf litter was randomly collected from the reserved sites of each habitat type at each location. Samples collected were bagged and returned to the laboratory for the counts of overwintering adults in each sampling unit.

In 1997-98 and 1998-99 winters, the study was conducted the same way as in 1996-97, except leaf litter was sampled twice a month from soybean and woodland and monthly from alfalfa, corn, and grassland. In addition, samples were collected from mid-October to late December.

Statistical Analyses. For soybean and woodland samples, mean ($\bar{x}$) and sample variance (s^2) were calculated over all locations on a sampling date, and expressed as number of adults per m^2. The methods used to describe the distribution of overwintering beetles were: Poisson distribution, negative binomial distribution, index of clumping, Green's index, Taylor's power law, and Iwao's regression. Methods for calculating and analyzing the beetle distribution followed those of Southwood (1978), Ludwig and Reynolds (1988), Davis (1994), and Pedigo and Zeiss (1996). Equations used in the calculations were:

Index of clumping (IC) (David and Moore 1954)

$$\mathrm{IC} = (s^2/\bar{x}) - 1 \tag{1}$$

Green's index (GI) (Green 1966)

$$\mathrm{GI} = [(s^2/\bar{x}) - 1]/(\mathrm{n}-1) = \mathrm{IC}/(\mathrm{n}-1) \tag{2}$$

Taylor's power law (Taylor 1961)

$$\log_{10}s^2 = \log_{10}a + b\log_{10}\bar{x} \tag{3}$$

Iwao's regression (Iwao 1968)

$$\overset{*}{x} = \alpha + \beta\,\bar{x} \tag{4}$$

Lloyd's (1967) mean crowding index calculation for Iwao's regression analysis

$$\overset{*}{x} = \bar{x} + (s^2/\bar{x}) - 1 \tag{5}$$

In addition, critical stop lines were calculated by using Kuno's formula (1969)

$$T_n = (\alpha + 1)/D_o^2 - [(\beta - 1)/n] \tag{6}$$

where α and β are coefficients from Iwao's regression, T_n is the cumulative number of insects, D_o is a fixed level of precision, and n is the number of samples taken.

Results

Habitats and Population Densities. In the 3-winter study, 9-26% of the overwintering adults were found hibernated in soybean fields, whereas 73-90% overwintered in woodlands (Table 2). Commonly, the overwintering beetles hibernated inside rolled soybean leaves in the soybean fields and rolled dicotyledous leaves in woodland litter. Frequently, beetles in the woodland litter were found inside acorns, which had previously been hollowed out by seed-feeding weevils. Few diapausing adults (<1%) overwintered in alfalfa fields, cornfields, and grasslands. Since >99% of the overwintering beetle population hibernated in soybean fields and woodlands, analyses were conducted and sampling plans were developed for soybean and woodland habitats.

Poisson Distribution. The chi-square test statistic of the Poisson distribution for populations in both soybean fields and woodlands were significant at $P<0.05$, except in soybean in 1997-98 (Table 3). Departure from the Poisson distribution is the standard test of non-randomness (Green 1966); therefore, the null hypothesis of a randomly dispersed population of overwintering adults was rejected.

Negative Binomial Distribution. As indicated by Anscombe (1949) and Taylor et al. (1978), the common k increased with the density of the species being studied. The chi-square estimation for each habitat type for each year was calculated by using the minimum k value and chi-square estimation at the 95% confident interval. The minimum k and chi-square estimation and the 95% CI for k are listed in Table 4. None of the chi-square estimations for populations in soybean fields and woodlands was significant at $P<0.05$. Thus, the null hypothesis of negative binomial distribution was not rejected. Therefore, it was assumed that the distribution of overwintering beetles was aggregated or clumped.

Index of Clumping and Green's Index. The calculated values for both the index of clumping and Green's index for populations in soybean fields and woodlands in the 3-winter study are listed in Table 5. All the calculated indices were > 0, which indicated a clumped dispersion of the overwintering populations. For the index of clumping, the maximum clumping value is equal to n – 1, whereas for Green's index, the value is equal to 1 (Ludwig and Reynolds 1988). Thus, none of the populations in this study showed maximum clumping.

Taylor's Power Law and Iwao's Regression. The slope coefficients from both Taylor's and Iwao's analyses for each year of the populations in soybean fields and woodlands were >1, although not all *t*-statistic analyses were significantly different in each year of the study (Table 6). However, pooled data of the 3-winter study showed high significance in the woodland populations for both Taylor's and Iwao's analyses and significance in the soybean populations for Iwao's regression. Therefore, these analyses also indicated a clumped dispersion of the overwintering beetles in both habitats.

Sampling Sizes and Critical Stop Line. The adequate number of sampling units to be taken depends on the degree of precision desired (Southwood 1978). For practical pest management programs, the recommended precision level (D_o) is 0.25, but it is 0.10 for population research (Pedigo 1999). During the 3-winter study, calculated by Kuno's formula, the maximum sample units for overwintering beetles in soybean and woodland at D_o = 0.1 and 0.25 are listed in Table 7.

The coefficients from Iwao's regression on the pooled data were used to calculate the critical stop line for sequential count plans. Iwao's regression was used because the R^2 values were greater for it than for Taylor's power law, and the coefficients for Iwao's regression were significant for both soybean and woodland populations (Table 6). The α and β values used for Kuno's formula were 1.78 and 1.19, respectively, for populations in soybean fields, and 5.04 and 1.39, respectively, for populations in woodlands.

The critical stop lines for both soybean and woodland sequential count plans, at specified precision levels, are shown in Fig. 1. In addition, the mean overwintering population densities during the 3-winter study are shown as examples for sequential count plans. For example, with the lowest population densities in soybean (2.49/m^2) and woodland (8.11/m^2), at $D_o = 0.25$, the maximum sampling units required were 21 and 19, respectively. At $D_o = 0.1$, however, the maximum sampling units required for soybean and woodland were 131 and 114, respectively.

Discussion

Habitats and Dispersion Analyses. During the study, > 99% of the beetles found were hibernating inside the rolled dicotyledous leaves, dried soybean pods, and hollowed acorns. The overwintering beetles seemed to prefer habitats with rolled dicotyledous leaves rather than grassy areas. Additionally, the overwintering adults seemed to behave thigmotactically inside the residue.

In this study, different mathematical analyses of dispersion were calculated to characterize the distribution of overwintering bean leaf beetle populations. As expected for most animal dispersions, most of the mathematical analyses indicated the distribution of overwintering adults was clumped in both soybean and woody habitats.

There is no universally accepted and satisfactory index for the spatial distribution of organisms. The range and value of index is greatly influenced by sample number, total number of individuals sampled (Lefkovitch 1966), sample size, and population density (Green 1966). Additionally, the spatial distribution of a biological population has complex patterns that are influenced by the heterogeneity of local habitat conditions and the characteristics of the species being studied (Iwao and Kuno 1971). This study indicated two aspects: One is the heterogeneity of the overwintering habitats that included an uneven distribution of the crop residue in the soybean fields or leaf litter of the woodland floor. The other aspect is the characteristics of the overwintering populations, which included the dispersal of adults in the overwintering sites, the aggregation of individuals, and the preference of the overwintering habitats. Further study should be conducted on the preference of hibernating sites by the overwintering beetles in woody areas, including tree species, soil humidity, and leaf litter thickness and temperature.

Sampling Plan. By using the population densities from the 3-winter study, it is feasible to develop a sequential count plan for estimating overwintering beetles. On average, the processing time for one sampling unit was 2 and 3h/person for soybean and woodland, respectively. As mentioned previously, at $D_o = 0.25$, with the lowest population densities during the study, the sampling units required were 21 and 19 for soybean and woodland populations, respectively (Fig. 1). Therefore, the processing time of sequential count samples for either soybean or woodland was less than 60 h. However, for population research, at $D_o = 0.10$, 5 to 6 times more time would be required. To use estimates of overwintered adults in a pest management program, further research should concentrate on how environmental factors affect winter mortality. Furthermore, a model to predict winter survival of the overwintering populations should be developed because these populations are closely related to the colonizing population in the next season.

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Table 1. Habitats for spatial distribution study of overwintering bean leaf beetle during 1996-99 winters

	Location[a]				
Habitat[b]	Curtiss farm	Heck farm[c]	Hinds farm	Johnson farm	Ross farm
Alfalfa	X	NA	NA	X	X
Corn	X	NA	X	X	X
Grassland	X	NA	X	X	NA
Soybean	X	X	X	X	X
Woodland	X	NA	X	NA	X

[a] All are located in Ames or within 4km from Ames (Story County), except the Heck farm is located 25km from Ames (Boone County).

[b] 3 replications for each habitat type at each location.

[c] Only studied during winter of 1996-97.

X: With habitat studied.

NA: No habitat studied.

Table 2. Population densities of overwintering bean leaf beetle adults in habitats studied during 1996-99 winters

Year	Habitat	Mean ± SE[a]	N [b]	%[c]
1996-97	Alfalfa	0	27	0
	Corn	0	27	0
	Grassland	0	27	0
	Soybean	2.49 ± 0.44	45	23.48
	Woodland	8.11 ± 2.55	27	76.52
1997-98	Alfalfa	0.15 ± 0.07	27	0.28
	Corn	0.06 ± 0.04	36	0.11
	Grassland	0	27	0
	Soybean	4.88 ± 0.43	60	9.23
	Woodland	47.84 ± 5.23	45	90.39
1998-99	Alfalfa	0.07 ± 0.07	27	0.25
	Corn	0.14 ± 0.06	36	0.48
	Grassland	0.07 ± 0.05	27	0.25
	Soybean	7.68 ± 0.73	60	26.24
	Woodland	21.31 ± 3.04	45	72.78

[a] Number of beetles ± SE per m^2, by year for all locations.

[b] Total number of samples.

[c] % of beetles among treatments in the same year.

Table 3. The chi-square test statistic of Poisson distribution on overwintering bean leaf beetle population in soybean and woodland (1996-99)

Year	Habitat	Total χ^2	df	χ^2 at $P<0.05$
1996-97	Soybean	33.9*	5	11.07
	Woodland	7984.52*	12	21.03
1997-98	Soybean	16.03	9	16.92
	Woodland	214552302.74*	30	43.77
1998-99	Soybean	893.69*	16	26.3
	Woodland	160098508.18*	26	38.89

* Significant at $P<0.05$; reject Poisson distribution.

Table 4. The test statistic of negative binomial distribution at 95% CI using minimum chi-square estimation of the overwintering bean leaf beetle populations in soybean and woodland (1996-99)

		Minimum χ^2 estimation				
Year	Habitat	k	χ^2	95% CI for k	df	χ^2 at P<0.05
1996-97	Soybean	1.845	5.51[a]	0.843, 4.99	4	9.49
	Woodland	0.59	12.79[a]	0.291, 1.073	11	19.68
1997-98	Soybean	5.455	4.83[a]	1.594, 53.78	8	15.51
	Woodland	1.828	33.83[a]	0.944, 3.046	29	42.56
1998-99	Soybean	1.57	13.92[a]	0.724, 3.129	15	25.0
	Woodland	1.535	28.29[a]	0.872, 2.288	25	37.65

[a] No significant difference at P<0.05; do not reject negative binomial distribution.

Table 5. The index of clumping and Green's index of the overwintering bean leaf beetle populations in soybean and woodland (1996-99)

Year	Habitat	n[a]	Index of clumping [b]	Green's index [c]
1996-97	Soybean	112	2.445	0.022
	Woodland	219	20.701	0.095
1997-98	Soybean	293	1.305	0.004
	Woodland	2153	24.865	0.012
1998-99	Soybean	461	3.172	0.007
	Woodland	959	18.497	0.019

[a] Total number of beetles.

[b] Index > 0 indicated clumped population; maximum clumping = n - 1.

[c] Index > 0 indicated clumped population; maximum clumping = 1.

