Natural enemies of the cottonwood leaf beetle in central Iowa

by

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CHAPTER ONE: GENERAL INTRODUCTION

Introduction

The United States, and indeed the world, is rapidly approaching a major energy crisis. According to the U.S. Department of Energy (http://nrelinfor.nrel.gov/documents/energy/re-bioms.html), 97% of the nation's transportation fuel needs are met by petroleum; 49.3% is imported from foreign countries. Our current dependence on a rapidly diminishing supply of non-renewable fossil fuels, combined with increasing damage to the environment, has researchers investigating alternative energy sources. The advantages of using renewable fuels include reducing national dependence on foreign oil, creating jobs, and reducing environmental damage and global climate change.

Alternative fuels under investigation include biodiesel from microalgae, electricity from biomass, and ethanol produced from nonfood agricultural energy crops, and household, industrial, agricultural, and forestry wastes. Emphasis on the use of ethanol has led to increasing interest in short rotation forestry. Fast growing trees (especially *Populus* hybrids) can be grown in short rotation (5 - 10 yrs) plantations for the production of biomass for energy and pulp production.

The U.S. Department of Energy has established goals for the next few years. Ethanol currently supplies about 1% of the nation's transportation fuel needs. By the year 2000, 10% of cars on U.S. highways will use alternative fuels; 25% by 2010. The current cost of converting biomass to ethanol is less than $1.00/gallon. By the year 2000, conversion costs will be reduced to $0.67/gallon.

In order to reduce the cost of ethanol, research needs to find ways of reducing the cost of growing hybrid *Populus*. Options include breeding pest resistant hybrids, using less competitive land (marginal agricultural land and riparian areas), and reducing the use of insecticides. Reduction of insecticide use involves developing improved chemical, cultural, and biological control methods.

One of the major insect pests of *Populus* hybrids in the U.S. is the cottonwood leaf beetle, *Chrysomela scripta* F. (Coleoptera: Chrysomelidae). The goals of this study were to: (1) identify the natural enemies of *C. scripta* in central Iowa, (2) quantify the impact of natural enemies on *C. scripta*, and (3) develop a temperature dependent model of development for *C. scripta*.

Thesis Organization

This general introduction is followed by three individual papers (one study per chapter) of the thesis, and a general summary. In Chapter Two, the natural enemy complex of *Chrysomela scripta* F. (Coleoptera: Chrysomelidae) in central Iowa is identified. In Chapter Three, partial life table analysis is used to evaluate the effectiveness of natural enemies and other mortality factors on *C. scripta*. In Chapter Four, the temperature dependent development of *C. scripta* is studied. These three papers follow the general guidelines of the Entomological Society of America for submission to scientific journals.

Literature Review

Populus Hybrids Used in Short Rotation Agroforestry

The continuing depletion of worldwide fossil fuel resources as well as environmental concerns over increasing atmospheric carbon and the effects of deforestation have led futurists to foresee a need for renewable energy sources, with special focus on the potential role of wood biomass. These resources are needed to increase carbon cycling rates, to supplement current lumber and fiber industry use, and also for conversion into alternative energy forms. The goal of current research is to increase early growth and reduce rotation length in order to counteract the higher expenses of intensive culture (Ranney et al. 1987).

Researchers have been concentrating on the use of selected hardwoods displaying characteristics that indicate success under conditions of intensive

culture: fast growth, wide site adaptability, good coppice growth, and pest tolerance. Other characteristics of importance include energy content, organic composition, specific gravity, moisture, and various residue products (Ranney et al. 1987). Selections of the genus *Populus* (primarily cottonwoods) show the greatest potential for short rotation agroforestry. Other potentially important selections include *Acer saccharinum* L. (silver maple), *Liquidambar styraciflua* L. (sweetgum), *Platunus occidentalis* L. (American sycamore), *Robinia pseudoacacia* L. (black locust), *Eucalyptus grandis* Hill ex Maiden, and *Salix* (willow) (Ranney et al. 1987).

Over 150 species of insects have been reported infesting poplars (Wilson 1976). Most of these are subeconomic pests. However, the nature of short rotation agroforestry creates an ecosystem in which there is a high potential for insect pests and pathogens to reach damaging levels; the planting of monocultures and use of silvicultural techniques have disrupted the natural interactions among plants and animal species (Wilson 1976).

The Cottonwood Leaf Beetle

Biology and Life History. The cottonwood leaf beetle, *Chrysomela scripta* Fabricius (Coleoptera: Chrysomelidae), is considered to be one of the most important defoliators of *Populus* in the north-central region of the United States (Burkot and Benjamin 1979, Harrell et al. 1982). It is also of considerable importance in the Pacific northwest and in the southeastern United States (E. R. Hart, ISU, personal communication). This native North American beetle feeds on the leaves of poplar trees throughout the United States and Canada (Burkot and Benjamin 1979, Drooz 1985). *Chrysomela scripta* is a multivoltine species, having three to five generations each season in the north-central region and up to seven in the southern region (Burkot and Benjamin 1979, Caldbeck et al. 1978). A field study conducted by Head and Neel (1973) from mid-April to mid-September in Mississippi found an average development period of 22 days per generation. An accompanying study of egg and larval development was performed in the

laboratory at room temperature and 30% R.H. An average egg incubation time of 4.2 days, a larval period of 10 days, a 2-day nonfeeding pre-pupal stage, a pupation period of 4-5 days, and an unspecified pre-oviposition feeding period were determined.

Adults overwinter under loose bark, debris, or grass clumps (Drooz 1985). They emerge in early spring, in synchrony with host plant leaf flush, and begin feeding on the expanding leaves and on the tender bark of twigs. Adults are oval with a length of about 6 mm. The head, thorax, and legs are black with the margins of the thorax being yellow or red. The elytra are usually bright yellow with black longitudinal markings (Drooz 1985). Although there is no record of chemical defense in adult *C. scripta*, their bright, distinctive coloration is typical of Chrysomelidae whose chemical defenses render them unpalatable (Pasteels et al. 1990).

Eggs of *C. scripta* are elongate-oval and yellow. They are usually oviposited on the underside of leaves in the upper one-third of the canopy in groups ranging from 15 to 75 eggs per mass (Bingaman and Hart 1992, Drooz 1985). Burkot and Benjamin (1979) report an average egg mass size of 64 eggs.

Upon hatching, larvae first feed on the egg chorion and then continue to feed gregariously on the epidermal leaf tissue. This causes windowpaning damage to the abaxial leaf surface. Newly hatched *C. scripta* are approximately 1 mm long, and are light tan to brown until the cuticle sclerotizes and they turn black. Each larva has two pair of thoracic and seven pair of abdominal tubercles containing eversible vesicles that secrete the defensive chemical, salicylaldehyde (Wallace and Blum 1969). However, in first instars, only the meso- and metathoracic tubercles secrete the chemical, while all nine pairs are functional in later instars. In addition, the functional tubercles of first instars produce smaller chemical droplets than later instars (Wallace and Blum 1969). Researchers have suggested that this limits the amount of salicylaldehyde available to individual first instars for defensive purposes. Thus, groups of young larvae have a greater

collective volume of salicylaldehyde to ward off predators (Wallace and Blum 1969).

Early second instars are black, but become lighter in color as they mature. The head of the third instar is black, but the body is tan. Also, there is a highly visible white area around the base of each thoracic tubercle in later instars (Wallace and Blum 1969). As larvae mature, they are increasingly mobile, and progressively less gregarious. Their feeding behavior changes from windowpaning to skeletonizing damage where all leaf tissue is consumed except the major veins and midrib. Older larvae tend to move upward on the stem toward more succulent foliage, and to feed on the upper leaf surface. Although larvae consume less tissue than adults, their feeding pattern results in the most severe damage to the host trees (Harrell et al. 1982) The third instar consumes approximately 87% of the total leaf biomass eaten per larva, making it the most damaging larval stage (Harrell et al. 1982).

Shortly before pupation, late third instars, or pre-pupae, cease feeding and move downward to locate a pupation site on the underside of leaves, branches, grass, weeds, or other litter at the base of the host tree (Drooz 1985). The pre-pupa attaches to the substrate with an adhesive caudal disc at the apex of the abdomen. The pupa hangs upside down within the third instar exuvia. It is black with two white "eye-spots" that are conspicuous in dorsal view. These spots are the white areas surrounding the base of the eversible glands of the metathoracic tubercles (Wallace and Blum 1985). During the larval-pupal ecdysis, the saclike glandular reservoir within the tubercles are shed with the integument. However, all these glands retain salicylaldehyde. Movement by a disturbed pupa forces secretion of the chemical, thus protecting the pupa from predation. The teneral adult also gains some measure of protection by clinging to the exuvia until fully sclerotized.

Response to Environmental Conditions. Temperature has an important influence on the rate of development and metamorphosis of poikilotherms (Laudien, H. 1973, Rockstein 1964, Tauber and Tauber 1987, Wigglesworth 1972).

In multivoltine species, developmental response to temperature affects the rate of increase and the number of generations per year. Understanding this response becomes especially important when dealing with insect pest species. Effective chemical, biological, and cultural control of many crop pests is dependent upon accurate timing of control measures.

Temperature dependent growth models have been developed for many major crop pests. Such models are developed from laboratory studies of development at a series of constant (and sometimes variable) temperatures within the ecological range of the species. The models can then be used to predict the occurrence of generations or specific life stages in the field.

Thus far, there is no generally accepted model for *C. scripta*. Burkot and Benjamin (1979) evaluated the effects of temperature on developmental times and survival at six temperatures. They found egg to adult developmental times of 42.5, 19.8, 18.1, 14.2, and 13.8 days at 17, 21, 23, 27, and 28°C respectively. The calculated minimal developmental threshold was 10.8 °C and highest survival occurred at 23 °C. They found an average of 257 degree-days above 10.8°C required for development from egg to adult. The difficulty in interpreting or applying the results of this study is that very little detail is given on rearing techniques or statistical design. Also, the length of individual stages was not calculated, making it difficult to predict the occurrence of specific life stages for a given generation.

The literature points to some variation in *C. scripta* life history traits. The number of generations each growing season varies in different regions. In Mississippi, *C. scripta* can have up to seven generations per year, while in central Iowa and Wisconsin there are three to four generations per year (Burkot and Benjamin 1979, Head and Neel 1973). Populations in other regions of North America may be adapted to local conditions. If this is the case, the usefulness of any model derived from the study of a single population may not have widespread application. It has been suggested that it might be useful to compare developmental response to temperature in geographically distinct populations.

Study of *C. scripta* from more than one area may add flexibility to a regional temperature dependent development model.

Impact of *C. scripta* on Hybrid Poplar. *Chrysomela scripta* adults display preferential selection among *Populus* clones, and among leaf age classes (Caldbeck et al. 1978). Both the larvae and adults of *C. scripta* feed in the immature leaf region, with larval feeding reported on leaf, apical bud, and stem tissues with a moisture content above 73% (Harrell et al. 1982). Bingaman and Hart (1992) have shown that the most-preferred leaf age class for *C. scripta* feeding was LPI 3, and that the most-preferred leaf age classes for oviposition behavior were LPI 3 and 4. The LPI system (or leaf plastochron index) is used to estimate physiological and morphological development of young leaves (Erickson & Michelini 1957, Larson & Isebrands 1971). It is based on the concept that an easily measurable leaf characteristic, such as lamina length, can be correlated with several developmental processes for each leaf on a stem. The size of the smallest leaf with maximum leaf expansion (LPI 0) is determined by plotting leaf lamina length over time. A lamina length of the LPI 0 leaf (index leaf) is 3.0 cm for *Populus* grown from cuttings (Bingaman 1991). Successive *Populus* leaves grow at the same relative rate, so leaves on a given stem are assigned an LPI designation by counting consecutively down the stem (apex to base) from the index leaf. Leaves apical to the index leaf are given consecutive negative numbers (Erickson & Michelini 1957, Larson & Isebrands 1971).