Table 6. Regression analysis of Taylor's power law and Iwao's regression on overwintering bean leaf beetle population in soybean and woodland (1996-99)

			Taylor's power law				Iwao's regression			
Year	Habitat	n[a]	R^2	a	$b \pm SE$	P[b]	R^2	α	$\beta \pm SE$	P[b]
1996-97	Soybean	15	0.478	<- 0.01	1.51 ± 0.44	0.27	0.622	- 0.58	$2.44 \pm 0.53^{*}$	0.02
	Woodland	9	0.873	0.08	$1.93 \pm 0.28^{**}$	0.01	0.937	4.22	$1.89 \pm 0.18^{**}$	<0.01
1997-98	Soybean	20	0.583	- 0.75	$2.29 \pm 0.46^{**}$	0.01	0.815	0.58	$1.33 \pm 0.15^{*}$	0.04
	Woodland	15	0.501	- 0.43	1.97 ± 0.54	0.1	0.794	6.37	1.31 ± 0.18	0.12
1998-99	Soybean	20	0.354	0.15	1.04 ± 0.33	0.92	0.786	2.14	1.13 ± 0.14	0.36
	Woodland	15	0.61	- 1.23	$2.55 \pm 0.57^{*}$	0.02	0.886	- 15.5	$2.39 \pm 0.24^{*}$	0.03
Total	Soybean	55	0.471	- 0.01	1.25 ± 0.18	0.18	0.749	1.78	$1.19 \pm 0.09^{*}$	0.05
	Woodland	39	0.749	0.01	$1.69 \pm 0.16^{**}$	<0.01	0.85	5.04	$1.39 \pm 0.1^{**}$	<0.01

[a] Number of data points in regression; each data point represents three $1 m^2$ sampling units.

[b] P value of t-statistic for H_o: $b = 1$ (Taylor's) or H_o : $\beta = 1$ (Iwao's).

* Significant at $P<0.05$; ** highly significant at $P< 0.01$.

Table 7. The maximum sampling units of soybean and woodland for overwintering beetles at fixed precision levels by using Kuno's formula during the 3-winter study (1996-99)

		Maximum sampling units required	
Year	Precision level	Soybean[a]	Woodland[b]
1996-97	0.1	131	114
	0.25	21	19
1997-98	0.1	55	52
	0.25	9	9
1998-99	0.1	76	68
	0.25	13	11

[a] The population density of the overwintering adults in soybean were 2.49 (96-97), 7.68 (97-98), and 4.88 (98-99) per m^2.

[b] The population density of the overwintering adults in woodland were 8.11 (96-97), 47.84 (97-98), and 21.31 (98-99) per m^2.

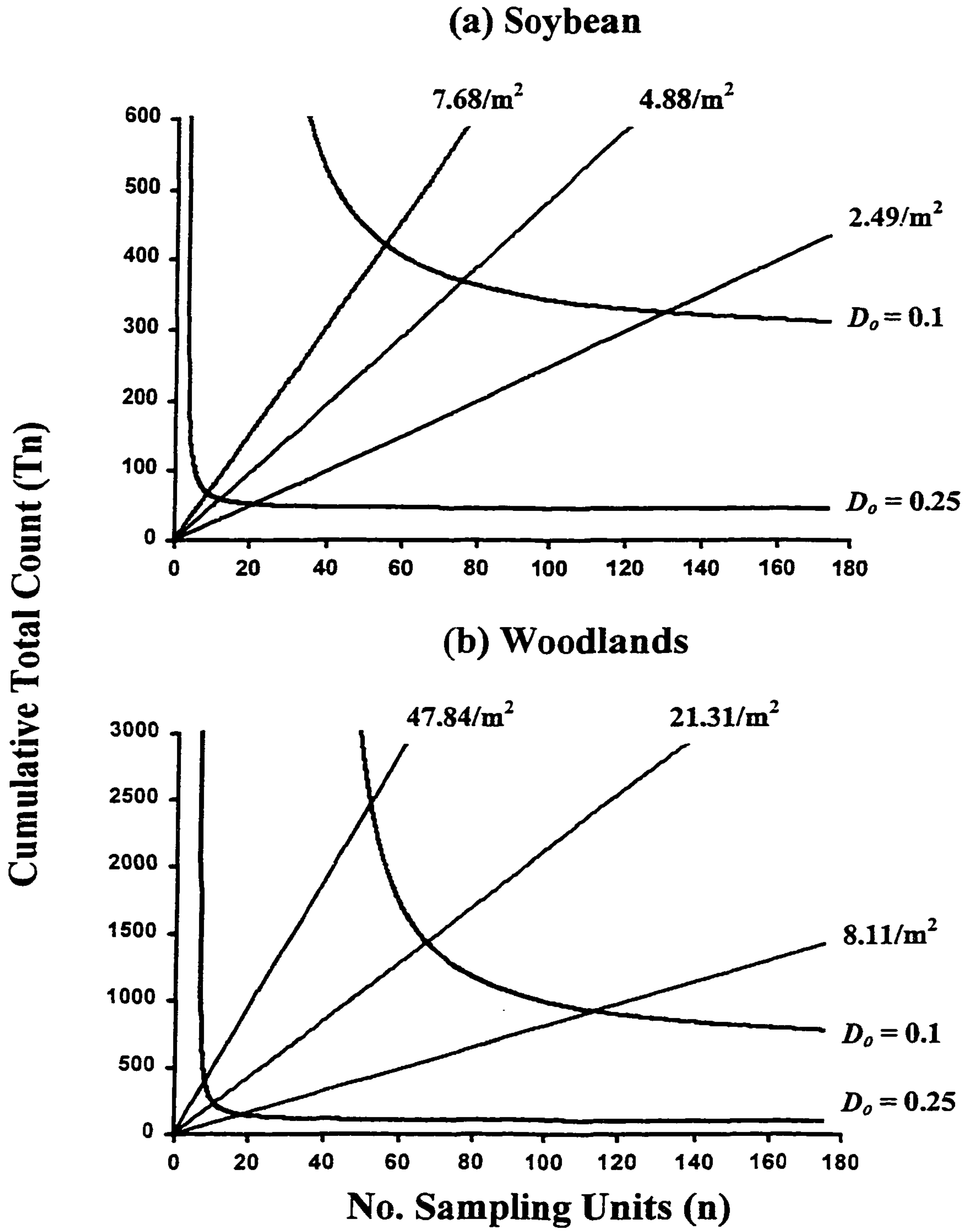

Fig. 1. Sequential count plans for density estimates of overwintering bean leaf beetle adults in a soybean (a) and woodland (b) for specified levels of precision. Precision level at 0.1 is used for intensive sampling in population research, whereas at 0.25 is sufficient for pest management sampling. The mean overwintering densities during the 3-winter study are shown as examples for sequential count plans.

CHAPTER 4. A PREDICTIVE MODEL FOR THE SURVIVAL OF OVERWINTERING BEAN LEAF BEETLES (COLEOPTERA: CHRYSOMELIDAE)

A paper to be submitted the Journal of Environmental Entomology

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Abstract

Survival of overwintering bean leaf beetle (*Cerotoma trifurcata* (Förster)) populations in woodland and soybean habitats was studied for 3 consecutive winters from 1996 through 1999. Four locations of soybean fields and 3 locations of woodlands were studied at Iowa State University farms near Ames, Iowa. In the 1996-97 winter, woodlands and soybean fields were sampled monthly from mid-November through mid-April of the following year, whereas in 1997-98 and 1998-99 winters, both habitats were sampled twice a month from mid-October through mid-April. On each sampling date, three sampling units (1 m^2) of leaf litter or crop residue was randomly collected from soybean fields and woodlands at each location. The collected samples were bagged and returned to the laboratory for absolute estimates of overwintering beetles. The beetles obtained from the samples were kept in individual sample bags at 24°C for 100 h to assess their survival. The beetles then were kept in a freezer at -15°C for later investigation of potential fungal pathogens and ectoparasitic mites. During the 3-winter study, the winter mortality of overwintering beetles in soybean fields (77.04-88.89%) was higher than those in woodlands (48.9-82.29%). The effects of winter temperature on beetle mortality and a predictive model for the overwintering beetle survivorship are discussed.

Introduction

Knowledge of seasonal adaptations and seasonal variability of pests, potential pests, and beneficial species can provide a basis for the prediction of survival populations in an agroecosystem (Tauber et al. 1986). In an ecosystem, populations of organisms interact with their biotic and abiotic environments, having the limiting factors likely to maintain the population densities in equilibrium (Morris 1959, Berryman 1993). These major factors, limiting the population growth and regulating population dynamics, can be used as the parameters to make quantitative forecasts of population fluctuations (Berryman 1997).

In most insects, ecological or behavioral means of avoiding low temperature are common ways to withstand the cold temperatures through winters (Danks 1991). Effective manipulation of insects in the agroecosystem requires an understanding of these insect behavioral mechanisms through the seasonal cycles (Tauber et al. 1986). Although the phenology of the bean leaf beetle, *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae), on soybean and alfalfa during the growing season was thoroughly studied by Waldbauer and Kogan (1976), Boiteau et al. (1979), and Smelser and Pedigo (1991), not much is known about the overwintering habitats and survival of the overwintered populations.

The bean leaf beetle, which is a native species in the eastern half of the United States (Pedigo 1994), is a serious pest in all soybean-growing regions (Hammond et al. 1991). The beetle larvae feed on the roots, root hairs, and nodules of soybean, whereas the adults defoliate the soybean leaves and feed on the external pod tissues (Pedigo and Zeiss 1996). In Iowa, the species develops 2 generations per year and the F_2 adults overwinter (Smelser and Pedigo 1991). However, the survival of overwintering stages, which is closely related to the initial colonizing population, plays the primary role in determining the status of the pest in the following year (Bale 1991). There is a need to understand the hibernating habitats and survival of the beetles through winter. The objectives of the study were to determine those factors that regulate the population densities and survivorship of the pests in hibernating habitats, so as to develop a predictive model for the mortality of overwintered populations.

Materials and Methods

Sampling Plan. Another study (Lam et al. 1999), conducted during the winters of 1996-99, showed that hibernating bean leaf beetle adults overwintered mostly in crop residue of soybean fields (≈20%) and leaf litter of woodlands (≈80%). A few (<1%) also overwintered in alfalfa fields, cornfields, and grasslands. Because the majority of the beetles hibernated mostly in soybean fields and woodlands, this study mainly concentrated on those habitats.

The study was conducted at 4 soybean fields and 3 woodlands near Ames, Iowa for 3 consecutive winters from 1996 through 1999. During the winter of 1996-97, 3 sites having 6 areas (10 x 10 m) at each habitat type were reserved randomly to sample overwintering beetles at each location. Woodland areas were reserved randomly in a 10-m border from the edge of an adjacent soybean field, whereas soybean fields were reserved randomly throughout the field. A 1-m^2 sampling unit of crop residue or leaf litter sample was sampled monthly from November through April of the following year in one of the reserved areas (10 x 10 m) at each site. Samples were bagged and returned to the laboratory for determining the number of overwintering adults.

During the winters of 1997-98 and 1998-99, the study was conducted the same way as in 1996-97, except 3 sites having 12 areas (10 x 10 m) at each habitat type were reserved randomly to sample overwintering beetles at each location. In addition, sampling was conducted from mid-October to mid-April of the following year and collected twice a month from both soybean fields and woodlands.

Survival of Overwintering Beetles. Beetles collected from the samples were placed individually in a sample bag and kept at 24°C (75.2°F) for 100 h. Subsequently, beetle activities were examined. If the beetles were able to stand and walk, they were considered alive, otherwise they were counted as dead (Boiteau et al. 1980, Slosser et al. 1996). All beetles then were stored in a refrigerator at -15°C (5°F) for the studies of entomogenous fungi and ectoparasitic mites.

Entomogenous Fungi Study. Overwintering beetles collected in the 1996-97 and 1997-98 winters were used for the study of entomogenous fungi. Beetles collected were surface-sterilized by a 3% (v/v) sodium hypochlorite solution for 3 min and rinsed 3 times consecutively in 5 ml of sterile deionized water. Then the beetles were placed into agar plates with modified Veen and Ferron's medium (Doberski and Tribe 1980) and incubated at 23°C (64.4°F) for 48 h or more. If no fruiting bodies were observed in the fungal colonies, the fungi were transferred to a low nutrient Sabouraud-dextrose agar at 23°C for another 48 h to enhance fructification. In addition, during the study of beetles from the 1997-98 winter, wings were removed carefully from the body before surface-sterilization.

Ectoparasitic Mites Study. Overwintering adults collected from the 1997-98 and 1998-99 winters were used to study mite ectoparasitism in overwintering populations.

Examination for parasitism was conducted by removing or lifting the elytra of the beetle to observe and count the ectoparasitic mites on the dorsum (Peterson et al. 1992).

Environmental Temperatures. A data set recorder (Model LI-1200S Minimum Data Set Recorder, LiCor, Lincoln, NE) was used to collect the daily meteorological data from mid-November to mid-April in 1996-97 and from mid-October to mid-April in 1997-98 and 1998-99. The data collected were analyzed for the relationship between the air temperature and the temperature beneath the leaf litter.