Reichenbacker (1994) examined the impact of defoliation on *Populus* hybrid growth. He found that defoliation by *C. scripta* of up to 25% of the leaf area of LPI 0 - 8 leaves may actually increase growth of some *Populus* hybrids. However, damage beyond 50% of LPI 0-8 leaves decreases growth rates and is therefore detrimental to biomass production (Reichenbacker 1994). Such defoliation can reduce height growth, radial growth, and volume (Kuhlman 1971, Wilson 1976). Feeding damage that removes tissue from the apical stem region can lead to tree deformity (Harrell et al. 1982, Head et al. 1977, Coulson & Witter 1984). Damage

also increases the risk of attack by secondary insect pests and pathogens (Kuhlman 1971, Coulson & Witter 1984, Barbosa & Wagner 1989).

Natural Enemies of *C. scripta*. The Catalogue of Predators and Parasitoids of Terrestrial Arthropods (Hertig & Simmonds 1973) reports on *Melasoma lineatopunctata* Forester, a synonym for *Chrysomela scripta* F. According to the catalogue, the predator *Perillus bioculatus* Fabricius (Heteroptera: Pentatomidae) attacks *C. scripta*. In addition, twelve species of insect predators of *C. scripta* have been reported in Mississippi (Head et al. 1977). *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) was the most abundant predator, representing 47-95% of total predators collected from first and second year trees from April to late September. Four other coccinellid species (*Neoharmonia venusta* Melsheimer, *Coccinella novemnotata* Herbst, *Olla abdominalis* Say, and *Hippodamia convergens* Guerin), *Stirentrus anchorago* Fab. and *Podisus maculiventris* Say (Hemiptera: Pentatomidae), and *Symmorphus washianus* Sauss. and *Stenodynerus* sp. (Hymenoptera: Eumenidae) were also included in the list of predators. A number of other predators were found to be present less frequently and were never observed feeding on *C. scripta*. These included *Arilus cristatus* L. (Hemiptera: Reduviidae), several *Chrysopa* species (Neuroptera: Chrysopidae), and *Stagmomantis carolina* Johannsen (Mantodea: Mantidae). In addition, Head et al. (1977) reported that the parasitoid *Schizonatus latus* Walker (Hymenoptera: Pteromalidae) and an unidentified Tachinid parasitoid (Diptera: Tachinidae) attacked *C. scripta* larvae.

Important causes of egg mortality in a study from Wisconsin (Burkot and Benjamin 1979) were predation by *C. maculata*, infertility, and "collapsed eggs" (an undefined term). Egg mortality was 42.9, 34.4, 57.0, and 76.2% in the first, second, third, and fourth generations respectively. The same study noted several predators of larvae, including the pentatomids *P. maculiventris* Say and *Perillus biocularus* Fab., and chrysopid and syrphid larvae. Mortality of first and second instar *C. scripta* was attributed to starvation (first generation) and unknown mortality factors.

The study showed mortality rates for first instar larvae to be 42.9, 22.3, 33.8, and 82.2% in the first through fourth generations. Second instar larvae suffered 37.5, 58.6, 52.7, and 69.7% in the first through fourth generations. Mortality of first generation third instar *C. scripta* was caused by starvation and unknown mortality factors (15.7%), while pupae were parasitized (8.2%), died while emerging (11.5%), or died of other, unspecified factors (18.0%). Second and third generation third instars were preyed on by Pentatomidae (1.4, and 1.7% respectively) or died of unknown factors (33.3, and 60.0%). Unknown mortality factors killed 54.2% of fourth generation third instars. Second, third, and fourth generation pupal mortality was caused by parasitism (3.2, 25.8, and 9.4%), predation (2.1, 6.1, and 25.0%), and failure to eclose (11.7, 6.1, and 17.7%). Predators of adult *C. scripta* included Pentatomidae and spiders.

Preliminary sampling and observations (E. R. Hart, Iowa State University, unpublished data) in a 2-year-old plantation in 1994 indicate that the natural enemy complex of *C. scripta* in central Iowa includes at least three coccinellid species (*C. maculata*, *H. convergens*, and *Coccinella septempunctata*), *Chrysoperla plorabunda* Fitch (Neuroptera: Chrysopidae) (identified by O. S. Flint, Smithsonian Institute), *P. maculiventris* Say (Hemiptera-Heteroptera: Pentatomidae) (identified by T. J. Henry, Systematic Entomology Laboratory, USDA), and the parasitoid *S. latus* (Hymenoptera: Pteromalidae) (identified by E. E. Grissell, Systematic Entomology Laboratory, USDA). Preserved specimens were identified at the Systematic Entomology Laboratory, USDA, Beltsville, Maryland. The preliminary work done in the 1994 field season indicates that many natural enemies interact with the *C. scripta* population in central Iowa

The impact of natural enemies on their prey is generally evaluated in the field by comparing host populations with and without natural enemies through use of introduction and augmentation, exclusion or inclusion, removal of natural enemies by insecticide or by hand, prey enrichment, direct observation, and evidence of natural enemy feeding (Luck et al. 1988). A second approach to evaluating the impact of natural enemies is the construction of life tables (Bellows

et al. 1992). Field studies may be augmented through laboratory and greenhouse studies designed to determine the effectiveness of a predator or parasitoid by measuring predation rates, or by feeding studies that test the suitability of prey.

Landscape Differences in Short Rotation Agroforestry and Natural Systems

Population dynamics are dependent on both abiotic agents, such as wind, rain, and temperature, and biotic agents such as predators, parasitoids, and pathogens. The size, distribution, and longevity of habitat patches (local areas of habitat) and the size of the local populations that they contain also help to determine the population dynamics of a species (Arms and Camp 1987). Naturally occurring habitat patches which are small or short-lived may be colonized by populations that grow rapidly and then crash when resources are exhausted. In other cases, pest populations may be held in check by biotic and abiotic natural control factors. In the natural landscape, stands of cottonwood trees are small and widely scattered along stream and river banks (Bradley and Smith 1986, Noble 1979, Wilson 1970), but may remain stable over decades.

In contrast, short rotation agroforestry plantations of *Populus* tend to be large, with regular spacing of equal-age, monoclonal trees. Such plantations tend to have a rotation age of 5 to 10 years. This resembles traditional monocultural agriculture systems, and pest problems may differ from those in natural areas. It is a generally accepted principle that pest problems increase when plants are grown in agricultural systems (Barbosa and Schultz 1987). This is caused by massive changes in landscape diversity. These changes include the enlargement of fields, aggregation of fields, increase in crop plant density, increase in the uniformity of crop population age structure and physical quality, and a decrease in inter- and intraspecific diversity within the planted field.

Some forestry work has examined the relationship of monocultures and pest outbreaks. In a recent review, Cromartie (1996) states that there are numerous examples where monoculture has been blamed for forest insect outbreaks.

Although factors such as poor site and poor sanitation may have contributed to the problem in some of these cases, the most important factor seemed to be the reduction in average distance between individuals of the same species and the same age in plantations, compared to natural forests. For example, outbreaks of spruce budworm in Canada are more likely to occur in stands with a high percentage of balsam fir (Mott 1963). Reduced diversity caused by the death of birch and poplar trees in balsam stands may have been another triggering factor of these outbreaks.

> "Agricultural crops are usually grown in monocultures ... or in relatively simple mixtures that are much less diverse in number of plant species present than the native vegetation they replace ... thus, modern crops are exposed to conditions very unlike the relatively rich associations of plants in which their wild ancestors probably evolved. Along with the plants ... the whole fauna (plant- associated insects, mites, spiders, nematodes, and so on) [are subjected] to the novel environmental conditions it creates ... agriculture reduces the multispecies, compound community of the forest or meadow to a single component community containing the crop plant and those herbivores, predators, and parasites adapted to living upon it, under the physical conditions created by cultivation."
>
> (Cromartie 1996 p. 183)

Although the lack of diversity in modern farming techniques is seen as the factor to blame for many insect pest problems, there are few studies that look specifically at monocultures and pest outbreaks. However, there is a great deal of evidence dealing with the influence of crop diversification on insect pest occurrence. Crop diversification can take many forms: row intercropping, strip intercropping, random mixture, undersown cover crops, or weed tolerance.

It is interesting to note that agricultural workers and ecologists tend to have different points of view when it comes to the role of fragmentation. Most landscape ecologists work in systems where fragmentation is of great concern; loss of habitat is leading to loss of species diversity, physical barriers and non-usable habitat are

inhibiting movement of organisms, etc. Agricultural workers on the other hand, work with flora and fauna which have been removed from the complex system in which they evolved; loss of fragmentation is causing the problems. The question of interest is whether by increasing the diversity of the agricultural system (by fragmenting it at a fine-grained scale) one can improve pest control by increasing natural enemy diversity and density, and/or by reducing pest populations.

Management of *Chrysomela scripta*

Many *Populus* hybrid plantation managers currently use chemical control methods to reduce *C. scripta* populations to prevent economic damage. However, there are serious consequences to this management method. The cost of insecticide application may be prohibitive. There is some indication that, under some circumstances, the cost of application may outweigh the economic loss, even when *C. scripta* populations are very high (Fang 1997). Also, the environmental cost of chemical insecticides is high. Many of these hybrid *Populus* plantations may be located on marginal agricultural land in riparian areas. The use of additional pesticides in these areas is unacceptable. Furthermore, application of chemical insecticides will affect all the fauna of the area, not just the pest population. This frequently disrupts any population regulation pressures from the natural enemy complex.

Bacillus thuringiensis is a bacterium that produces an insecticidal protein crystal. The advantage of *B. thuringiensis* formulated as a biological insecticide is that it tends to be very selective and therefore causes limited direct mortality of insect parasitoids and predators (Bauer 1990). *Bacillus thuringiensis* var. san diego, M-one (Mycogen Corporation, San Diego) has been shown to be effective against *C. scripta* (Bauer 1990, Ramachandran et al. 1993). If M-one, or the newer Mycogen product M-trac, is applied to suppress *C. scripta* populations, there should be minimal impact on the natural enemy complex. However, this product is

only effective against early instar *C. scripta*, so the timing of application is critical. Also, there is a question of the continued availability of this product.

Augmentative releases of adult *C. maculata* (collected from overwintering sites in Mississippi) and *H. convergens* (obtained commercially from California) have been made in *Populus* plantations in Mississippi (Neel and Solomon 1985). *Hippodamia convergens* did not remain in the release area for more than 7 d, while *C. maculata* remained in the plantation for up to 14 d. Van Driesche et al. (1996) gives a brief overview of the pest status of *C. scripta* and possibilities for biological control.

Research continues to produce *Populus* hybrids that are more resistant to insect pests and pathogens. A wide range of natural resistance to the cottonwood leaf beetle has been reported for selected *Populus* (Caldbeck et al. 1978, Harrell et al. 1982). Recently, several secondary leaf chemicals have been isolated that act as feeding stimulants (Lin 1997). This finding could potentially be used in breeding and selection programs to reduce the susceptibility of selections, or to produce highly susceptible *Populus* hybrids that could be used as trap crops, or even to formulate a bait for use in traps. Finally, more information about the natural enemy complex of *C. scripta* is needed in order to evaluate the potential for biological control. It may be possible to use several of these methods in an integrated pest management program.

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CHAPTER TWO: SURVEY OF THE NATURAL ENEMY COMPLEX OF *CHRYSOMELA SCRIPTA* (COLEOPTERA: CHRYSOMELIDAE) IN CENTRAL IOWA

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Abstract

A survey of the natural enemies of the cottonwood leaf beetle, *Chrysomela scripta* Fabricius (Coleoptera: Chrysomelidae) was conducted in central Iowa in 1995 and 1996. An absolute sampling method, visual observations, and yellow sticky traps were used in selected *Populus* plantation and natural sites. Natural enemies found in absolute and visual samples from plantation sites were *Coleomegilla maculata*, *Hippodamia convergens*, *H. tredecimpunctata*, and *Neoharmonia venusta* (Coleoptera: Coccinellidae), *Podisus maculiventris* (Heteroptera: Pentatomidae), Chrysopidae (Neuroptera), Cantharidae, Formicidae, Lampyridae, Nabidae, Araneae and Opiliones. Predators in absolute and visual samples from natural sites were *C. maculata*, *P. maculiventris*, Cantharidae, Formicidae, Lampyridae, Reduviidae, Araneae, and Opiliones. Additional coccinellid species captured by yellow sticky traps were *Coccinella septumpunctata*, *Adalia bipunctata*, *H. parenthesis*, *Cycloneda munda*, *Harmonia axyridis*, and three undetermined species. Sticky traps also captured Neuroptera and Carabidae.

Key words: *Chrysomela scripta*, *Populus* hybrids, natural enemies

Introduction

Luck et al. (1988) suggested a sequence of experiments to demonstrate changes in pest densities caused by natural enemies. These experiments require a carefully designed sampling scheme for determining predator-prey and parasitoid-host identity and densities during the season. Direct observation is useful for initial identification of predator-prey associations and locations.

Population dynamics of both pests and natural enemies are dependent on abiotic and biotic factors. The size, distribution, and longevity of local areas of habitat (habitat patches) and the size of populations within those patches also helps to determine the population dynamics of a species (Arms and Camp 1987). Small, short-lived habitat patches tend to be colonized by populations that grow rapidly and then crash when local resources are exhausted. Natural stands of cottonwood trees (*Populus deltoides* Marsh) that are seeded in as small and widely scattered patches along stream and river banks are good examples (Bradley and Smith 1986, Noble 1979, Wilson 1970). In contrast, short rotation (5 - 10 years) agroforestry plantations of *Populus* hybrids tend to be large, evenly spaced plantings of equal-age, monoclonal trees. These short rotation plantations resemble orchard systems. Since pest problems increase when plants are grown in agricultural systems (Barbosa & Schultz 1987) it is possible that both pest and natural enemy populations in plantations may differ from those in naturally occurring stands.

The cottonwood leaf beetle, *Chrysomela scripta* Fabricius (Coleoptera: Chrysomelidae), is one of the most important defoliators of *Populus* in the north-central region of the United States (Burkot and Benjamin 1979, Harrell et al. 1982). It is native to North America and feeds on the leaves of poplar trees throughout the United States and Canada (Burkot and Benjamin 1979, Drooz 1985). Larvae and adults of *C. scripta* feed on immature leaf material and often damage the meristem tissue. Feeding damage can lead to tree deformity and increases the risk of attack

by secondary insect pests and pathogens (Head et al. 1977, Harrell et al. 1982, Kuhlman 1971).

The Catalogue of Predators and Parasitoids of Terrestrial Arthropods (Hertig & Simmonds 1973) reports on *Melasoma lineatopunctata* Forester, a synonym for *Chrysomela scripta* F. According to the catalogue, the predator *Perillus bioculatus* Fabricius (Heteroptera: Pentatomidae) attacks *C. scripta.* Twelve species of insect predators of *C. scripta* have been reported in Mississippi (Head et al. 1977). *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) was the most abundant predator, representing 47-95% of all predators collected from April to September. Four other coccinellid species (*Neoharmonia venusta* Melsheimer, *Coccinella novemnotata* Herbst, *Olla abdominalis* Say, and *Hippodamia convergens* Guerin), two pentatomid species: *Stirentrus anchorago* Fab and *Podisus maculiventris* Say (Heteroptera: Pentatomidae), and two eumenid species: *Symmorphus washianus* Sauss. and *Stenodynerus* sp. (Hymenoptera: Eumenidae) were also predators of *C. scripta.* In addition, Head et al. (1977) reported the parasitoid *Schizonatus latus* Walker (Hymenoptera: Pteromalidae) and an unidentified tachinid parasitoid (Diptera: Tachinidae) attacking *C. scripta* larvae.

In Wisconsin, predation by *C. maculata* was determined to be an important cause of egg mortality (Burkot and Benjamin 1979). The same study noted several predators of larvae, including the pentatomid species *P. maculiventris* Say and *Perillus biocularus* Fab., and chrysopid and syrphid larvae. Predators of adult *C. scripta* included pentatomids and spiders.

Preliminary sampling (E. R. Hart, unpublished data) in a plantation of *Populus* hybrids in central Iowa in 1994 found three coccinellid species (*C. maculata*, *H. convergens*, and *Coccinella septempunctata*), *Chrysoperla plorabunda* Fitch (Neuroptera: Chrysopidae), *P. maculiventris* Say (Heteroptera: Pentatomidae), *S. latus* (Hymenoptera: Pteromalidae), and an unidentified Braconidae parasitoid (Hymenoptera). Early season infestations of *C. scripta* have been reported as heaviest in the southwestern part of the plantation. This

observation led us to hypothesize that *C. scripta* densities (as well as densities of natural enemies) might differ depending on location in the plantation; *C. scripta* densities might be higher in the southwestern part of the plantation.

The objectives of this study were to (1) identify what natural enemies of *C. scripta* are present, and when they occur, (2) determine whether different natural enemies occur in natural versus plantation sites, and (3) determine if densities of *C. scripta* and natural enemies differed by location within the plantation.

Materials and Methods

Field site selection

Plantation sites A 16.2 ha plantation of the *Populus* hybrid 'Eugenei' (*P. deltoides* x *P. nigra*) was established in 1990 at the Ames Municipal Water Pollution Control Facility (AMWPCF) located 16 km southeast of Ames, Story Co., Iowa (Fig 1). There are approximately 2785 trees per ha; spacing is 1.25 m between trees in a row and an average of 3 m between rows arranged in strips. Each strip consisted of six rows of trees with a 15.2 m planting of corn, soybeans, or switch grass separating it from the next tree-strip. After establishment (yrs 1-4) weeds were allowed to grow among tree-strips. The trees were planted in six plantation replicates. Plantation replicates 1 & 2, 3 & 4, and 5 & 6 were planted in 1990, 1991, and 1992 respectively (Fig. 1). The areas to the north and east of the plantation are agricultural (planted in corn or soybeans). Directly west of the plantation is a gravel road that runs along a wooded streambed. This wooded streambed curves around to the southwest of the plantation. Also southwest of the plantation is an area where field trials of a variety of *Populus* clones were planted.

Study sites were located within plantation replicate 5. The average tree height in plantation replicate 5 was 2.53 m in 1995 and 2.64 m in 1996 (C. W. Mize, ISU, personal communication). Plantation study sites were chosen so as not to interfere with previously established long-term research plots. Each plantation study site consisted of 30 trees, with 5 trees in each of the 6 rows. There were nine

such plantation study sites; these were arranged in three blocks with three experimental replicates in each block.

Natural Sites. Natural sites were selected to include a small grouping of young cottonwood trees (10 - 30 trees) separated from other cottonwoods by at least 100 m. Four stands of *P. deltoides* were selected within 1 km of the plantation at AMWPCF (Fig. 1). **Site A** consisted of 11 trees located in the far northwest corner of the plantation along a wooded drainage ditch. When measured in the spring of 1997, the trees ranged from 3.4 to 9.0 m tall and from 2.5 to 20.1 cm dbh. The mean height was 8.61 ± 5.1 m (X ± SD), and mean dbh was 7.28 ± 1.99 cm. These trees are mixed with a variety of other trees, as well as shrubs and weeds. **Site B** was a stand of mixed age located along a drainage ditch southwest of the plantation. There seemed to be trees from two distinct seedings. One group (n = 10) had a mean height of 5.96 ± 1.2 m and dbh of 6.15 ± 2.25 cm. The other had a mean height of 2.04 ± 0.73 m and dbh of 1.25 ± 0.47 cm (n = 13). **Site C** was located on top of a hill southwest of the plantation. There were 11 trees with a mean height of 4.54 ± 1.76 m (range of 3.0 - 7.8 m) and mean dbh of 5.63 ± 3.3 cm (range of 2.5 - 12.7 cm). **Site D** was also located southwest of the plantation. It consisted of 20 young trees. The mean height was 3.89 ± 1.12 m and mean dbh was 2.92 ± 1.41 cm. Sites B, C, and D were all separated from the plantation by a wooded area that runs along a drainage ditch west and then south of the plantation.

Sites E and **F** were southwest of Boone, Boone Co., Iowa. These two sites were only used for one sampling date in 1995 and then eliminated because of time constraints. Three additional natural sites were located in the Des Moines river valley about 16 km northwest of Boone, Iowa on the south side of E47. **Site G** consisted of 10 trees in the roadside ditch west of the Des Moines river. The trees at this site were removed before they were measured. However, the site was similar to site C. **Site H** was on the west bank of the Des Moines river. The mean height was 4.9 ± 1.49 m and dbh was 4.85 ± 2.43 cm (n = 10). **Site I** was in the roadside ditch east of the river, and was also removed before trees were

measured. The site was made up of about 15 young trees comparable in size to site D.

Absolute Sampling

1995. Sampling of arthropods present in the tree canopy was accomplished by physical knockdown and collection. There were 30 trees in each of nine sample sites within the plantation. In each site, trees were numbered 1 through 30, and three trees were selected at random (using a random numbers table). Thus, a total of 27 trees were sampled in the plantation on each sampling date. Selected trees were flagged for use throughout the season. If a tree declined (e.g. leaves turned yellow or lost leaves because of disease or girdling) during the season, it was replaced with the nearest unflagged tree. Natural-site sampling used sites A, B, C, D, G, H, and I (Table 1). At each of these sites, three trees were selected at random and flagged for sampling.

On each sampling date, a single lateral branch with an actively growing terminal was chosen. Selected branches had to be easily reached from the ground for sampling. This requirement meant that branches on all trees were used on multiple sampling dates. However, re-sampling of branches on consecutive weeks was avoided because, while most of the natural enemies found in samples are mobile, *C. scripta* eggs and early larvae are not. These less mobile stages are relatively brief, so it was assumed that samples would be representative of population densities as long as samples were not taken from the same branch on consecutive weeks. To avoid this, all trees were approached from the same direction on a given sampling date, and the following week, a different direction of approach was used.

To sample a branch, a white plastic bag (0.46 x 0.20 x 1.1 m) was placed over the selected branch. The bag was held closed around the branch and the end was held away from the terminal (to avoid damage to the terminal) and the branch was shaken vigorously. The bag was removed from the branch, closed, labeled, placed in a cooler, and transported back to the laboratory. Leaves on the selected

branch were inspected for the presence of eggs or other stationary life stages of *C. scripta* or other organisms. These were placed in small plastic bags for later identification. Sampling was done once each week from 19 May to 11 August. Collected arthropods were frozen and stored for later identification.

The second generation *C. scripta* population increased notably over the first generation. Therefore, M-trac, *Bacillus thuringiensis* var. san diego, (Mycogen Corporation, San Diego) was applied to the plantation trees on 4 July 1995. While this microbial insecticide should not have affected natural enemy populations directly, the population of *C. scripta* was reduced. No sampling was done after Lorsban 4E (0.47 L per 0.4 hectares) was applied at the plantation on 13 August to control the third generation *C. scripta* population and grasshoppers.

1996. Sampling was performed as in 1995. Because trees in block 3 of the plantation were in poor condition, a randomly selected tree may have been dead, or may have had no suitable lateral branches. In such cases, the nearest usable tree was used for sampling. Also, sites A, G, H, and I were eliminated because very few *C. scripta* were found at these sites in 1995. Sampling was done once each week from 17 June to 5 August 1996. Sampling earlier in the season was not done because few *C. scripta* were present in plantation replicate 5. Adult *C. scripta* were present in high numbers in plantation replicates 1 & 2 where there was heavy feeding on adventitious shoots. This type of growth is unusual and the trees in that area were much larger than those in plantation replicate 5. Therefore, no sampling was performed until adult *C. scripta* moved into plantation replicate 5. Sampling was terminated after Lorsban 4E (0.47 L per 0.4 hectares) was applied at the plantation for control of third generation *C. scripta* and grasshoppers on 6 August 1996.