Data Analysis. Time-mortality of overwintering adults in both soybean and woodland habitats during the 3-winter study were calculated. Regression analyses were used to observe the relationships between air mean temperature with leaf-litter mean temperature and mortality rate. A predictive model for the mortality of overwintering bean leaf beetle adults was developed by accumulating air mean temperature below freezing (air mean subfreezing temperature) throughout winter.

Results

Survival of Overwintering Beetles. The number of overwintering beetles collected and the time-mortality of the beetles in woodlands and soybean fields through the 3-winter study are presented in Tables 1 and 2, respectively. During the study, the number of beetles recovered from the residue in both habitats was highest in early winter (October through November), and the number recovered decreased as winter progressed. It was assumed that the beetles collected in the early winter samples represented the initial overwintering population density of a particular year. The total number of beetles collected in November 1996 was considered to represent the initial overwintering density for that winter. However, for 1997-98 and 1998-99 winters, the mean of the first 3 samples was calculated as the initial populations for those winters. The numbers of missing beetles in Tables 1 and 2 were calculated by using the initial density minus the total beetles found in the same month, whereas the assumed dead beetles were the sum of the dead beetles found and the missing beetles calculated. The time-mortality (%) was calculated by using the number of assumed dead beetles divided by the initial overwintering density times 100. During the 3-winter study, the time-mortality of the beetles in soybean fields (77.04-88.89%) was higher than those in woodlands (48.9-82.29%).

Entomogenous Fungi Study. The prominent fungi and other microorganisms isolated from the overwintering beetle populations are presented in Table 3. All beetles collected in the 1996-97 winter were used for the entomogenous fungi study, whereas in 1997-98, 240 (12 pr from each sampling date) and 540 beetles (27 pr from each sampling date) were randomly selected to conduct the study from soybean and woodland samples, respectively.

Among those fungi observed, *Beauveria* spp. was the only fungus suspected to be entomopathogenic. However, the fungus was found only in 1 year of the study, and <8% of overwintering adults of that year were infected. Other prominent fungi isolated from the overwintering beetles, either suspected to be saprophytic or pathogenic to soybean, were *Alternaria* spp., *Cladosporium* spp., *Aspergillus* spp., *Mucor* spp., and *Penicillium* spp. Additionally, >20% of the beetles studied had no microorganisms isolated from both habitats.

Ectoparasitic Mites Study. In the 1997-98 and 1998-99 studies of parasitic mites, 240 and 540 beetles were randomly selected for analysis from soybean and woodland samples, respectively. None of the overwintering beetles had ectoparasitic mites located on the dorsum. These results implied that the ectoparasitic mites of bean leaf beetles would not overwinter on the beetle and have no impact on the dynamics of the overwintering population.

Environmental Temperatures. Scattergrams between air mean and leaf-litter mean temperatures of the 3-winter study are presented in Fig.1. Regression analyses indicated that there was a strong relationship between these temperatures when air temperatures were at -10°C or above. However, below -10°C, the leaf-litter mean temperature only had low correlation to air mean temperature (Table 4). Characteristics of the 3-winter temperatures between air and leaf litter in woodlands are summarized in Table 5.

Analyses of Overwintering Population Mortality. Since there was a strong relationship between the air mean and leaf-litter mean temperatures (Table 4) during the study, it was assumed that measuring the air mean temperatures would indicate the temperature in the litter residue through winter. Therefore, for simple calculation, it is proposed that the accumulation of air mean temperature below the freezing point (air mean subfreezing temperature) through winter could indicate the cold temperature in the leaf residue of the hibernating sites. In the analyses, the winter temperature of each year was calculated by accumulating the air mean subfreezing temperature from early October through mid-April of the following year. For example, in October, there were only 2 d with air mean temperature below freezing, one was –2 and the other was –3°C. Then, the accumulated air mean subfreezing temperature of October was equal to –5°C.

The accumulated air mean subfreezing temperatures on each sampling date of the month, corresponded to the mortality of the beetles in both woodlands and soybean fields during the study, are listed in Table 6. The regression analyses of the pooled data in Table 6 for the time-mortality of overwintering beetles in woodlands ($y = -0.0954x + 12.752$; $R^2 = 0.7857$) and soybean fields ($y = -0.0982x + 22.153$; $R^2 = 0.7206$) are showed in Fig. 2.

Discussion

Survival of Overwintering Beetles. The overwintering bean leaf beetles hibernated in the residue of soybean fields and woodlands, and the crop residue or leaf litter in the habitats seemed to act as an insulating layer for them. Indeed, during the 3-winter study, the leaf-litter mean temperature in woodlands never dropped below -7°C (Table 5). However, the cold temperatures in the litter through the winter still killed over 50% of the overwintering beetles (Table 6). This result was similar to a study in North Carolina, in which mean mortality of the beetles was estimated to be 65% in protected overwintering habitats and reached 100% in exposed sites (Boiteau et al. 1980).

Entomogenous Fungi Study. The only suspected entomogenous fungi found associated with the overwintering beetles was *Beauveria* (<8.0%) and was isolated in only 1 year of the study. It might be that the fungus was operating in a density-dependent fashion because it was isolated only when the overwintering population density was very high. This result agreed with the study by Payah and Boethel (1986) in Louisiana that the fungus may function in a density-dependent manner. However, our data indicated that the pathogen played a minor role in the mortality of overwintering beetles in Iowa because only a relatively low percentage of fungal infection was found. On the other hand, in Louisiana, the study indicated 65% of the overwintering beetles were infected (Payah and Boethel 1986). In addition, a study in North Carolina (Marrone et al. 1983) indicated that the percentage of overwintering beetles infected by *Beauveria* was 22%. The differences in fungal infection among overwintering beetles in different states may be due to differences in climate.

The prominent nonentomopathogenic fungus isolated from beetles in this study was *Alternaria* spp. (≈40%), which is similar to the study in Illinois (Shortt et al. 1982). However, in Illinois no entomopathogenic fungi were isolated. Furthermore, *Cladosporium* spp. was isolated from beetles in this study, which had not reported in other studies.

Ectoparasitic Mites Study. Ectoparasitic mites seemingly have no effect on overwintering beetles as they were not found overwintering on the dorsum of the insects. This result strongly agreed with the study by Peterson et al. (1992) in which parasitic mites, *Trombidium hyperi* Vercammen-Grandjean, Van Driesche, and Gyrisco and *Trombidium newelli* Welbourn and Flessel parasitized overwintered bean leaf beetles after the beetles entered the soybean and F_1 adults during the growing season. No mites were found on overwintered beetles sampled in alfalfa or on F_2 adults sampled in both soybean and alfalfa. Therefore, it seems likely that the mites only parasitized the overwintered beetles after they had emerged and moved into soybean and on F_1 adults.

Predictive Model for Overwintering Populations. The biotic factors of this study, including the entomogenous fungi and ectoparasitic mites, seemed to have little or no impact on the overwintering populations. Therefore, it was concluded that the major factor having a limiting effect on the population dynamics of bean leaf beetle is winter temperature. A predictive model for the estimation of winter survival of bean leaf beetle populations was developed. By substituting the accumulated air mean subfreezing temperature throughout winter into the equations obtained by the regression analyses (Fig. 2), we can estimate the time-mortality of the overwintering beetles in both soybean fields and woodlands (Table 7).

As mentioned earlier, ≈80% of the overwintering beetles hibernated in woodlands and ≈20% in soybean fields (Lam et al. 1999). The estimated overwintering population mortality of the beetles should be the sum of the mortality in woodlands times 0.8 and the mortality in soybean fields times 0.2 (Table 8).

This model could predict the survival of overwintered beetle adults by accumulating the air mean subfreezing temperature throughout winter. Therefore, knowing the previous year beetle density in soybean, the relative density of the overwintered population in the next early season can be estimated. Since the colonizing population is closely related to the level of survival of overwintered stages (Bale 1991), the model seems to have a good potential to forecast the relative density of colonizing beetles during the early season for management.

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Table 1. Mortality of overwintering bean leaf beetle in woodlands through the 3-winter study (1996-99)

		Overwintering bean leaf beetles						
Year	Month[a]	Live	Dead	Total	Initial density[b]	Missing beetles[c]	Assumed dead[d]	Time Mortality (%)
96-97	Nov	92	4	96	96			4.17
	Dec	54	4	58		38	42	43.75
	Jan	26	7	33		63	70	72.92
	Mar	27	27	54		42	69	71.88
	Apr	17	23	40		56	79	82.29
97-98	Oct	474	5	479				1.04
	Nov-A	430	12	442				2.71
	Nov-B	513	16	529	483.33 ± 43.66			3.02
	Dec-A	323	31	354		129.33	106.33	33.17
	Dec-B	319	17	336		147.33	164.33	34
	Jan-A	307	12	319		164.33	176.33	36.48
	Jan-B	201	14	215		268.33	282.33	58.41
	Feb-B	333	5	338		145.33	150.33	31.1
	Mar-B	248	11	259		224.33	235.33	48.69
	Apr	247	9	256		227.33	236.33	48.9
98-99	Oct	199	2	201				1
	Nov-A	207	5	212				2.36
	Nov-B	206	10	216	209.67 ± 7.77			4.63
	Dec-A	172	4	176		33.67	37.67	17.97
	Dec-B	147	9	156		53.67	62.67	29.89
	Jan-A	129	30	159		50.67	80.67	38.47
	Jan-B	119	23	142		67.67	90.67	43.24
	Feb-A	116	23	139		70.67	93.67	44.67
	Feb-B	111	36	147		62.67	98.67	47.06
	Mar-A	72	16	88		121.67	137.67	65.66
	Mar-B	59	27	86		123.67	150.67	71.86
	Apr	55	21	76		133.67	154.67	73.77

Table 1. (continued)

[a] Samples A and B of the same month represented samples of the first-half and second-half of the month, respectively.

[b] In the 96-97 study, represented only by the November sample; in the 97-98 and 98-99 studies, represented by the mean ± SD of the first 3 samples.

[c] The initial overwintering density minus the total beetles found in the same month.

[d] The missing beetles plus the number of dead beetles in the same month.

Table 2. Mortality of overwintering bean leaf beetles in soybean fields through the 3-winter study (1996-99)

		Overwintering bean leaf beetles						
Year	Month[a]	Live	Dead	Total	Initial density[b]	Missing beetles[c]	Assumed dead[d]	Time Mortality (%)
96-97	Nov	39	6	45	45			13.33
	Dec	18	19	37		8	27	60
	Jan	12	19	31		14	33	73.33
	Mar	10	26	36		9	35	77.78
	Apr	5	9	14		31	40	88.89
97-98	Oct	68	1	69				1.45
	Nov-A	69	4	73				5.48
	Nov-B	50	4	54	65.33 ± 10.02			7.41
	Dec-A	38	4	42		23.33	27.33	41.84
	Dec-B	46	8	54		11.33	19.33	29.59
	Jan-A	31	7	38		27.33	34.33	52.55
	Jan-B	23	3	26		29.33	42.33	64.4
	Feb-B	20	0	20		45.33	45.33	69.39
	Mar-B	26	0	26		39.33	39.33	60.2
	Apr	15	0	15		50.33	50.33	77.04
98-99	Oct	127	7	134				5.22
	Nov-A	92	8	100				8
	Nov-B	106	10	116	116.67 ± 17.01			8.62
	Dec-A	76	2	78		38.67	40.67	34.86
	Dec-B	68	2	70		46.67	48.67	41.71
	Jan-A	61	30	91		25.67	55.67	47.71
	Jan-B	43	12	55		61.67	73.67	63.14
	Feb-A	56	17	73		54.67	60.67	52
	Feb-B	49	13	62		54.67	67.67	58
	Mar-A	35	9	44		72.67	81.67	70
	Mar-B	32	20	52		64.67	84.67	72.57
	Apr	24	18	42		74.67	92.67	79.43

Table 2. (continued)

[a] Samples A and B of the same month represented samples of the first-half and second-half of the month, respectively.

[b] In the 96-97 study, represented only by the November sample; in the 97-98 and 98-99 studies, represented by the mean ± SD of the first 3 samples.

[c] The initial overwintering density minus the total beetles found in the same month.

[d] The missing beetles plus the number of dead beetles in the same month.