Visual Observations

Visual observations were added to the natural enemy survey in 1996. This sampling was done weekly, in conjunction with absolute sampling. A tree adjacent to each bag-sampled tree (the next tree in the row, or one row over) was chosen.

These trees were observed for 1 min by two individuals. The number, and kind (to species level if possible) of natural enemies were noted. Because of the high numbers of *C. scripta*, the presence of the different life stages was noted, but not counted.

Sticky Traps

Scentry® Multigard yellow sticky traps were placed in the field in both 1995 and 1996. These traps remain effective in row crops for about 2 weeks (Robin Prisoner, ISU, personal communication). Traps were placed around tree trunks or lateral branches, at a height of approximately one meter above the ground, and were secured using twist-ties.

1995. One trap was placed in each location (Fig. 1) and traps were left in the field for approximately 2 weeks (Table 2). For plantation replicates 5 and 6, three traps were placed in the middle pair of rows of block 1, with one trap placed at least 50 m from the north and south ends of the rows and one trap at the junction of plantation replicates 5 and 6. One trap each was placed at the junctions of plantation replicates 1 and 2, and of plantation replicates 3 and 4. One trap was placed on a tree near the center of each natural site. Collected traps were wrapped in plastic wrap, returned to the laboratory, and stored in a freezer. Adult *C. scripta* and coccinellids were later removed from the traps using mineral spirits and then placed in alcohol.

1996. One trap was placed in each location from 11 May until 30 September (Fig. 1). For plantation replicates 5 and 6 (or blocks 1, 2, and 3), six traps were placed in each block with one pair of traps (one on each of the outside rows, approximately 16.5 m apart) placed at least 50 m from the north and south ends of the rows and one pair of traps at the junction of plantation replicates 5 and 6. A pair of traps was placed parallel to each of these locations in the wooded strip to the west of the plantation (traps in each pair were approximately 16 m apart). Three traps were placed in each of the six tree-strips in blocks 4 and 5. In each of those tree strips, one trap was placed at least 50 m from the north and south ends of the plantations

and one was placed at the junction of plantation replicates. Two traps were placed in each of sites B, C, and D, as well as two each in the wooded area and the field trial area southwest of the plantation. Traps in each of these locations were at least 16 m apart. A total of 52 traps were used. One week after placement, all adult *C. scripta* and Coccinellidae were removed from the traps and placed in vials of BioT® Biochem Systems, Golden, CO). Approximately 2 wk after placement, traps were collected (as in 1995) and replaced. Adult *C. scripta* and Coccinellidae were later removed from the traps using BioT® and then placed in alcohol. Chrysopidae were counted, but could not be removed from the traps. The same traps were in the field from 31 July until 24 August because of supply problems. However, Lorsban 4E was applied to the plantation on 6 August. Traps remained effectively sticky for longer than 2 wk in the plantation, i.e. insects continued to be trapped.

Data Analysis

Plantation sites were arranged in a complete block design (three blocks and three replicates per block) in order to test for differences among blocks and replicates on each sampling date; data from absolute sampling and visual observations in plantation sites were analyzed using analysis of variance (SAS Institute, 1987). Data from absolute sampling and visual observations in natural sites were tested for differences between sites on each sampling date using general linear models (SAS Institute, 1987). Data were separated into categories for analysis: *C. scripta* adults, early larvae (instar I and early instar II), late larvae (late instar II and all instar III), egg masses, lady beetles, and total predators.

Data from sticky traps were separated into the following categories for analysis: *C. scripta* adults, *C. maculata* adults, *H. convergens* adults, other lady beetles, and other predators. Traps in blocks 1, 2, 3, 4, 5 (in the plantation), and along a road (natural vegetation between a road and a streambed) were set up together to test for movement of *C. scripta* into and out of the plantation from the plantation and roadside (Fig. 1). Pairs of traps located along the road were set up

parallel to pairs of traps in the plantation. Traps in these locations were analyzed together using analysis of variance. Traps from the natural sites (B, C, and D), wooded area, and field trials were analyzed using general linear models to test for differences between sites (SAS Institute 1987).

Analyses with probabilities > 0.05 are not reported. Voucher specimens have been deposited in the Iowa State University Insect Collection, Department of Entomology, Ames, Iowa.

Results

The sampling techniques used in this study captured low numbers of organisms. This was especially true of absolute sampling, where most of the samples contained zero organisms. This made analysis and interpretation of results difficult; all variance was between zero and one. Thus, although there were some statistically significant differences in the number of organisms in various locations, these differences have no biological meaning.

Absolute Sampling

1995 Plantation Sites. Predators collected in plantation sites included *Coleomegilla maculata*, *Hippodamia convergens*, and *H. tredecimpunctata* (Coleoptera: Coccinellidae), as well as *Podisus maculiventris* (Heteroptera: Pentatomidae), Cantharidae, Formicidae, Lampyridae, Neuroptera, and Araneae (Table 3, Fig. 2 and 3). Lady beetle adults were collected on 19 and 24 May, 22 June, and 7 July. Other predators were collected on all sampling dates except 7 July. On 8 June, there was a significant difference in total predators between blocks and replications, as well as a significant interaction between blocks and replications ($F = 16.0$; d. f. 8, 26; $p = 0.0001$); the mean number of predators found in block 1, replicate 2 was 1.33 ± 0.58, and 0 in all other sites.

Chrysomela scripta egg masses were collected on 19 & 24 May, 22 June, 3, 7, 11, & 25 July. The occurrence of egg masses indicates that first generation eggs

were laid in mid to late May, and second generation eggs were laid in late June (Fig. 2). Early larvae were collected on 3, 8, 13, & 22 June, and 3, 7, 11, & 25 July (Fig. 3). The number of early larvae peaked on 3 June (4.46 ± 1.8 larvae per sample), 3 July (4.93 ± 1.54 larvae per sample), and 25 July (1.3 ± 0.89 larvae per sample). This seems to represent the peak of first, second, and third generation early larvae (Fig. 3). Late larvae were collected on 3, 8, 13, and 22 June, and 3, 7, 11, and 25 July with the number of first generation late larvae peaking (3.22 ± 0.82 larvae per sample) on 8 June 1997 (Fig. 3). Adult *C. scripta* were collected in absolute samples on all sample dates, except 11 August.

1995 Natural Sites. Predators collected in natural sites included the coccinellid *C. maculata*, the pentatomid *P. maculiventris*, Cantharidae, Formicidae, Lampyridae, Reduviidae, and Araneae (Table 4, Fig. 4 and 5). Coccinellidae were collected on 19 May, 8, 13, and 22 June, and 7 July; coccinellids and *C. scripta* egg masses were found together only on 19 May and 8 June. Other predators were collected on all sampling dates.

Egg masses of *C. scripta* were collected in absolute samples from natural sites on 19 May, 8 June, and 11 July (Fig. 4). Early and late larvae were collected on 3, 8, 13, and 22 June, 3, 7, and 11 July (Fig. 5). A significant difference in *C. scripta* larvae between sites ($F = 3.11$; d. f. 6, 20; $p = 0.0376$) was seen on 11 July. Sites A, G & I had no larvae, sites C & D each had 3.33 larvae, and sites B & H each had 0.33 larvae. There were very few adult *C. scripta* found in samples from natural sites. Adults were collected from site D on 19 May, 8 and 22 June, 25 July, and 1 August. Adult *C. scripta* were also collected from site C on 3 and 25 July.

1996 Plantation Sites. Predators collected from plantation sites included the coccinellids *C. maculata*, and *N. venusta*, the pentatomid *P. maculiventris*, Chrysopidae, Formicidae, and Araneae (Table 5, Fig. 6 and 7). Adult Coccinellidae were collected on 19 July (0.04 ± 0.04 per sample), and on 5 August ((0.07 ± 0.07 per sample); on 5 August, both lady beetles and *C. scripta* egg

masses (0.41 ± 0.14 per sample) were present. Coccinellids were only collected from block 1, replicate 2. Other predators (0.07 ± 0.05 predators per sample), were collected on 17, 26 June, and 10 July, and (0.11 ± 0.06 predators per sample) on 30 July; there was little difference in the mean number of other predators (Fig. 7).

Egg masses of *C. scripta* were collected on 10 and 30 July, and 5 August. There was a significant difference between blocks (F = 10.47; d. f. = 2, 26; p = 0.0010) on 30 July (Table 5); there were more egg masses in samples from block 1. Early larvae were collected on all sampling dates after 2 July, and late larvae were collected on all dates except 2 July. The mean number of early and late larvae per sample increased after 2 July (Table 5). On 5 August, there was a significant difference in *C. scripta* larvae between blocks (F = 3.80; d. f. = 2, 26; p = 0.0419); more larvae were in samples from block 1. The mean number of *C. scripta* early larvae for each sample tree over the whole season showed a significant difference between blocks (F = 6.31; d. f. = 2, 26; p = 0.0024). This analysis also shows a significant difference in late larvae between replicates (F = 4.71; d. f. = 2, 26; p = 0.0106). Adults were collected on all dates except 17 June, with the highest number occurring on 30 July (2.48 ± 0.58 adults per sample) and 5 August (2.0 ± 0.42 adults per sample).

1996 Natural Sites. Predators collected in natural sites included various Formicidae and Araneae (Table 6, Fig. 8). No lady beetles were found in any samples. No *C. scripta* egg masses were collected in the natural site samples. Early larvae were collected on 10 July, while late larvae were collected on 10 and 19 July. Adult *C. scripta* were only collected on 19 July (0.11 ± 0.11 adults per sample).

Visual Observations

Plantation Sites. Predators observed in plantation sites included *C. maculata* adults and larvae, and *H. convergens* adults (Coleoptera: Coccinellidae), *P. maculiventris* (Heteroptera: Pentatomidae) adults and nymphs, Chrysopidae larvae, and Cantharidae, Formicidae, Nabidae, Araneae, and Opiliones adults

(Table 7). On 17 June, there was a significant interaction for other predators between blocks and replicates ($F = 3.00$; d. f. = 4, 26; $p = 0.0464$). Predators were observed in block 1 replicate 2, and in block 3 replicate 1. On 26 June we observed a total of 12 damaged *C. scripta* egg masses, and one *C. maculata* adult feeding on an egg mass. On 8 July, a total of 5 egg masses were observed to be damaged. Chrysopidae larvae were observed feeding on egg masses on 8 and 30 July.

Egg masses of *C. scripta* were seen on all dates except 19 July. Early and late larvae were observed on all sampling dates. Pre-pupae were observed only on 2 and 8 July, while pupae were seen on all dates except 26 June. Adult *C. scripta* were observed on all dates.

Natural Sites. Predators observed in natural sites included adult *P. maculiventris* (Heteroptera: Pentatomidae), Cantharidae, Formicidae, Araneae, and Opiliones (Table 8). Predators were not observed attacking *C. scripta*.

Chrysomela scripta egg masses were observed in natural sites on 26 June, and 2, 8, and 30 July. Early larvae were seen on 26 June, and 2, and 8 July, while late larvae were seen on 2, 8, and 30 July. Pupae of *C. scripta* were only seen on 17 June, and adults were never observed in natural sites.

Sticky Traps

1995. Adult *C. scripta* were captured on yellow sticky traps throughout the 1995 season (Table 9). Peak numbers were on 18 and 30 May in the plantation, and 18 May in the natural sites. Several coccinellid species were also captured: *C. maculata*, *H. convergens*, *H. tredecimpunctata*, *C. septumpunctata*, and *Adalia bipunctata*. *Coleomegilla maculata* and *H. convergens* were the most abundant coccinellids in the plantation and the natural sites and were found on most sampling dates. More *C. maculata* and *H. convergens* were captured in the plantation than were in the natural sites. *Hippodamia tredecimpunctata* and *C. septumpunctata* were in both the plantation and the natural sites, but were

captured on only a few dates. *Adalia bipunctata* was only found on 11 June in the natural sites.

1996. Coccinellids captured included *C. maculata*, *H. convergens*, *H. tredecimpunctata*, *N. venusta*, *C. septumpunctata*, *H. parenthesis*, *N. venusta*, *C. septempunctata*, *Cycloneda munda*, *Harmonia axyridis*, and three undetermined, predatory coccinellid species. In addition, traps occasionally captured Carabidae and Neuroptera.

Traps in blocks 1-5 and along the road. *Chrysomela scripta* adults were captured on all sampling dates except 20 September and 29 September 1996 (Fig. 9). There seemed to be a peak trap catch of overwintering adults on the first few sampling weeks (11 May to 6 June) that indicates that adults had emerged from overwintering sites before traps were placed in the field. There is also a peak in the number of adults per trap at the end of July (second generation adults) and a third peak in adults at the end of August (third generation). The population should have dropped at the beginning of August because of the application of Lorsban 4E. However, the traps were not changed between 31 July and 23 August, so there is no measure of how much the population dropped.

Adult *C. scripta* were distributed throughout the plantation on the first few sampling weeks. Numbers then dropped off in blocks 1, 2, and 3 until 16 July. Only two *C. scripta* adults were ever found along the road. Significant differences between means of traps in blocks were shown on 18 May ($F = 4.28$; d.f. = 5, 41; $p = 0.0063$) and 17 June ($F = 8.12$; d.f. = 5, 41; $p = 0.0001$). In both cases, means were higher in block 5. Another significant difference between blocks was shown on 16 July ($F = 4.12$; d.f. 5, 41; $p = 0.0077$). On 31 July there was a significant difference between blocks and replicates, as well as an interaction between block and replicate ($F = 5.71$; d.f. 17, 41; $p = 0.0001$). A significant difference between blocks was also shown on 23 August ($F = 4.53$; d.f. 4, 41; $p = 0.0048$).

Coleomegilla maculata adults were collected throughout the season, but numbers dropped off dramatically after 24 June (Fig. 10). Figure 10 shows two distinct peaks that may represent overwintering adults from 11 May to 24 May, and

first generation adults from 6 June to 24 June. Adult *C. maculata* were distributed throughout the plantation for most of the season. On 18 May there was a significant difference between replicates and an interaction between block and replicate ($F = 3.13$; d.f. = 17, 41; $p = 0.0053$). A significant difference between blocks was shown on 24 May ($F = 8.74$; d.f. = 5, 41; $p = 0.0001$) where the highest means were shown in block 1 (9.5 per trap) and block 5 (5.11 per trap). Block 5 continued to show significantly higher numbers of *C. maculata* on 17 June ($F = 8.12$; d.f. = 5, 41; $p = 0.0001$), 24 June ($F = 5.66$; d.f. = 5, 41; $p = 0.0014$), and 9 July ($F = 5.26$; d.f. = 5, 41; $p = 0.0021$).

Hippodamia convergens adults were present on traps from all dates except 11 May, 16 July, 31 July, and 23 August. On 9 September, a significant interaction between block and replicate was shown ($F = 2.70$; d.f. = 10, 41; $p = 0.0223$).

By looking at all the Coccinellidae as total lady beetles, a series of differences in distribution is seen. On 18 May there were significant differences on all levels ($F = 10.67$; d.f. = 17, 41; $p = 0.0001$). There was a statistical difference in distribution of adult Coccinellidae among blocks on 24 May ($F = 5.47$; d.f. = 5, 41; $p = 0.0017$) with the highest number of Coccinellidae present in block 1. An interaction between block and replicate was seen on 31 May ($F = 3.76$; d.f. = 10,41; $p = 0.0038$). Statistical differences between blocks for total lady beetles were seen on 6 June ($F = 3.14$; d.f. = 5, 41; $p = 0.0254$), 17 June ($F = 6.30$; d.f. = 5, 41; $p = 0.0007$) where the number of lady beetles was highest in blocks 1 and 3, and on 24 June ($F = 4.78$; d.f. 5, 41; $p = 0.0036$) and 9 July ($F = 3.31$; d.f. = 5, 41; $p = 0.0206$) when the highest mean number of lady beetles was in block 5. Significant differences in the number of lady beetles were seen at all levels on 24 July ($F = 5.36$; d.f. = 17, 41; $p = 0.0001$) where the mean was higher in traps along the road. Finally, significant differences were shown between blocks on 23 August ($F = 2.77$; d.f. = 5, 41; $p = 0.0409$), 30 August ($F = 7.58$; d.f. = 5, 41; $p = 0.0002$) where the mean was highest in block 3, and 9 September ($F = 2.77$; d.f. = 5, 41; $p = 0.0411$).

Other predators (i.e., Neuroptera and Carabidae) were found on all dates except 31 May, 6 June, and 20 September. On 24 July there was a significant difference between both blocks ($F = 3.35$; d.f. = 5, 41; $p = 0.0196$) and replicates ($F = 4.14$; d.f. = 2, 41; $p = 0.0286$). There was also a significant difference between replicates on 29 September ($F = 4.00$; d.f. = 2, 41; $p = 0.0317$), as well as an interaction between block and replicate ($F = 2.93$; d.f. 10, 41; $p = 0.0149$).

Traps outside the plantation. *Chrysomela scripta* adults were not found on traps in the natural sites after 9 September. In addition, they were not found on 31 May, or 17 June. On 11 May, adults were found only in site D and in the field trials; there was a significant difference between sites ($F = 19.0$; d.f. = 9; $p = 0.0032$). There was also a significant difference among sites on 18 May ($F = 7.52$; d.f. = 9; $p = 0.0241$) when adults were found only in field trial areas (Fig. 1).

Coleomegilla maculata adults were collected on traps for all dates except 16 July, 24 July, and 31 July. There were significant differences between sites on 18 May ($F = 56.0$; d.f. = 9; $p = 0.0002$), 24 May ($F = 12.59$; d.f. = 9; $p = 0.0080$), and 17 June ($F = 10.18$; d.f. = 9; $p = 0.0128$). *Hippodamia convergens* adults were not found in traps for 31 May, 6 June, 16 July, 24 July, 31 July, 20 September, or 29 September. Other predators were not caught in traps from 11, 18, or 31 May, 6 June, 9, 16, or 31 July, or 20 September.

Discussion

The natural enemy complex

Natural enemies found in absolute and visual samples from both the plantation and natural sites were *Coleomegilla maculata*, (Coleoptera: Coccinellidae), *Podisus maculiventris* (Heteroptera: Pentatomidae), Cantharidae, Formicidae, Lampyridae, Araneae and Opiliones. Samples from plantation sites also included *Hippodamia convergens*, *H. tredecimpunctata*, and *Neoharmonia venusta* (Coleoptera: Coccinellidae), Chrysopidae (Neuroptera), and Nabidae. Reduviidae were found only in natural sites. Sticky traps captured Carabidae,

Neuroptera, and several additional coccinellid species: *Coccinella septumpunctata*, *Adalia bipunctata*, *H. parenthesis*, *Cycloneda munda, Harmonia axyridis*, and three undetermined predatory species. Many of the same families of natural enemies were found with both the absolute and the visual sampling methods. Absolute samples contained two coccinellid species not seen in the visual samples (*H. tredecimpunctata* and *N. venusta*). Nabidae and Opiliones were seen only in the visual sampling. Both the absolute and the visual sampling methods have problems. The absolute sampling method requires a larger time commitment than the visual sampling. However, the visual sampling method does not allow identification below the family level for most organisms. High numbers of adult *C. scripta* tended to occur in both the absolute samples and the sticky traps.

Agricultural systems tend to have more pest problems than orchards or natural stands of vegetation. They also tend to have a lower diversity of natural enemies because resources vital to natural enemy survival are not present (Barbosa and Schultz 1987, Cromartie 1996). Therefore, our original hypothesis was that there would be fewer families of natural enemies represented in the plantation as compared with the natural sites. This is not supported by the data. The same families of natural enemies tend to be represented in both plantation and natural sites. In fact, fewer coccinellid species, and no Neuroptera or Nabidae were observed in natural sites. Plantation sites contained no Reduviidae. A possible explanation is that the plantation sampled in this survey is a diversified system; weeds are allowed to grow among the trees after establishment, and the tree-strips are interspersed with corn, soybeans, and switch grass. The diversity of crops and weeds within the plantation creates an agroforestry system that resembles orchard systems.

The natural enemy complex found in central Iowa is similar to that reported in other areas. Studies from Mississippi (Head et al. 1977) and Wisconsin (Burkot & Benjamin 1979) report the coccinellids *C. maculata*, *N. venusta*, *C. novemnotata*, *H. convergens,* and the pentatomid *P. maculiventris*, as well as chrysopid larvae and spiders as predators of *C. scripta*. Our observations indicate that coccinellids

are primarily egg predators, while pentatomid nymphs and adults, neuropteran larvae, and spiders may feed on all life stages. In addition, we saw egg predation by Formicidae. Thus, we are confident that these are natural enemies of *C. scripta.* However, *A. bipunctata*, *H. parenthesis*, *C. munda*, *H. axyridis* and the three undetermined coccinellid species, as well as the Carabidae, Cantharidae, Nabidae, Lampyridae, and Opiliones were never observed feeding on *C. scripta*, and therefore cannot be included in the natural enemy complex of *C. scripta* in central Iowa.

The seasonal occurrence of predators of *C. scripta* in Wisconsin was not addressed by Burkot & Benjamin (1979). However, Head et al. (1977) state that coccinellids tend to be present early in the season in Mississippi. This was observed in Iowa in 1995, but not in 1996. The presence of coccinellids in the plantation later in the 1996 season may have been because of a prolonged oviposition period for overwintering *C. scripta* adults caused by below average temperatures in May, 1996 (2.2 °C below normal) and total rainfall that was 8.55 cm above normal (Iowa Climatological Data 1996). Both Head et al. (1977) and Burkot and Benjamin (1979) found that the first generation *C. scripta* was the generation most affected by natural enemies -- primarily egg predation by coccinellids. *Chrysomela scripta* egg masses and adult coccinellids occurred simultaneously (Fig. 2, 4, and 6). A life table study performed in conjunction with the natural enemy survey found that predation by coccinellids killed 40.7, 73, and 57.3% of eggs in first and second generation 1995, and second generation 1996, respectively (see Chapter Three). The number of Pentatomidae and Neuroptera tends to increase later in the season.

Researchers working at the AMWPCF plantation prior to 1995 noted that *C. scripta* numbers were higher in plantation replicate 5 than elsewhere in the plantation early in the season. This led to the hypothesis that *C. scripta* adults overwintered along the wooded streambed west of the plantation, and moved into the plantation from the southwest in the spring. Data from the absolute sampling do not support this hypothesis. There were very few occasions when there were

significant differences in distribution of *C. scripta* or natural enemies within the plantation. The few instances where differences were observed form no pattern consistent with adult movement into or out of the plantation. In addition, sticky trap data from 1996 show *C. scripta* adults to be distributed throughout the plantation from the first sampling date on 11 May (Fig. 9).

Implications

This study has found a natural enemy complex of *C. scripta* in a central Iowa plantation that is similar to that found in other studies. Predators found in Wisconsin and Mississippi, but not in this survey, included the coccinellids *Coccinella novemnotata* Herbst and *Olla abdominalis* Say, the pentatomids, *Perillus biocularus* Fab., and *Stirentrus anchorago* Fab., and Eumenidae (Burkot & Benjamin 1979, Head et al. 1977). Predatory coccinellid species are the most commonly occurring egg predators in Wisconsin, Mississippi and Iowa.