Table 3. Microorganisms isolated from the overwintering bean leaf beetles in the 1996-97 and 1997-98 winters

	Percentage of beetles with fungi isolated in the same habitat (%) [a]			
	96-97		97-98	
Microorganisms	Woodland	Soybean	Woodland	Soybean
Beauveria spp.	0	0	7.78	6.67
Alternaria spp.	60.86	48.78	37.59	38.75
Cladosporium spp.	5.34	4.27	23.7	21.25
Aspergillus spp.	10.68	7.93	5.93	6.25
Penicillium spp.	6.41	5.48	6.48	7.5
Mucor spp.	5.69	4.88	1.11	2.08
Septate mycelia	6.76	8.53	8.89	7.92
Bacteria	2.14	9.14	4.81	5.42
No microorganisms	19.22	32.32	22.41	23.75

[a] Total % in the same column >100% because >1 microorganism might be isolated from 1 beetle.

Table 4. Regression analyses of air mean and leaf-litter mean temperatures in woodland during the 3-winter study (1996-99)

Year [a]	Temperature range (°C) Air mean	Leaf-litter mean	Regression analysis [b]	R^2
96-97	14.18 to –10.0	10.73 to -4.24	$y = 0.0196x^2 + 0.3608x + 0.1391$	0.767
	-10.01 to -22.76	-1.05 to -6.67	$y = 0.0104x^2 + 0.4674x + 1.0948$	0.1795
			$y = 0.1247x - 1.5509$	0.1685
97-98	16.49 to -10.0	13.86 to -2.63	$y = 0.0191x^2 + 0.4889x + 2.0463$	0.8543
	-10.01 to -16.24	0.76 to -4.03	$y = 0.2263x^2 - 6.2349x - 42.936$	0.2398
			$y = 0.218x - 3.9855$	0.0726
98-99	19.22 to -10.0	17.12 to -3.67	$y = 0.0135x^2 + 0.5795x + 2.315$	0.8959
	-10.01 to -22.93	-0.07 to -5.38	$y = 0.0234x^2 + 0.7518x + 4.1864$	0.0403
			$y = 0.0005x - 1.5801$	<0.0001

[a] 96-97: from mid-November to mid-April; 97-98 and 98-99: from mid-October to mid-April.

[b] Polynomial and linear regression analyses were shown for air mean <-10°C in each year.

Table 5. Comparison of air mean and leaf-litter mean temperatures in woodland during the 3-winter study (1996-99)

	Lowest temperature (°C)		No. of days below 0°C (days)		Mean ± SD below 0°C (°C) [b]	
Year [a]	Air mean	Leaf-litter mean	Air mean	Leaf-litter mean	Air mean	Leaf-litter mean
96-97	-22.76	-6.67	95	84	-7.96 ± 6.38	-1.97 ± 1.62
97-98	-16.24	-4.03	101	15	-3.9 ± 3.58	-1.56 ± 1.06
98-99	-22.93	-5.38	63	28	-6.69 ± 5.74	-1.57 ± 1.52

[a] 96-97: from mid-November to mid-April; 97-98 and 98-99: from mid-October to mid-April.

[b] Mean ± SD of those days below 0°C.

Table 6. Relationship between air mean temperature and mortality of overwintering bean leaf beetles in woodland and soybean fields during the 3-winter study (1996-99)

Year	Sampling date[a]	Accumulated air mean subfreezing temperature (°C)[b]	Mortality (%)	
			Woodland	Soybean
96-97	Nov	-80.8	4.17	13.33
	Dec	-173.13	43.75	60
	Jan	-504.61	72.92	73.33
	Mar	-828.9	71.88	77.78
	Apr	-837.25	82.29	88.89
97-98	Oct	0	1.04	1.45
	Nov-A	-5.33	2.71	5.48
	Nov-B	-38.79	3.02	7.41
	Dec-A	-63.23	33.17	41.84
	Dec-B	-92.36	34	29.59
	Jan-A	-129.64	36.48	52.55
	Jan-B	-255.55	58.41	64.8
	Feb-B	-314.05	31.1	69.39
	Mar-B	-396.29	48.69	60.2
	Apr	-396.73	48.9	77.04
98-99	Oct	0	1	5.22
	Nov-A	-0.75	2.36	8
	Nov-B	-2.24	4.63	8.62
	Dec-A	-4.16	17.97	34.86
	Dec-B	-50.89	29.89	41.71
	Jan-A	-238.5	38.47	47.71
	Jan-B	-344.64	43.24	63.14
	Feb-A	-371.44	44.67	52
	Feb-B	-396.73	47.06	58
	Mar-A	-415.18	65.66	70
	Mar-B	-421.43	71.86	72.57
	Apr	-421.43	73.77	79.43

Table 6. (continued)

[a] Samples A and B of the same month represented samples of the first-half and second-half of the month, respectively.

[b] All data were recorded by data set recorder (Model LI-1200S Minimum Data set Recorder, LiCor, NE) at one of the overwintering sites studied; except November 1996, when data were recorded from a weather station ≈13 km WSW from Ames.

Table 7. Estimation of accumulated overwintering bean leaf beetle mortality by accumulated air mean subfreezing temperature during winter

Accumulated air mean subfreezing temp. (°C)	Accumulated overwintering bean leaf beetle mortality (%)	
	Woodland [a]	Soybean [b]
-100	22.94	31.97
-200	32.41	41.79
-300	41.87	51.61
-400	51.33	61.43
-500	60.79	71.25
-600	70.25	81.07
-700	79.71	90.89
-800	89.17	100
-900	98.63	
-1000	100	

[a] $y = -0.0954x + 12.752$.

[b] $y = -0.0982x + 22.153$.

Table 8. Predictive model for overwintering bean leaf beetle mortality by the accumulation of air mean subfreezing temperature during winter

Accumulated air mean subfreezing temp. (°C)	Accumulated overwintering bean leaf beetle mortality (%)		
	Woodland[a]	Soybean[b]	Total[c]
-100	18.36	6.4	24.75
-200	25.93	8.36	34.29
-300	33.5	10.32	43.82
-400	41.06	12.29	53.35
-500	48.63	14.25	62.88
-600	56.2	16.22	72.41
-700	63.77	18.19	81.94
-800	71.34	20	91.34
-900	78.9	20	98.9
-1000	80	20	100

[a] (Mortality of beetles in Table 7)*(0.8).

[b] (Mortality of beetles in Table 7)*(0.2).

[c] Sum of beetle mortality in woodland and soybean.

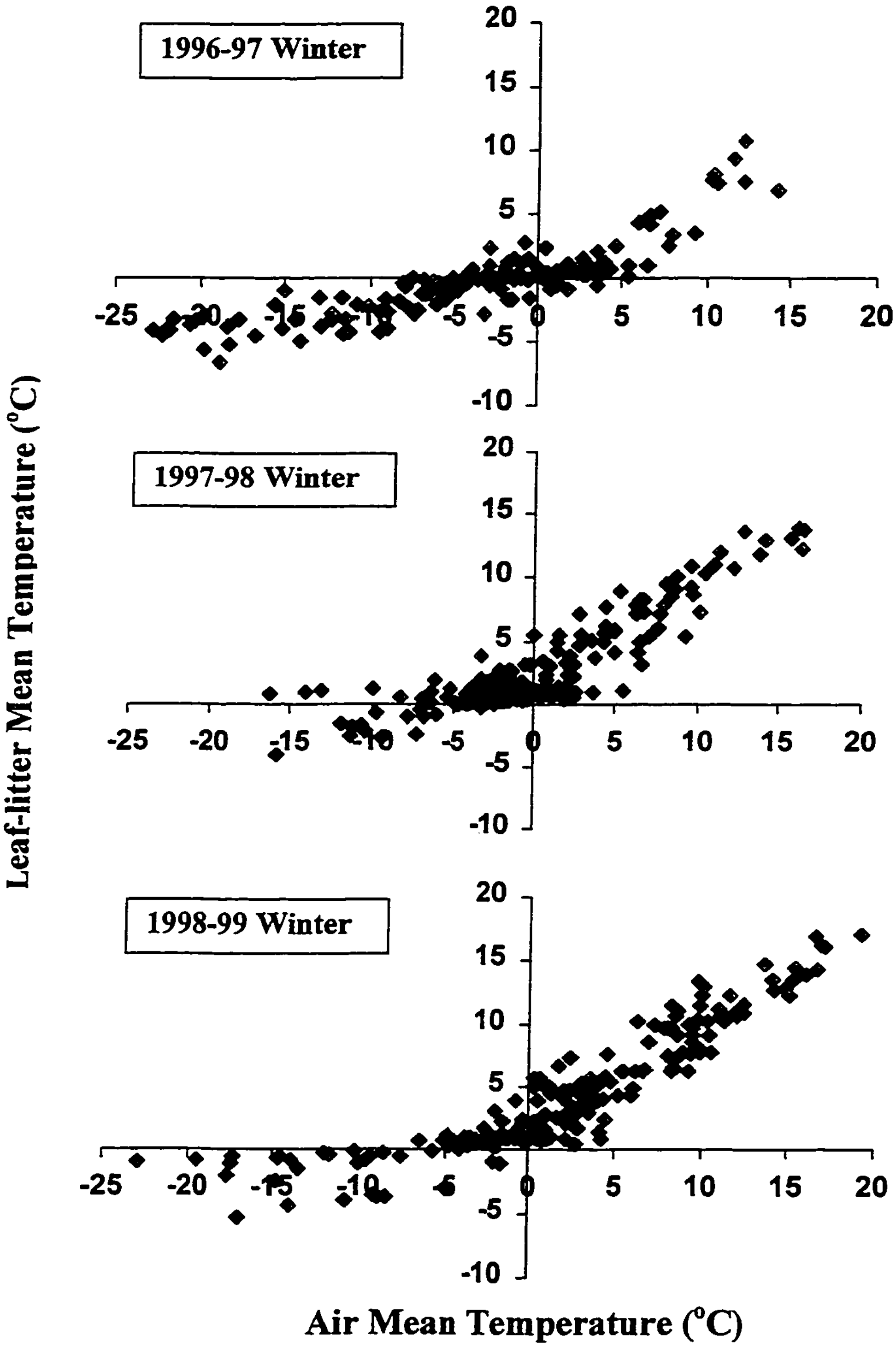

Fig. 1. Relationship between air mean temperature and leaf-litter mean temperature in a typical woodland habitat near Ames, Iowa during the 3-winter study (1996-99)

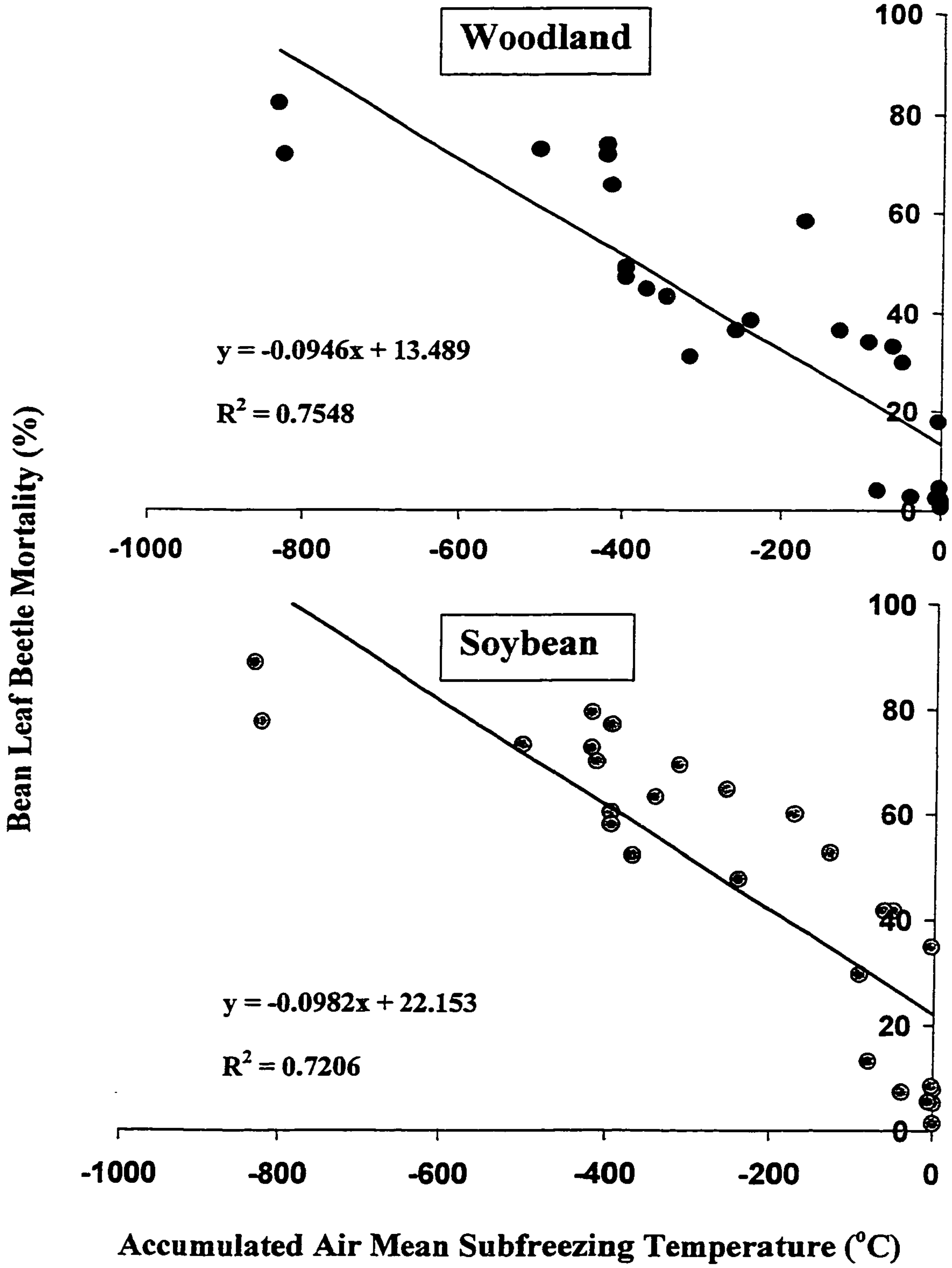

Fig. 2. Regression analyses of pooled data during the 3-winter study for the time-mortality of overwintering bean leaf beetle populations in woodland and soybean habitats (1996-99)