Fang (1997) evaluated the relationship between *C. scripta* egg mass density and the amount of damage to growing terminals and found that 0.5 egg masses per terminal will result in death of the terminal. Data from absolute sampling show that the number of egg masses per terminal was above that threshold on 22 June 1995 (0.52 ± 0.16) and on 30 July 1996 (0.63 ± 0.19). It may be possible to develop a program for plantation management using a method similar to the absolute sampling of this study to monitor egg mass densities. Also, no research has yet determined the relationship between density of adult *C. scripta* and damage to plantation trees.

Chrysomela scripta is currently considered to be the most important defoliating insect pest of *Populus* in the midwest region. This study has given us a better understanding of the natural enemy complex of *C. scripta.* However, this study and previous studies by Burkot and Benjamin (1977), and Head et al. (1979) have not evaluated the role of nocturnal predators; these may also be an important component in the natural enemy complex.

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Table 1. Natural sites used for absolute sampling, 1995

Date	Natural Sites Sampled[1]
19 May	A, B, C, D
24 May	A, B, C, D
3 June	A, B, C, D, E, F, G, H
8 June	A, B, C, D, G, H, I
13 June	A, B, C, D, G, H, I
22 June	A, B, C, D, G, H, I
3 July	A, B, C, D, G, H, I
7 July	A, B, C, D, G, H, I
11 July	A, B, C, D, G, H, I
18 July	A, B, C, D, G, H, I
25 July	A, B, C, D, G, H, I
1 August	A, B, C, D, G, H, I
11 August	A, B, C, D, G, H, I

[1] sites A, B, C, & D were located at the AMWPCF, sites E & F were southwest of Boone, IA, and sites G, H, & I were northeast of Boone, IA

Table 2. Scentry® Multigard yellow sticky trap field placement schedule, 1995

Date	Sites Used	Number of traps
18 May	A, B, & C	1 per site
	Block 1	3
30 May	A, B, & C	1 per site
	Block 1	3
11 June	A, B, C, & D	1 per site
	Block 1	3
22 June	A, B, C, & D	1 per site
	Block 1	3
18 July	A, B, C, D, G, H, & I	1 per site
	Block 1	3
	Block 4 & 5	2 per block
5 August	A, B, C, D, G, H, & I	1 per site
	Block 1, 4, & 5	2 per block
20 August	A, B, C, D, G, H, & I	1 per site
	Block 1, 4, & 5	2 per block
13 September	A, B, C, D, G, H, & I	1 per site
	Block 1, 4, & 5	2 per block
30 September	A, B, C, D, G, H, & I	1 per site
	Block 1, 4, & 5	2 per block

Table 3. Mean (± SE) lady beetles, other predators, and *C. scripta* (CLB) in 1995 weekly absolute samples from 3 trees in each of 9 plantation sites

Date	Lady beetles[1]	Other predators[2]	CLB egg masses	CLB early larvae	CLB late larvae	CLB adults
19 May	0.15	0.11	0.41	0.00	0.00	0.81
	±0.07	±0.06	±0.12	±0.00	±0.00	±0.19
24 May	0.04	0.07	0.11	0.00	0.00	0.22
	±0.04	±0.05	±0.08	±0.00	±0.00	±0.08
3 June	0.00	0.04	0.00	4.46	1.54	0.04
	±0.00	±0.04	±0.00	±1.80	±0.65	±0.04
8 June	0.00	0.15	0.00	1.22	3.22	0.30
	±0.00	±0.09	±0.00	±0.31	±0.82	±0.19
13 June	0.00	0.04	0.00	0.15	1.81	0.04
	±0.00	±0.04	±0.00	±0.09	±0.67	±0.04
22 June	0.07	0.37	0.52	0.22	0.22	1.48
	±0.05	±0.12	±0.16	±0.11	±0.12	±0.60
3 July	0.00	0.56	0.19	4.93	0.22	0.48
	±0.00	±0.38	±0.11	±1.54	±0.11	±0.17
7 July	0.04	0.00	0.04	0.92	0.33	0.52
	±0.04	±0.00	±0.04	±0.39	±0.17	±0.12
11 July	0.00	0.15	0.22	0.59	0.11	0.19
	±0.00	±0.09	±0.08	±0.22	±0.06	±0.09
18 July	0.00	0.04	0.00	0.00	0.00	0.04
	±0.00	±0.04	±0.00	±0.00	±0.00	±0.04
25 July	0.00	0.78	0.04	1.30	0.07	0.07

Table 3 continued

	±0.00	±0.56	±0.04	±0.89	±0.07	±0.05
1 August	0.00	0.04	0.00	0.00	0.00	0.11
	±0.00	±0.04	±0.00	±0.00	±0.00	±0.06
11 August	0.00	0.07	0.00	0.00	0.00	0.00
	±0.00	±0.05	±0.00	±0.00	±0.00	±0.00

[1] Lady beetles include adult *C. maculata*, *H. convergens*, and *H. tredecimpunctata*

[2] Other predators include Araneae and Pentatomidae

Table 4. Mean (± SE) lady beetles, other predators, and *C. scripta* (CLB) in 1995 weekly absolute sampling from 3 trees in each natural site

Date	Lady beetles[1]	Other predators[2]	CLB egg masses	CLB early larvae	CLB late larvae	CLB adults
19 May	0.17	0.17	0.08	0.00	0.00	0.17
(n=4)	±0.11	±0.11	±0.08	±0.00	±0.00	±0.11
24 May	0.00	0.17	0.00	0.00	0.00	0.00
(n=4)	±0.00	±0.11	±0.00	±0.00	±0.00	±0.00
3 June	0.00	0.46	0.00	0.88	0.79	0.00
(n=8)	±0.00	±0.16	±0.00	±0.67	±0.57	±0.00
8 June	0.05	0.48	0.05	1.57	0.14	0.05
(n=7)	±0.05	±0.34	±0.05	±1.57	±0.08	±0.05
13 June	0.05	0.52	0.00	0.05	0.90	0.00
(n=7)	±0.05	±0.28	±0.00	±0.05	±0.68	±0.00
22 June	0.05	1.14	0.00	1.62	0.10	0.05
(n=7)	±0.05	±0.45	±0.00	±1.62	±0.10	±0.05
3 July	0.00	0.43	0.00	0.10	0.05	0.10
(n=7)	±0.00	±0.16	±0.00	±0.07	±0.05	±0.07
7 July	0.05	0.76	0.00	0.05	0.05	0.00
(n=7)	±0.05	±0.32	±0.00	±0.05	±0.05	±0.00
11 July	0.00	0.86	0.05	0.48	0.52	0.00
(n=7)	±0.00	±0.35	±0.05	±0.21	±0.34	±0.00
18 July	0.00	0.71	0.00	0.00	0.91	0.00
(n=7)	±0.00	±0.21	±0.00	±0.00	±0.86	±0.00

Table 4 continued

25 July	0.00	1.14	0.00	0.00	0.00	0.33
(n=7)	±0.00	±0.50	±0.00	±0.00	±0.00	±0.29
1 August	0.00	1.00	0.00	0.00	0.00	0.05
(n=7)	±0.00	±0.48	±0.00	±0.00	±0.00	±0.05
11 August	0.00	0.62	0.00	0.00	0.00	0.00
(n=7)	±0.00	±0.22	±0.00	±0.00	±0.00	±0.00

n is the number of natural sites sampled, with three samples per site

[1] Lady beetles include adult *C. maculata*, *H. convergens*, and *H. tredecimpunctata*

[2] Other predators include Araneae, Pentatomidae, and Reduviidae

Table 5. Mean (± SE) lady beetles, other predators, and *C. scripta* (CLB) in 1996 weekly absolute samples from 3 trees in each of 9 plantation sites

Date	Lady Beetles[1]	Other Predators[2]	CLB egg masses	CLB early larvae	CLB late larvae	CLB adults
17 June	0.00	0.07	0.00	0.00	0.04	0.00
	± 0.00	± 0.05	± 0.00	± 0.00	± 0.04	± 0.00
26 June	0.00	0.07	0.00	0.00	0.04	0.15
	± 0.00	± 0.05	± 0.00	± 0.00	± 0.04	± 0.09
2 July	0.00	0.00	0.00	0.63	0.00	0.44
	± 0.00	± 0.00	± 0.00	± 0.59	± 0.00	± 0.14
10 July	0.00	0.07	0.26	4.85	4.52	0.26
	± 0.00	± 0.05	± 0.09	± 2.56	± 1.94	± 0.11
19 July	0.04	0.00	0.00	3.00	5.22	0.15
	± 0.04	± 0.00	± 0.00	± 1.53	± 1.57	± 0.07
30 July	0.00	0.11	0.63	3.07	0.81	2.48
	± 0.00	± 0.06	± 0.19	± 2.14	± 0.29	± 0.58
5 August	0.07	0.00	0.41	3.52	2.44	2.00
	± 0.07	± 0.00	± 0.14	± 1.52	± 1.35	± 0.42

[1] Lady beetles include *C. maculata* and *N. venusta*

[2] Other predators include Araneae, Pentatomidae, Formicidae, and Neuroptera

Table 6. Mean (± SE) predators and *C. scripta* (CLB) in 1996 weekly absolute samples from 3 trees in each of 3 natural sites

Date	Predators[1]	CLB egg masses	CLB early larvae	CLB late larvae	CLB adults
17 June	0.89	0.00	0.00	0.00	0.00
	±0.68	±0.00	±0.00	±0.00	±0.00
26 June	0.56	0.00	0.00	0.00	0.00
	±0.18	±0.00	±0.00	±0.00	±0.00
2 July	0.33	0.00	0.00	0.00	0.00
	±0.17	±0.00	±0.00	±0.00	±0.00
10 July	0.22	0.00	0.22	0.22	0.00
	±0.15	±0.00	±0.15	±0.22	±0.00
19 July	0.44	0.00	0.00	0.22	0.11
	±0.24	±0.00	±0.00	±0.22	±0.11
30 July	0.67	0.00	0.00	0.00	0.00
	±0.29	±0.00	±0.00	±0.00	±0.00
5 August	0.56	0.00	0.00	0.00	0.00
	±0.34	±0.00	±0.00	±0.00	±0.00

[1] Predators include Araneae, and Formicidae

Table 7. Mean (± SE) lady beetles and other predators in 1996 visual observations from 3 trees in each of 9 plantation sites

Date	Lady Beetles[1]	Other Predators[2]
17 June	0.15 ± 0.09	0.11 ± 0.06
26 June	0.41 ± 0.22	0.07 ± 0.05
2 July	0.11 ± 0.08	0.15 ± 0.07
8 July	0.04 ± 0.04	0.04 ± 0.04
19 July	0.04 ± 0.04	0.44 ± 0.25
30 July	0.04 ± 0.04	0.26 ± 0.09

[1] Lady beetles include *C. maculata*, *H. convergens*, and an unidentified species (2 specimens on 2 July)

[2] Other predators include Araneae, Opiliones, Formicidae, Cantharidae, Nabidae, Pentatomidae, and Neuroptera

Table 8. Mean (± SE) lady beetles and other predators in 1996 visual observations from 3 trees in each of 3 natural sites

Date	Lady Beetles	Other Predators
17 June	0.00 ± 0.00	0.67 ± 0.37
26 June	0.00 ± 0.00	1.56 ± 0.82
2 July	0.00 ± 0.00	2.00 ± 1.09
8 July	0.00 ± 0.00	0.33 ± 0.17
19 July	0.00 ± 0.00	16.56 ± 7.06
30 July	0.03 ± 0.02	1.65 ± 0.70

[1] Lady beetles include *C. maculata*, *H. convergens*, and an unidentified species (2 specimens on 2 July)

[2] Other predators include Araneae, Opiliones, Formicidae, Cantharidae, Nabidae, Pentatomidae, and Neuroptera

Table 9. Mean (± SD) *C. scripta* (CLB) and Coccinellidae per sticky trap, 1995

Date	Adult CLB		*C. maculata*		*H. convergens*		*H. tredecim-punctata*		*C. septem-punctata*		*A. bipunctata*	
	P[1]	N[2]	P	N	P	N	P	N	P	N	P	N
18 May	35	2.3	15	1.3	12	0.3	1.3	0	0	0.3	0	0
30 May	54	0.7	2.3	3	1.7	1	0.7	0	0.7	0.3	0	0
11 June	6.7	0	6	3	2.3	0	0	0.8	0	0	0	0.3
22 June	9.3	0.8	5	7.8	1	2.3	2	0.3	0	0	0	0
18 July	6.1	0.4	0.4	0.3	0.9	0	0	0	0	0	0	0
5 August	1.8	0.6	0	0.6	0	0.1	0.2	0	0	0	0	0
20 August	0.7	0	0.3	0	0	0	0	0	0	0	0	0
13 September	0	0	0.2	0.3	0	0	0	0	0	0	0	0
30 September	0	0	0	2.3	0	0	0	0	0	0.1	0	0

[1] plantation sites

[2] natural sites

Fig. 1. Sticky trap locations, AMWPCF plantation (16 km SE Ames, IA), 1995 and 1996. The plantation replicates 1, 2, 3, 4, 5, and 6, and the road are drawn in proportion to one another (each plantation replicate is 100 m wide by 182 m long, and the road is approximately 15 m wide). Sites A, B, C, D, the woods area, and the field trial area show relative locations, but are not drawn to scale.

Fig. 2. Coccinellidae (*C. maculata*, *H. convergens*, and *H. tredecimpunctata*) and *C. scripta* (CLB) egg masses in weekly absolute samples (mean ± SE) from 3 trees in each of 9 sites in the AMWPCF plantation, 1995

Fig. 3. Predators (Araneae, and Pentatomidae), Coccinellidae, and *C. scripta* (CLB) in weekly absolute samples ($\bar{x}$ ± SE) from 3 trees in each of 9 sites in the AMWPCF plantation, 1995

Fig. 4. Coccinellidae (*C. maculata*, *H. convergens*, and *H. tredecimpunctata*), and *C. scripta* (CLB) egg masses in weekly absolute samples ($\bar{x}$ ± SE) from 3 trees in each natural site, 1995

Fig. 5. Predators (Araneae, Pentatomidae, and Reduviidae), and *C. scripta* (CLB) in weekly absolute samples ($\bar{x}$ ± SE) from 3 trees in each natural site, 1995

Fig. 6. Coccinellidae (*C. maculata* and *N. venusta*), and *C. scripta* (CLB) egg masses in weekly absolute samples ($\bar{x}$ ± SE) from 3 trees in each of 9 sites in the AMWPCF plantation, 1996

Fig. 7. Predators (Araneae, Pentatomidae, Formicidae, and Neuroptera), and *C. scripta* (CLB) in weekly absolute samples ($\bar{x}$ ± SE) from 3 trees in each of 9 sites in the AMWPCF plantation, 1996

Fig. 8. Predators (Araneae, and Formicidae), and *C. scripta* (CLB) in weekly absolute samples ($\bar{x} \pm SE$) from 3 trees in each of 3 natural sites near the AMWPCF plantation, 1996

Fig. 9. Adult *C. scripta* (CLB) ($\bar{x}$ per sticky trap, n = 6) in each of 5 blocks in the AMWPCF plantation and along the road west of the plantation, 1996

Fig. 10. Adult *C. maculata* ($\bar{x}$ per sticky trap, n = 6) in each of 5 blocks in the AMWPCF plantation and along the road west of the plantation, 1996

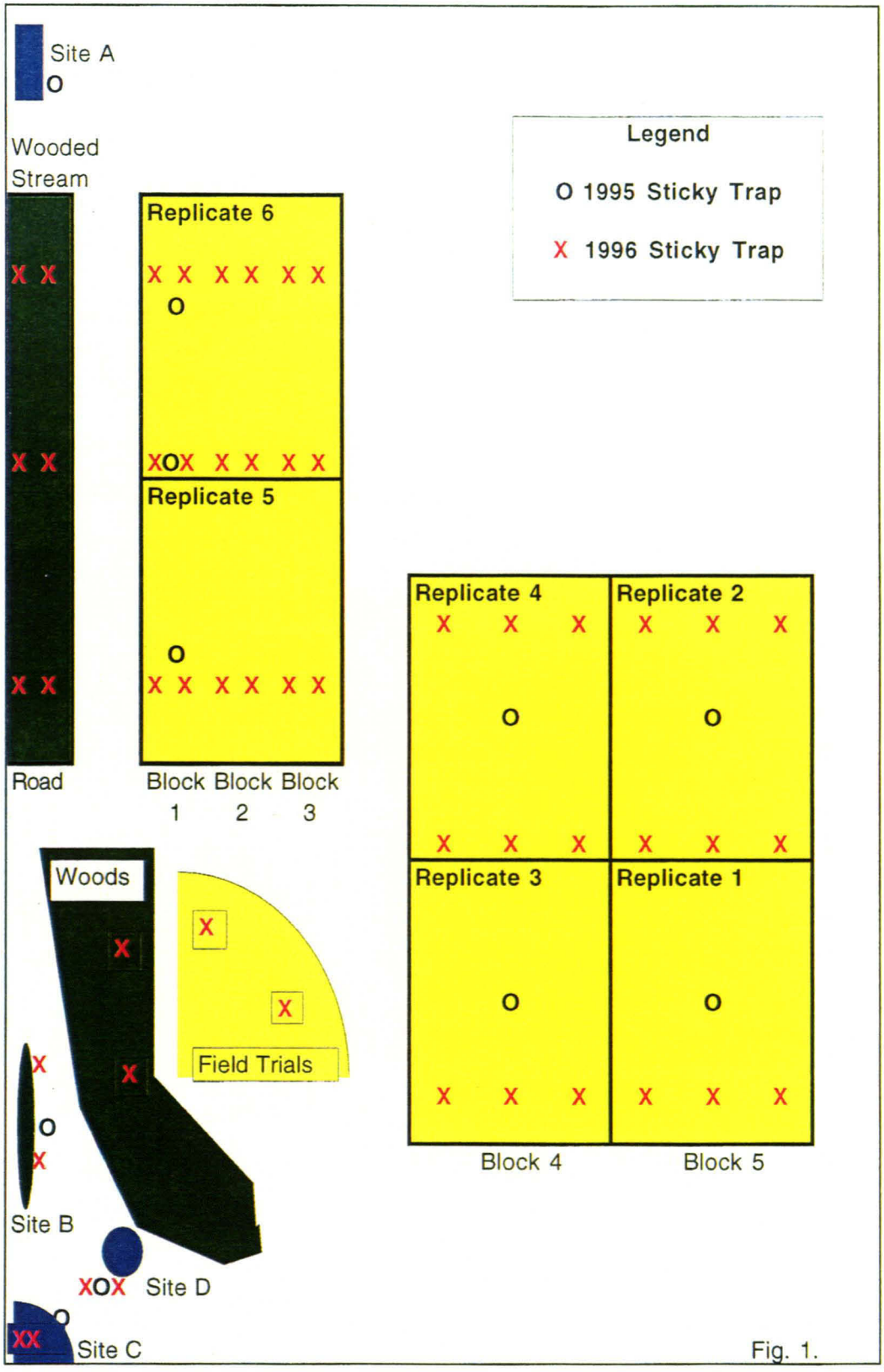

Site A
O
Wooded
Stream
Legend
O 1995 Sticky Trap
X 1996 Sticky Trap
Replicate 6
Replicate 5
Replicate 4
Replicate 2
Replicate 3
Replicate 1
Road
Block 1
Block 2
Block 3
Block 4
Block 5
Woods
Field Trials
Site B
Site D
Site C

Fig. 1.

Fig. 2.

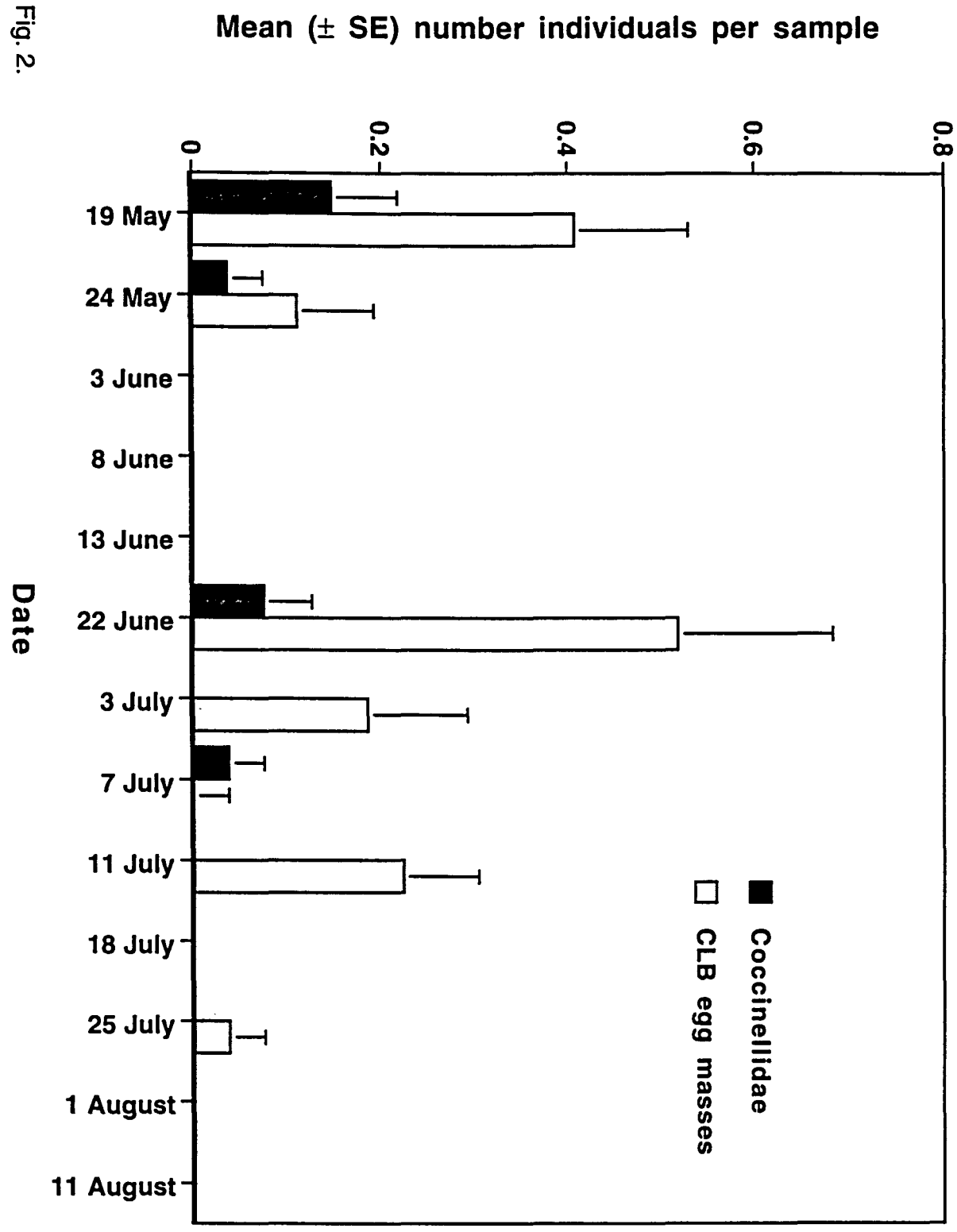
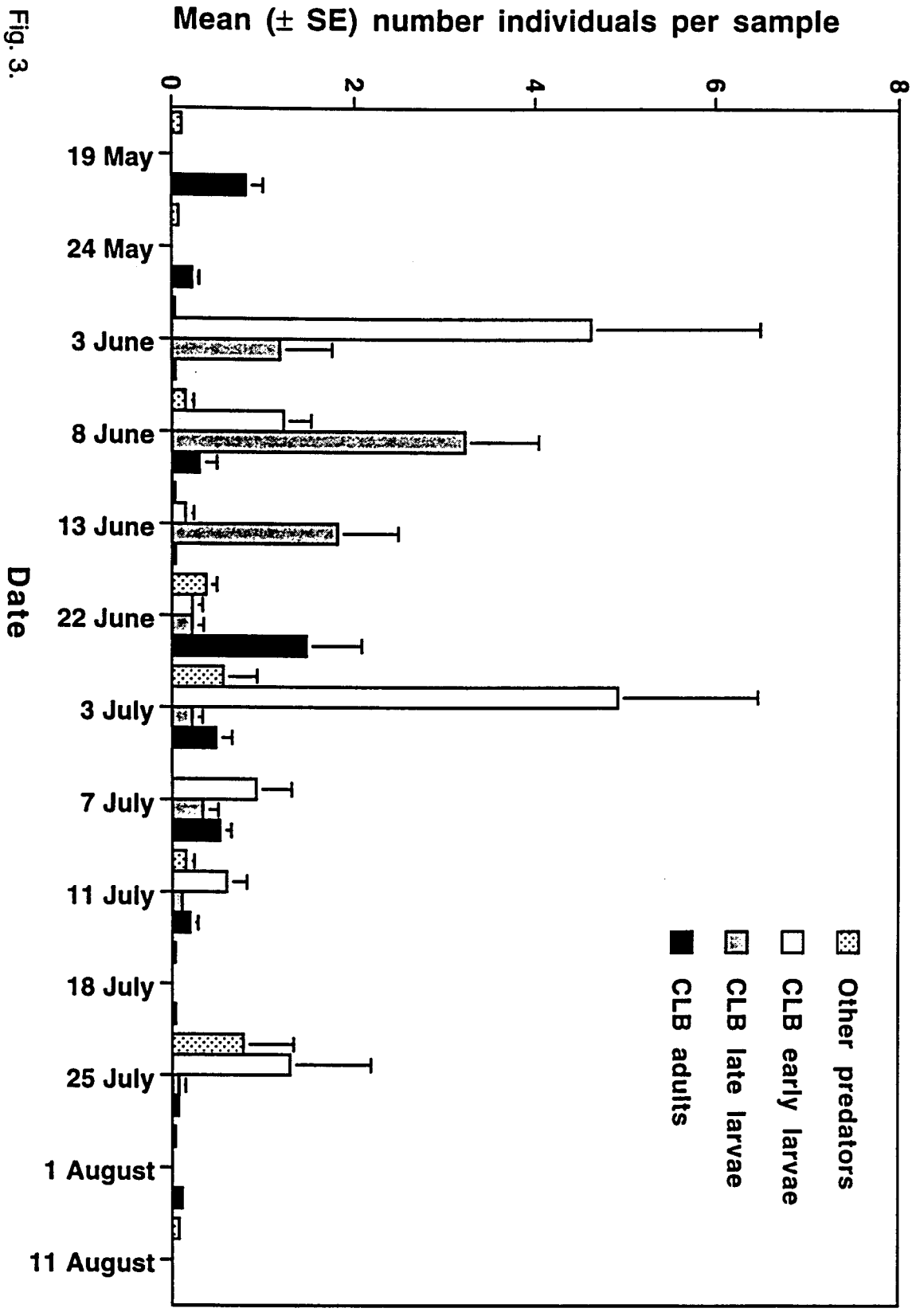