CHAPTER 5. POPULATION DYNAMICS OF BEAN LEAF BEETLES (COLEOPTERA: CHRYSOMELIDAE) IN CENTRAL IOWA

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Abstract

Linear regression and multiple regression stepwise selection procedure analyses were used to relate population fluctuations of bean leaf beetle, *Cerotoma trifurcata* (Förster), in soybean, *Glycine max* (L.) Merrill, to climatic factors. The study was conducted at Iowa State University Johnson farm near Ames, Iowa for 10-yr (1989 through 1998, inclusively). Bean leaf beetle adults were sampled twice weekly from emergence to mature stages of soybean. Linear regression showed a strong relationship between first- and second-generation adult densities during the growing season, whereas multiple regression analyses indicated a strong relationship between the second-generation beetle population densities and environmental factors, including temperature and precipitation. The major factors that affected the population dynamics of bean leaf beetle and regression models for population prediction are discussed.

Introduction

Understanding the population dynamics of organisms depends on the identification of the major factors that relate to population fluctuations (Morris 1959, Price 1997). A combination of factors, including inherited properties of individuals and environmental attributes (Clark et al. 1967), usually influence rate responses of insect activities, such as feeding, development, egg laying, and dispersal. Those environmental attributes, including temperature, humidity, and light intensity, are density-independent factors that affect the dynamics of populations. Preventive management of insect pests is primarily based on the understanding of the effects of these ecological factors on the pests' status (National Research Council 1996, Pedigo 1999).

Although the biology of the bean leaf beetle, *Cerotoma trifurcata* (Förster), (McConnell 1915, Eddy and Nettles 1930, Isely 1930) and the population fluctuations of the insect in soybean (*Glycine max* (L.) Merrill) and alfalfa (*Medicago sativa* L.) is known (Waldbauer and Kogan 1976, Boiteau et al. 1979, Smelser and Pedigo 1991, Pedigo and Zeiss 1996), not much is understood about the regulation and ecological changes of the pest populations. For management of the insect, there is a need for the understanding of environmental effects on the dynamics of the beetle populations.

The bean leaf beetle is an important soybean pest in the southern United States (Kogan et al. 1980, Hammond et al 1991) and an occasional pest in the Midwest (Smelser and Pedigo 1991). During spring, the overwintered adults move from the overwintering sites to wild hosts (Helm et al. 1983) and alfalfa, until soybean emerge, then the beetles colonize the soybean during early vegetative stages (Smelser and Pedigo 1991). The insect has 2 generations per year in Iowa. The second-generation (F_2) adults overwinter mostly in the crop residue of soybean and the leaf litter of woodlands (Lam et al. 1999). In addition, cold temperature, especially below freezing, has a strong effect on the survival of overwintering beetles (Lam and Pedigo 1999a and b). Environmental factors, including temperature (Zeiss et al. 1996) and precipitation during the growing season and winter temperature, certainly has a great impact on the dynamics of the beetle densities. The objectives of this study were to describe the population dynamics of bean leaf beetle adults, relate the insect densities to important climatic factors, and to determine the role of those factors in the population fluctuations of the pest.

Materials and Methods

Plot Location and Experimental Design. The study was conducted at the Iowa State University Johnson Farm, located ≈5 km (3 miles) south of Ames (Story County), for 10 consecutive years (1989 through 1998, inclusively). Soybean plots (30 by 120 m) were located at sites immediately adjacent to alfalfa, which attracted and promoted movement of overwintered adults into soybean. All plots were planted with soybean c.v. *Corsoy 79* at a rate of 40 seeds/m and 76-cm (30-in.) row spacings. Three plots were conducted in 1989 and 1990, whereas four plots were studied from 1991 through 1998. In the study, all soybean were planted on or before May 15, except the years 1993 (May 21), 1995 (May 26), and 1996 (June 12).

Bean Leaf Beetle Populations. The bean leaf beetle adult populations were sampled twice weekly from early development through maturity of the soybean. Samples were taken between 1100 and 1400 h on each sampling date. However, during the early soybean stages, the plants were short and not adequate for sweeping. From emergence until soybean stage V3, 4 in situ counts of a 5-m length of row were sampled in each plot. When the plants were at stage V4 or older, four 50-sweep sampling units were taken in each plot by using a 38-cm-dia. sweep net (Zeiss and Klubertanz 1994). All sweep-net samples were bagged and returned to the laboratory for counting adults. In addition, soybean maturity was estimated according to the maturity stage of Fehr et al. (1971) on each sampling date.

Data Analysis. Climatic data from a weather station (Station ID: 130200) ≈13 km (8 miles) west-southwest of Ames were used for analyses. Relative mean densities of different beetle generations were obtained by calculating the mean of samples collected during population peak periods of a season (Lam and Pedigo 1998). Linear regression and multiple regression stepwise selection procedure analyses (SAS Institute 1996) were used to relate population fluctuations of the beetles with winter temperature, summer temperature, and precipitation (McManus and Giese 1968). The F_2 bean leaf beetles, which cause direct damage of soybean pods, is the most important population for management (Smelser and Pedigo 1992). Therefore, regression analyses were mainly concentrated on the prediction of F_2 density.

In the analyses, the winter temperature of each year was calculated by accumulating the air mean, below freezing temperature (accumulated air mean subfreezing temperature), from October through mid-April of the following year (Lam and Pedigo 1999b). For example, in October of a particular year, there were only 2 d with air mean temperature below freezing, one was -2, and the other was -3°C. Then, the accumulated air mean subfreezing temperature of October of that year was equal to -5°C. During the growing season, summer temperature and precipitation were divided into 2 periods corresponding to the development of the first-generation (F_1) and F_2 beetle generations. The following variables were chosen and used for the multiple regression analyses:

WT = accumulated air mean subfreezing temperature from 1 October through 15 April (°C)

ST1 = accumulated air mean summer temperature from 15 May through 30 June (°C)

ST2 = accumulated air mean summer temperature from 1 July through 10 August (°C)

ST3 = accumulated air mean summer temperature from 15 May through 10 August (°C)

SP1 = accumulated summer precipitation from 15 May through 30 June (cm)

SP2 = accumulated summer precipitation from 1 July through 10 August (cm)

SP3 = accumulated summer precipitation from 15 May through 10 August (cm)

BLBDC = mean density of overwintered beetles (direct counts of 5-m row from VE to V3 soybean stages)

BLBSP = relative mean density of overwintered beetles (per 50-sweep)

F_1 = relative mean density of first-generation beetles (per 50-sweep)

F_2 = relative mean density of second-generation beetles (per 50-sweep)

pF_2 = relative mean density of second-generation beetles of pervious growing season (per 50-sweep)

Results and Discussion

Bean Leaf Beetle Population Fluctuations. Three seasonal peaks of adult beetle populations were observed in soybean plots during each year of the study. Fluctuations of populations in 1997 are presented as an example of the changes during a typical growing season (Fig.1). The first peak represents the overwintered beetles that moved into soybean, whereas the 2^{nd} and 3^{rd} peaks represent the F_1 and F_2 beetle densities, respectively. Commonly, the F_2 beetles have relatively greater population densities than the F_1.

The density changes of overwintered populations, F_1, and F_2 beetles, together with the climatic factor fluctuations, are presented in Figs. 2 to 4. During the 10-yr study, 2 major peaks of population densities were observed, one approximately during the period 1990 to 1992 and the other approximately between 1997 and 1998. Additionally, the 1998 F_2 beetle population had the highest density during the study.

The results of linear regression between beetle populations during the study are presented in Table 1. The analysis showed the strongest relationship between F_1 and F_2 adult densities. The relative mean density of F_2 generation was ≈4 times the F_1 generation ($y = 3.8x - 6$). Such a prediction would be particularly useful for bean leaf beetle management, however, this prediction requires an estimate of the F_1 generation.

In the study, regression analyses showed no strong relationship between the overwintered populations and either the F_1 or F_2 generations. During spring, the beetles emerged from overwintering sites and moved to different environments, including wild host habitats (Helm et al. 1983), alfalfa fields, and early emerged soybean fields (Smelser and Pedigo 1991). Sampling the beetles in soybean fields during the early season only estimated those overwintered beetles that moved into soybean. In addition, the abundance of overwintered beetles in the field is highly related to soybean planting date (Pedigo and Zeiss 1996), further reducing the value of the overwintering population as predictor.

Population Densities and Climatic Factors. None of the beetle population densities was highly correlated to any individual climatic factor by linear regression analysis. It seemed that population fluctuations of the beetle were affected by combinations of environmental factors. Subsequently, multiple regression analyses were applied to characterize impact of these factors on F_2 beetle densities.

Different combinations of time were used to separate the summer temperature and precipitation into 2 periods, corresponding to the development of the F_1 and F_2 beetle generations. The times considered for the first set of periods were 1 May to 30 June, 15 May to 15 June, 15 May to 30 June, and 15 May to 15 July. While the times considered for the second set of periods were 1 July to 30 August, 1 July to 15 August, 1 July to 10 August, and 16 July to 15 September. These periods were used to calculate the accumulated air mean summer temperature and precipitation for the analyses. Furthermore, the accumulated temperature and precipitation of different periods, winter temperature, and different beetle densities were used for the multiple regression analyses.

Results of the analyses of 15 May to 30 June (first period) and 1 July to 10 August (second period) were acceptable in describing the dynamics of the F_2 populations. Four models with acceptable R^2 and P values were obtained (Table 2). The results indicated a strong relationship between the F_2 populations and the climatic factors. Among those models, only model A is a linear equation, all other models contain quadratic elements. As expected, when variables were added in the model, the R^2 value increased.

However, Model A, with only 2 variables in a linear equation, was quite acceptable for the prediction of F_2 density. Model C was selected to represent those models with quadratic elements and compared in the predictability with Model A. Thus, these were the two models selected for further investigation.

To investigate individual variables, constant values of the mean during the study were substituted, leaving a single independent variable in the models. By calculating the means of the pF_2 and substituting into the equations, Models A and C became:

$$F_2 = 55.18 - 1.5(SP2) \qquad \text{(Model AA)}$$

$$F_2 = 0.002(WT)^2 + 2.18(WT) - 0.013(ST2)^2 + 26.36(ST2) - 11798 \qquad \text{(Model CC)}$$

The relationship in Fig. 5 was obtained by substituting the data of SP2 during the study into Model AA. In the graph, the line intercepts x-axis at 37cm of precipitation. It indicated that if the accumulated precipitation was >37cm, a negative number of beetles would be obtained. Hence, the domain of the variable SP2 in Model A would be considered as:

$\{0 < SP2 < 37\}$

Moreover, the range of overall mean value of SP2 within one standard deviation during the 10-yr study was 17.44 ± 12.63, which was included in the domain of SP2 in the model. Therefore, it seemed that Model A has a good potential to predict the F_2 density and also seems biologically realistic.

By calculating the means of the second-period summer temperature and substituting into Model CC, the model became:

$$F_2 = 0.002(WT)^2 + 2.18(WT) + 664 \qquad \text{(Model CC1)}$$

The curve in Fig. 6 was obtained by substituting the data of WT during the study into Model CC1. The curve intercepts the x-axis at -500 and -800°C. The curve indicated that when the accumulated air mean subfreezing temperature was above –500°C, there is a good potential to predict the F_2 density. However, when the accumulated subfreezing temperature was between –500 and -800°C, the predicted F_2 densities were negative. Furthermore, when the accumulated subfreezing temperature was below -800°C, the predicted insect density increased, as the winter temperature decreased. These predictions of the beetle densities are unrealistic.

By calculating the means of the winter temperature and substituting into Model CC, the model became:

$$F_2 = -\,0.014(ST2)^2 + 26.36(ST2) - 12457 \qquad \text{(Model CC2)}$$

Substituting the data of ST2 during the study into Model CC2, the curve in Fig. 7 was obtained. The curve intercepts the x-axis at 900 and 1000°C (by calculation). Beyond the range 900 to 1000°C, the predicted F_2 densities were negative, which also is unrealistic. Therefore, the domain of the variables in Model C would be considered as:

$\{WT \geq -500; \quad 900 \leq (ST2) \leq 1000\}$

The range of overall mean value of WT within one standard deviation during the 10-yr study was –593.5 ± 151.55, which fell outside the domain of WT in Model C. Moreover, the overall mean value of ST2 within one standard deviation during the study was 919.96 ± 37.59, which also was fell outside the domain of ST2 in the model. Hence, the potential of Model C for use in prediction of beetle density seemed less reliable than that of Model A.

To further investigate the models, a graph of original data and the predicted F_2 densities from Models A and C are presented in Fig. 8. In 6 out of 9 years of prediction (90, 93, 94, 95, 96, and 97), the predicted values of Model A were better than that from Model C. Because of the unrealistic nature of the variables, less predictability, and reliable in prediction, Model C was dropped from further consideration. All other models with quadratic elements were considered to behave similarly to Model C. Model A was accepted as the model for the prediction of F_2 density in late August and early September. Therefore, the model (Model A) that included only linear elements, including previous season F_2 beetle density and summer precipitation, is the one recommended for the prediction of F_2 density and management consideration.

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Table 1. The relationship between bean leaf beetle population densities during the growing season in the 10-yr study (1989-98)

Relationship [a]				
y	x	Regression analysis [b]	R^2	*P*
F_1	BLBDC	$y = 0.5x + 7$	0.02	0.71
F_2	BLBDC	$y = -2x + 30$	0.02	0.7
F_1	BLBSP	$y = 0.3x + 8$	0.01	0.77
F_2	BLBSP	$y = -2x + 29$	0.02	0.7
F_2	F_1	$y = 3.8x - 6$	0.73	<0.01
F_2	pF_2	$y = 2.2x - 4$	0.56	0.02

[a] BLBDC: Mean density of overwintered beetles (direct counts of 5-m row from VE to V3 soybean stages); BLBSP: Relative mean density of overwintered beetles (per 50-sweep); F_1: Relative mean density of first-generation beetles (per 50-sweep); F_2: Relative mean density of second-generation beetles (per 50-sweep); pF_2: Relative mean density of second-generation beetles of pervious growing season (per 50-sweep).

[b] Regression coefficients were rounded by using Kelley's rule (1924).

Table 2. Acceptable models obtained from multiple regression stepwise selection procedure analysis during the 10-yr study (1989-98)

Model [a]	Multiple regression stepwise selection procedure analysis [b]	R^2	P
A	$F_2 = 2.3(pF_2) - 1.5(SP2) + 21$	0.8	<0.01
B	$F_2 = 0.0022(WT)^2 + 2.8(WT) - 0.022(ST2)^2 + 41(ST2) - 18000$	0.9	0.01
C	$F_2 = 1.1(pF_2) + 0.00175(WT)^2 + 2.2(WT) - 0.014(ST2)^2 + 26(ST2) - 11800$	0.98	<0.01
D	$F_2 = 1.04(pF_2) + 1.21(F_1) + 0.00116(WT)^2 + 1.37(WT) + 0.73(ST2) + 0.0244(SP1)^2 - 297$	>0.99	<0.01

[a] All P values of the coefficients of variables in the models are ≤ 0.05.

[b] Regression coefficients were rounded by using Kelley's rule (1924); F_2: No. beetles per 50-sweep; pF_2: previous year F_2; WT: accumulated air mean subfreezing temperature from 1 October through 15 April (°C); ST2: accumulated air mean summer temperature from 1 July through 10 August (°C); SP1: accumulated summer precipitation from 15 May through 30 June (cm); SP2: accumulated summer precipitation from 1 July through 10 August (cm).

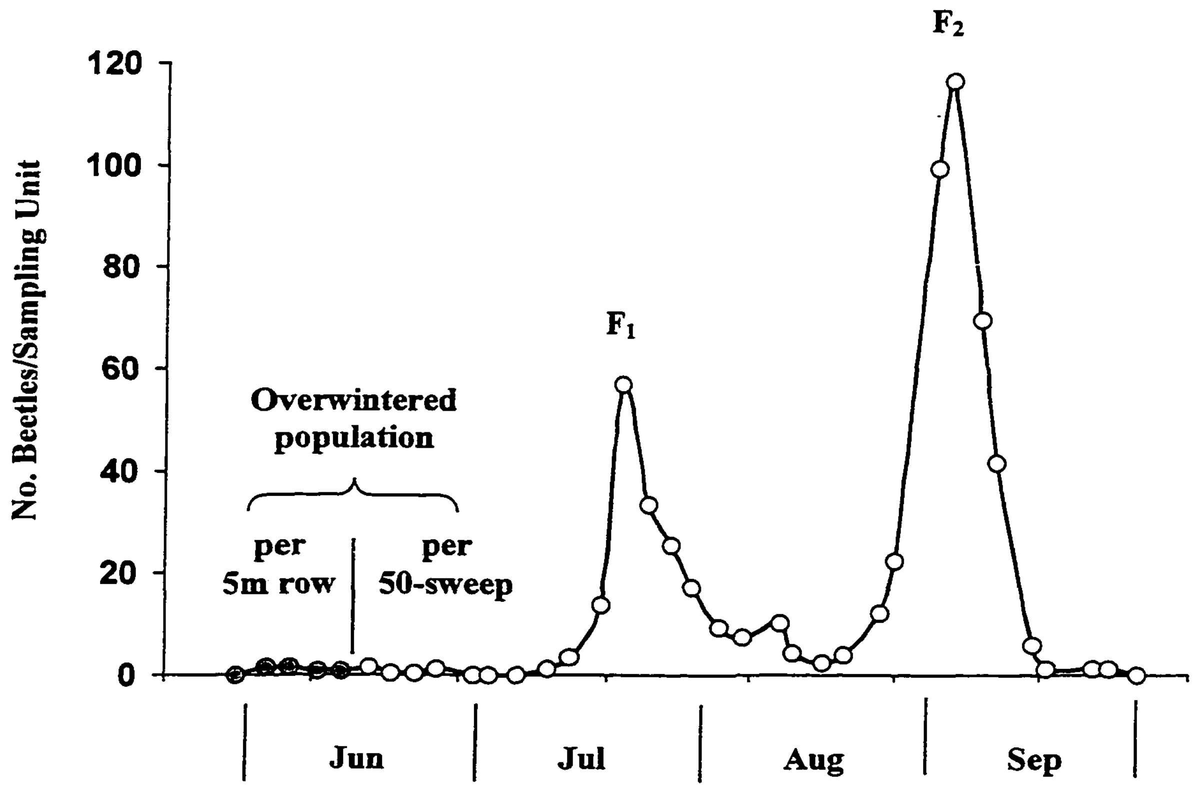

Fig. 1. Bean leaf beetle population fluctuation in 1997. Overwintered population was sampled by 2 methods: in situ counts (5m row) when soybean stages were V3 or below and sweeping (50-sweep) when soybean were V4 or older. Sampling units for both F_1 and F_2 were 50-sweep.

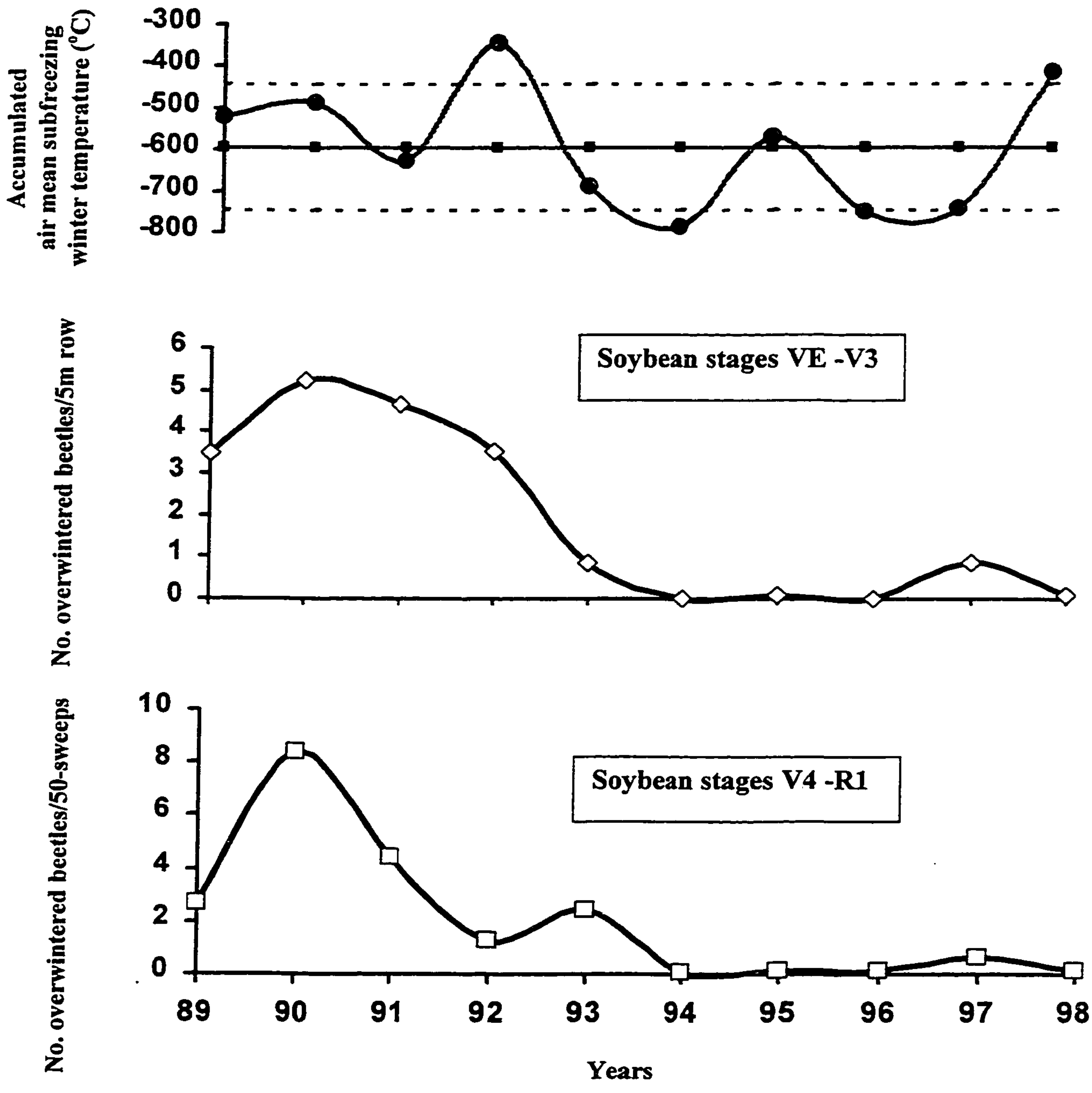

Fig. 2. Overwintered population fluctuations, including in situ counts and sweeping samples, compared to accumulated air mean subfreezing winter temperature (1 October through 15 April) of central Iowa for the 10-yr study (1989-1998). Mean ± SD of the winter temperature is presented.

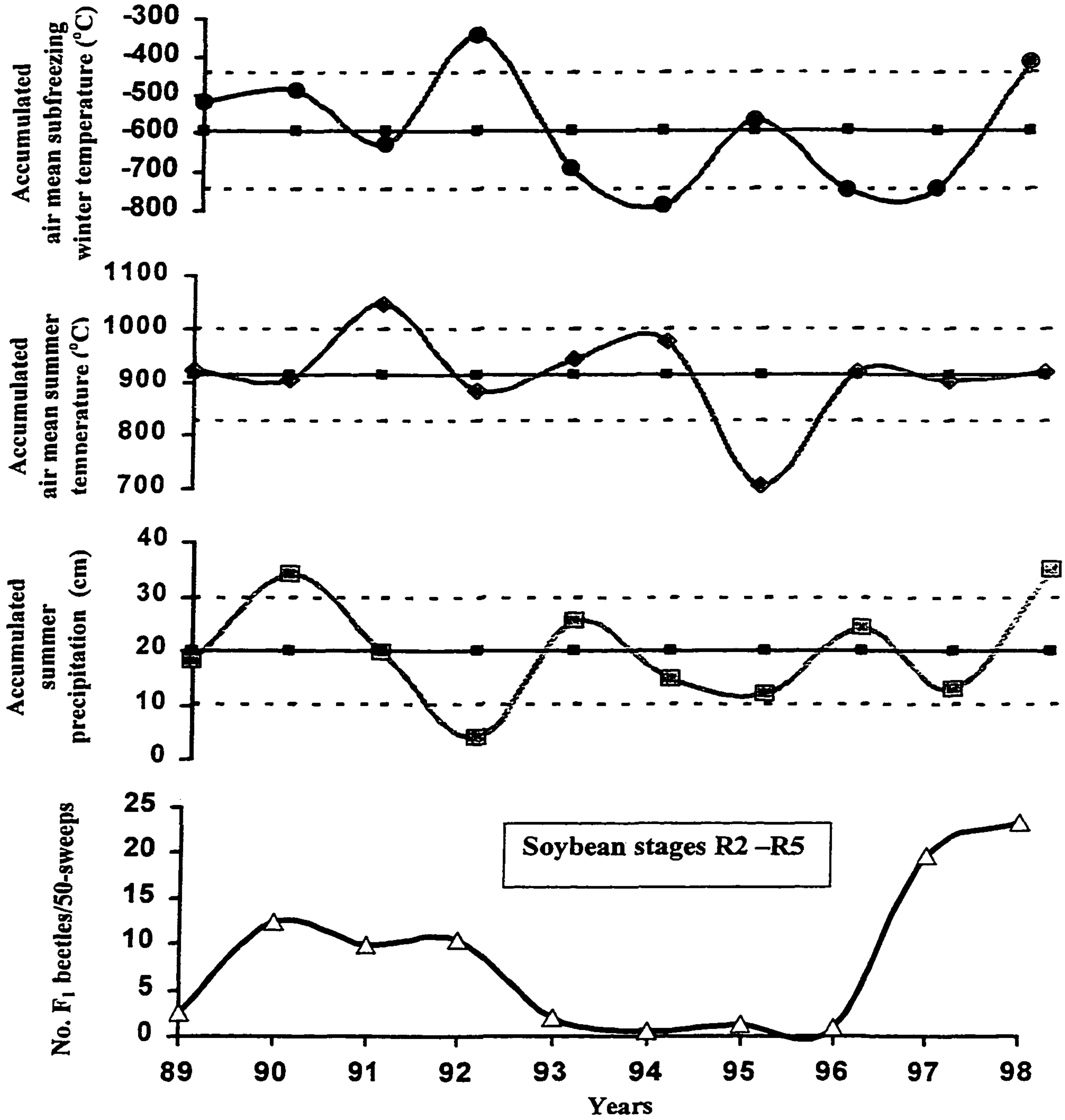

Fig. 3. The F_1 population fluctuations compared to accumulated air mean subfreezing winter temperature (1 October through 15 April), accumulated air mean summer temperature (15 May through 30 June), and accumulated summer precipitation (15 May through 30 June) of central Iowa for the 10-yr study (1989-98). Mean ± SD of the climatic factor is presented on each graph.

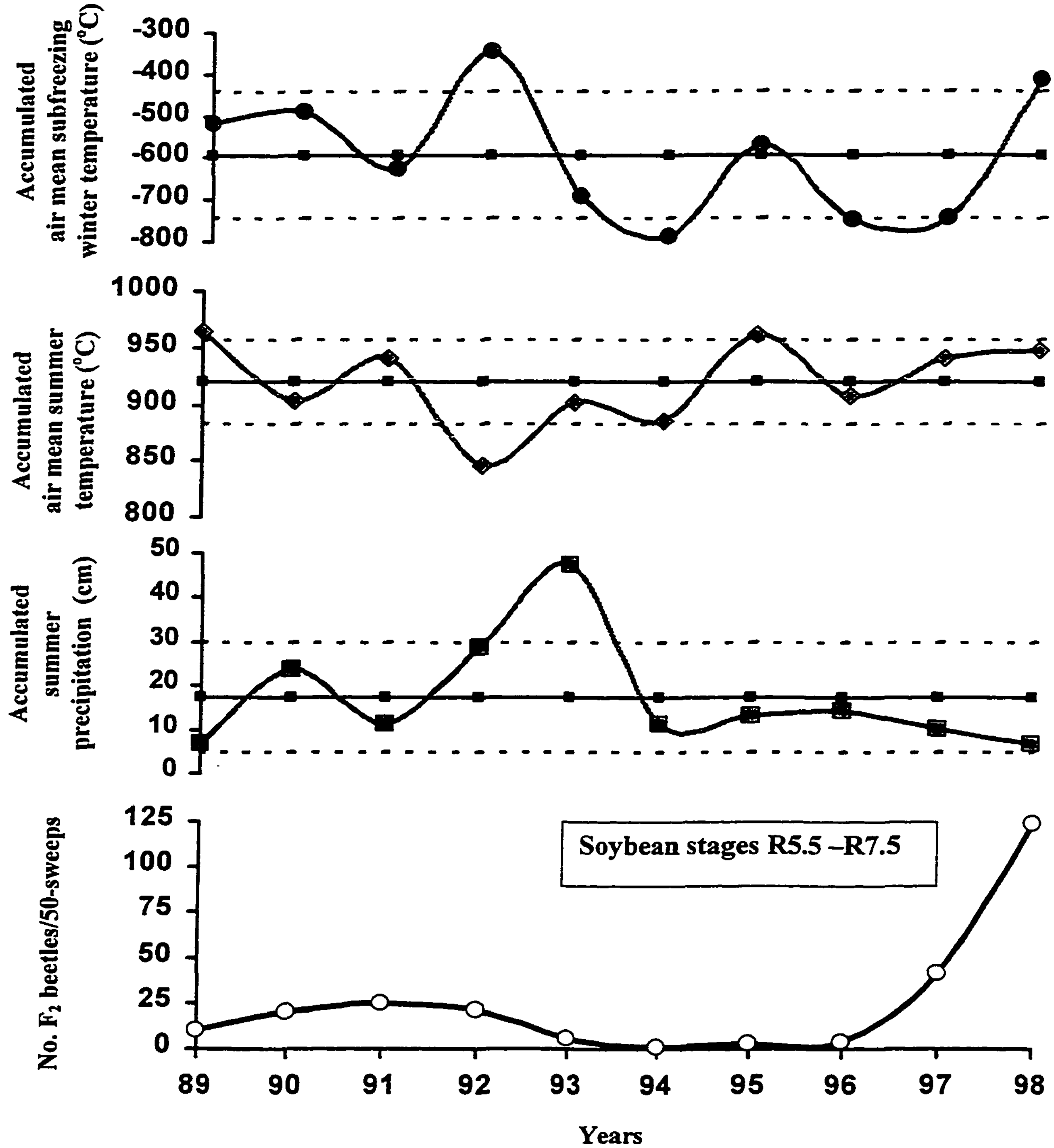

Fig. 4. The F_2 population fluctuations compared to accumulated air mean subfreezing winter temperature (1 October through 15 April), accumulated air mean summer temperature (1 July through 10 August), and accumulated summer precipitation (1 July through 10 August) of central Iowa for the 10-yr study (1989-98). Mean ± SD of the climatic factor is presented on each graph.

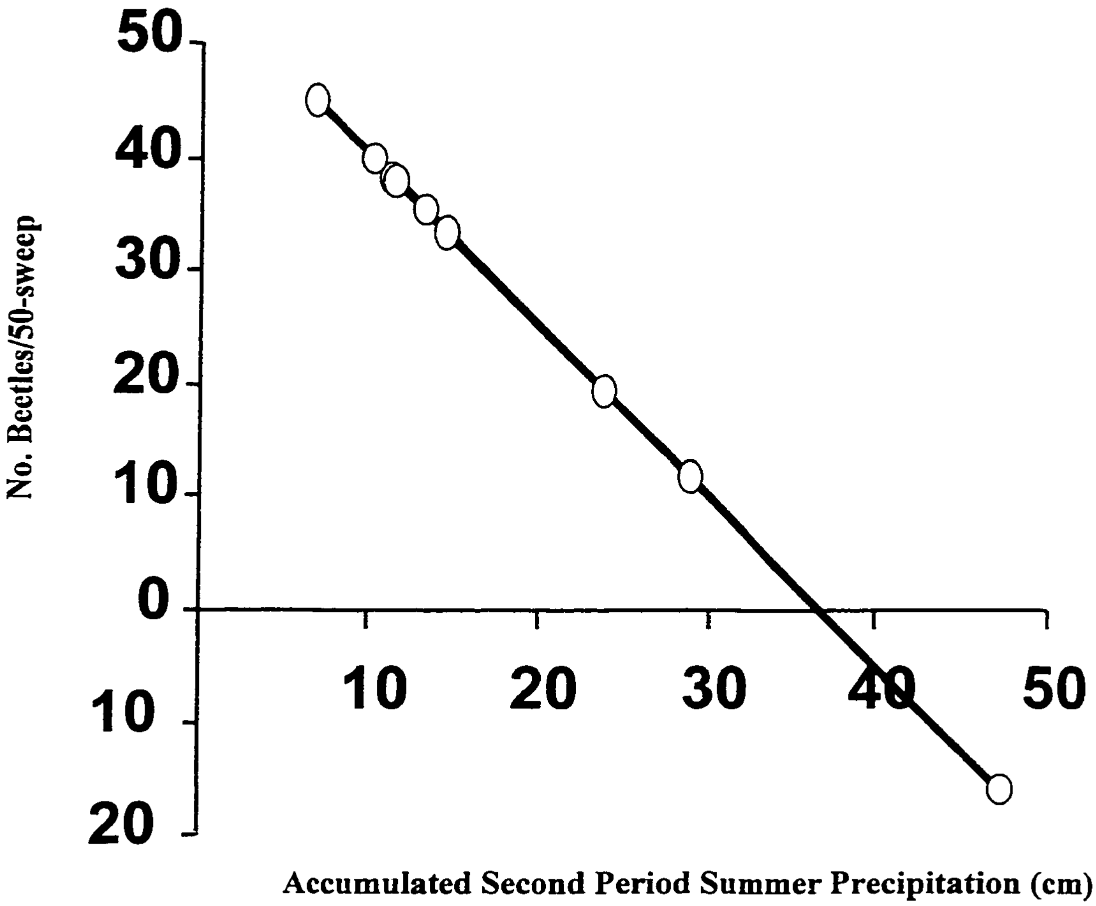

Fig. 5. The predicted number of F2 adults per 50-sweep by substituting the accumulated second period summer precipitation during the study into Model AA.

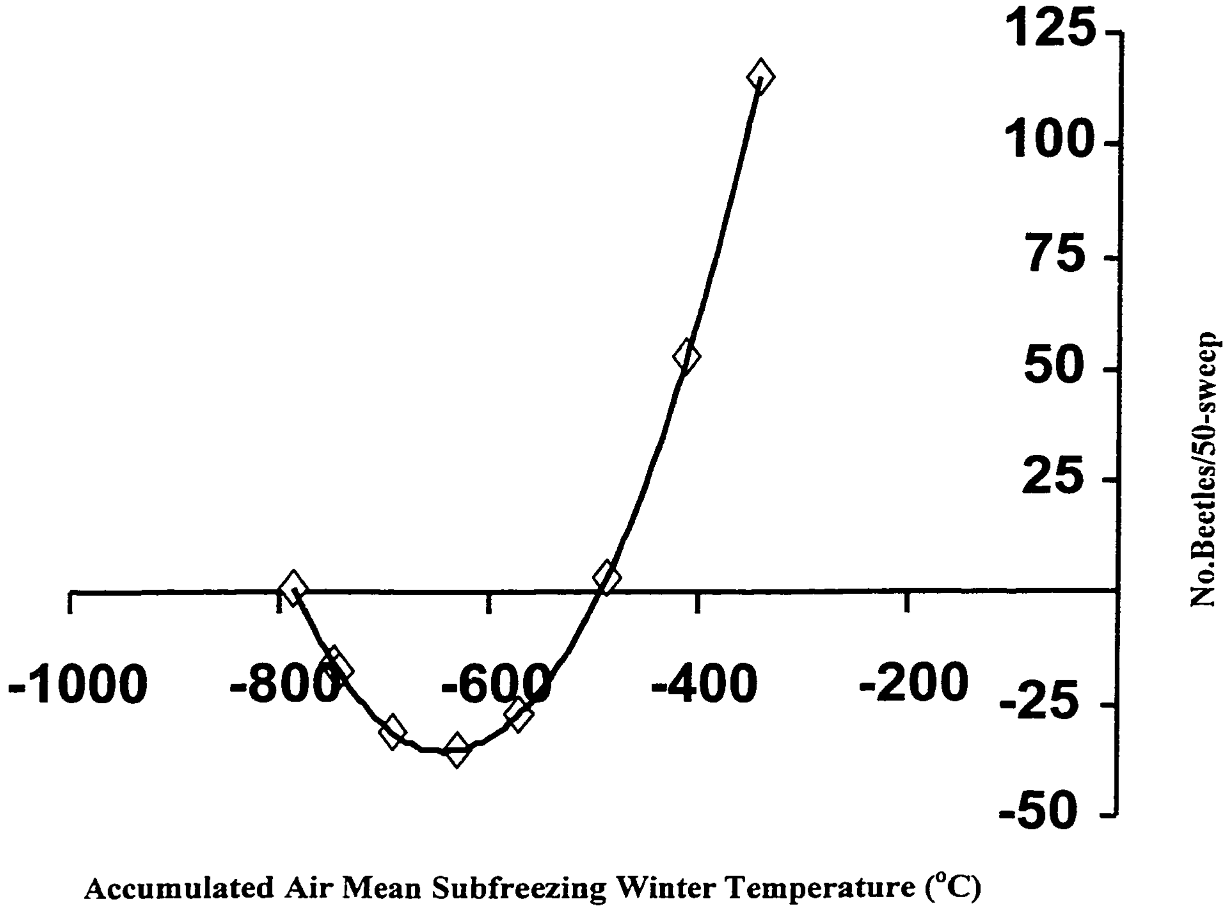

Fig. 6. The predicted number of F_2 adults per 50-sweep by substituting the accumulated air mean subfreezing winter temperature during the study into Model CC1.

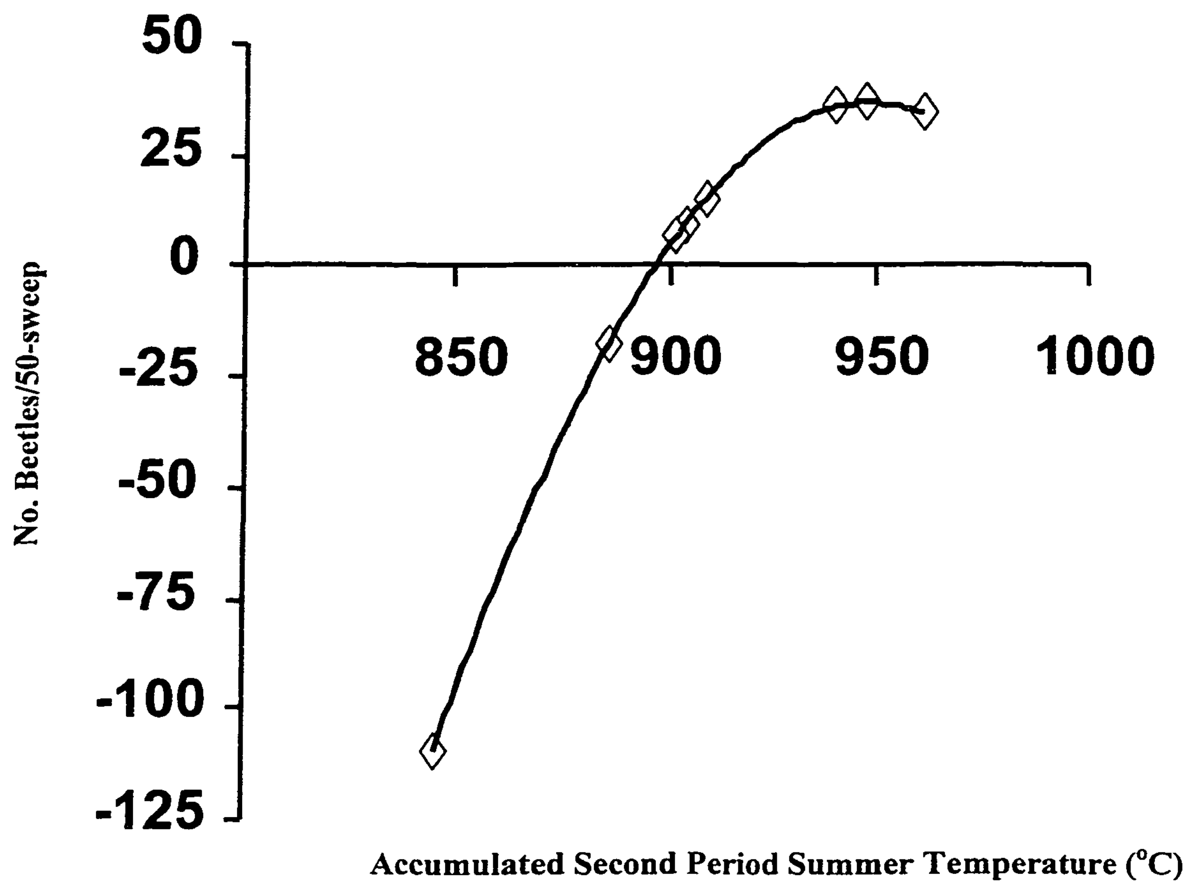

Fig. 7. The predicted number of F_2 adults per 50-sweep by substituting the accumulated second period air mean summer temperature during the study into Model CC2.

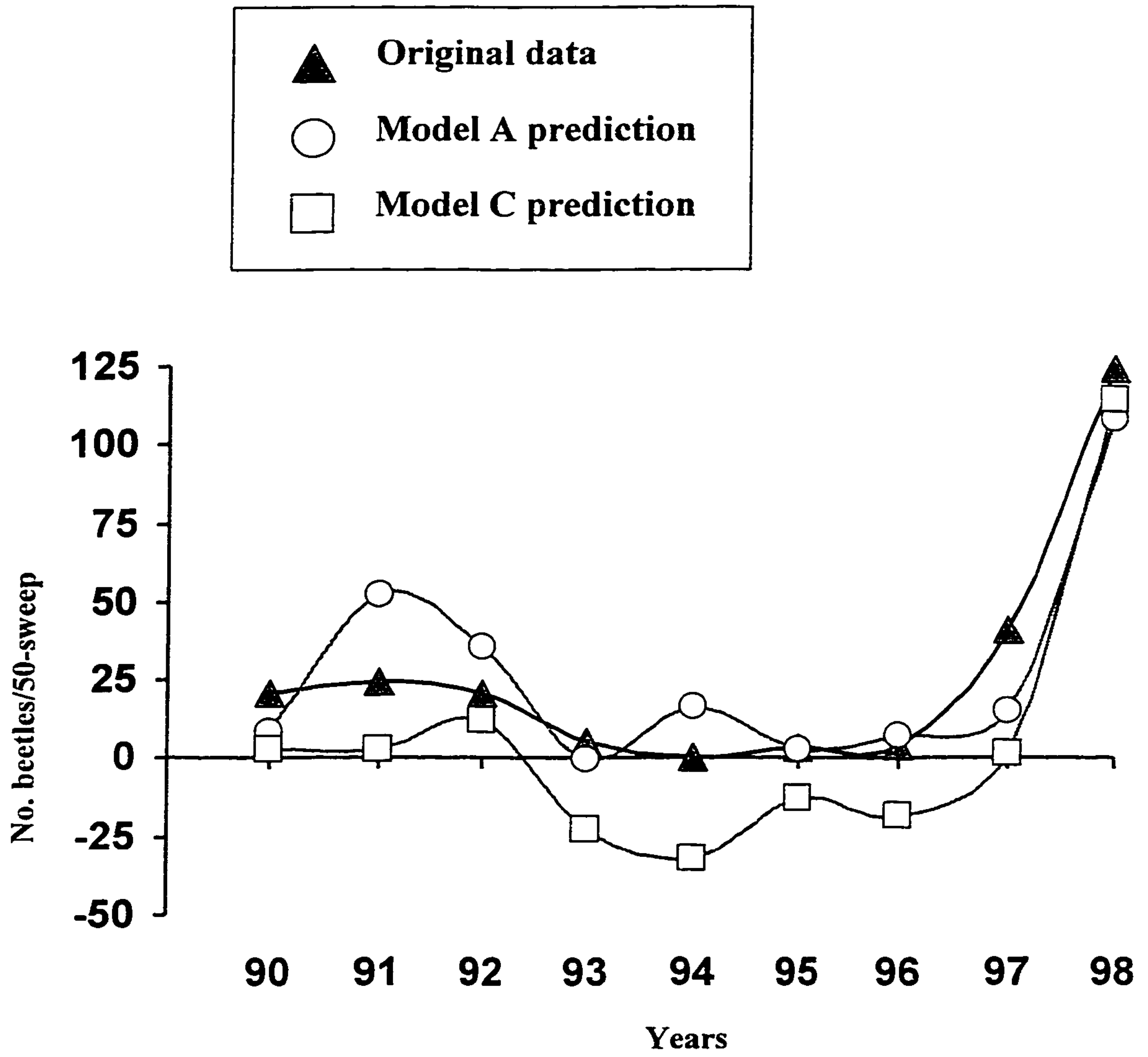

Fig. 8. The original data of F_2 densities and the calculated predicted numbers of F_2 adults from Models A and C during the 10-yr study (1989-98).

CHAPTER 6. GENERAL CONCLUSIONS

The ecological study of bean leaf beetle adults indicated that the population dynamics of the beetle during seasonal cycles were highly affected by the winter temperature and mid summer precipitation. Cold temperature has a strong effect on the survival of the overwintering populations, whereas summer precipitation seemed to be a major factor affecting the population fluctuations of the beetle during growing season in central Iowa.

Laboratory study on the cold tolerance of the overwintering adults showed that most of the beetles survived more than hundreds of hours at -5°C or above. However, most of the beetles died at -10°C or below. These results indicate that cold temperature has a strong effect on the winter survival of the overwintering populations.

Study on the spatial distribution of the overwintering beetles showed that the overwintering adults aggregated in the residue of the overwintering sites, including soybean fields and woodlands. Additionally, only a few beetles overwintered in alfalfa fields, cornfields, and grasslands. Sequential count plans of sampling the overwintering beetles in both soybean and woodland habitats were developed. The results of the study indicated that it is feasible to sample the overwintering populations for pest management programs.

Although the residue in the overwintering habitats served as an insulating layer for the beetles, over 50% mortality of the overwintering adults was observed during the 3-yr study on the overwintering populations in central Iowa. The result showed a strong relationship between the air mean temperature and leaf-litter mean temperature in woodlands. In addition, the mortality of the overwintering beetle is highly correlated to the cold temperature of the winter. A predictive model for the mortality of the overwintering populations in central Iowa was developed.

Analyses on the linear regression showed that the first- and second-generation adult densities during a growing season were highly correlated. Likewise, multiple regression stepwise selection procedure indicated that the second-generation adult density was strongly related to the previous year beetle density and mid summer precipitation. Based on these correlation two predictive models were developed for the second-generation adult density. One of the models was simply based on the linear regression of the first-generation adult density, whereas the other model was based on the multiple regression of the previous second-generation adult density and mid summer precipitation. The models indicated that during the growing season the second-generation beetle density, which is the economic population, could be predicted in early August.

As a whole, this study indicated that the fluctuation of bean leaf beetle densities in soybean fields is strongly related to the history of beetle populations and the climatic factors, including winter temperature and summer precipitation.

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