Mean (± SE) number individuals per sample
0
0.2
0.4
0.6
0.8
Date
19 May
24 May
3 June
8 June
13 June
22 June
3 July
7 July
11 July
18 July
25 July
1 August
11 August
Coccinellidae
CLB egg masses

Fig. 3.


Mean (± SE) number individuals per sample
0
2
4
6
8
Date
19 May
24 May
3 June
8 June
13 June
22 June
3 July
7 July
11 July
18 July
25 July
1 August
11 August
CLB adults
CLB late larvae
CLB early larvae
Other predators

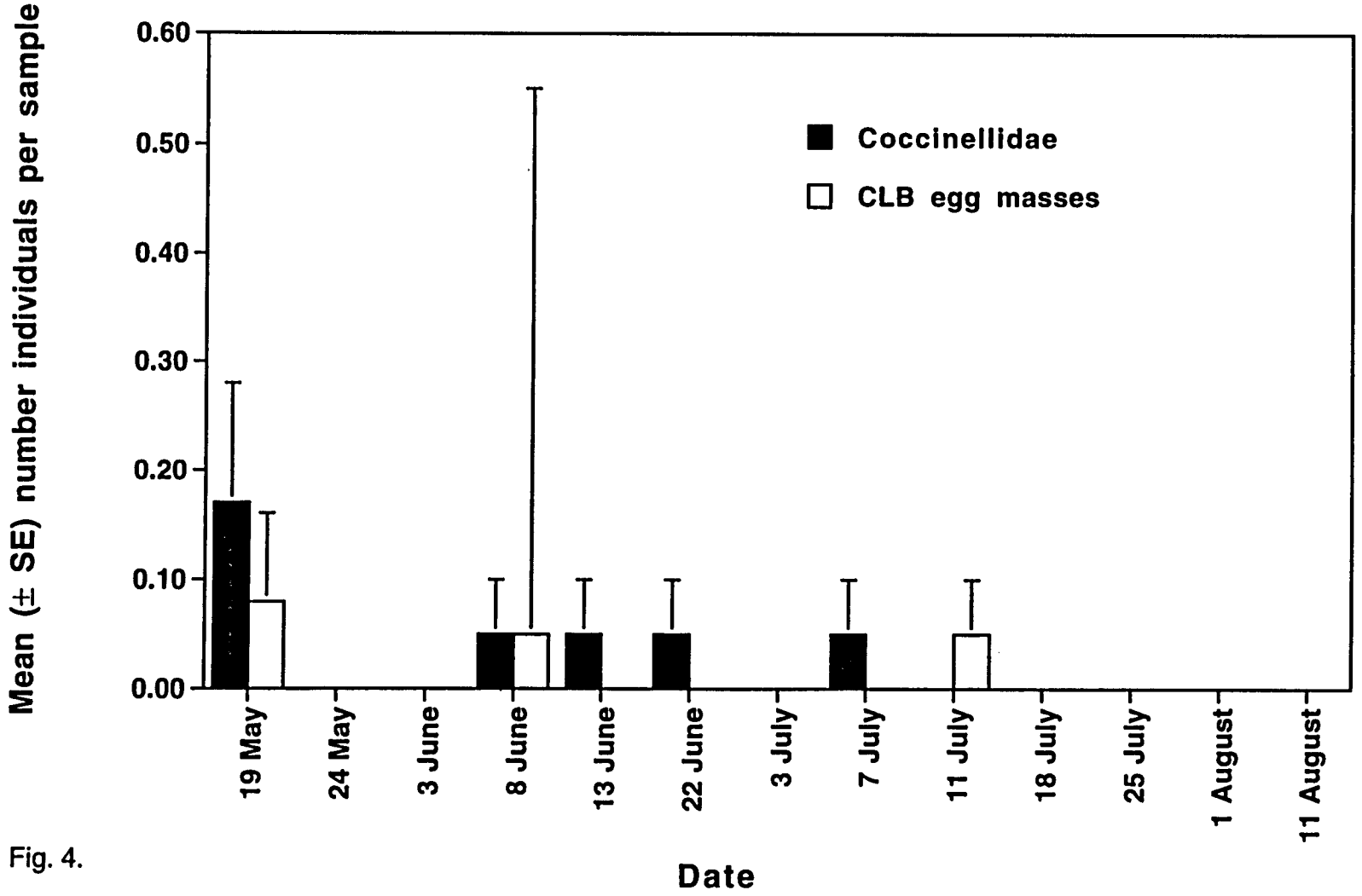
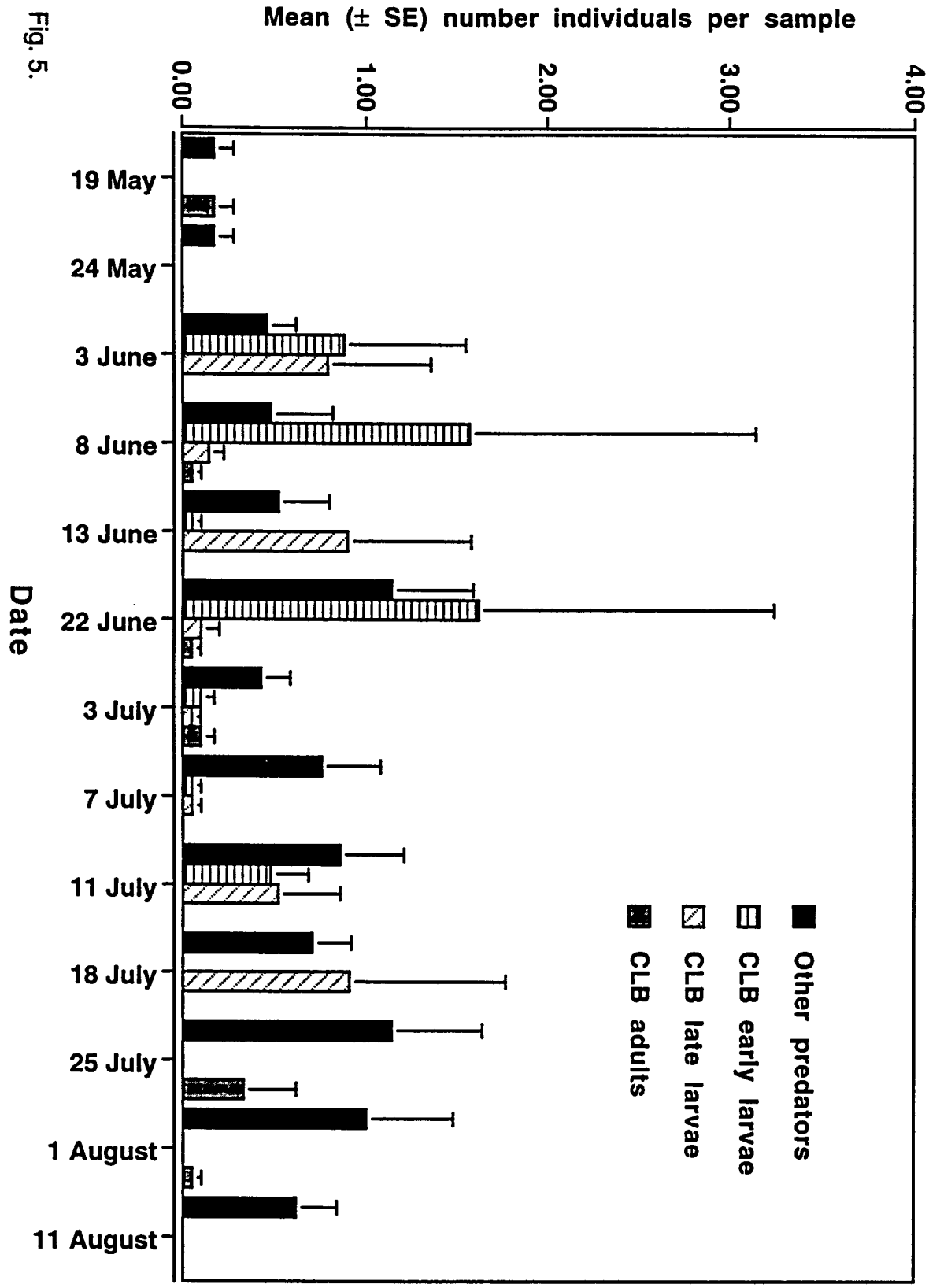

Mean (± SE) number individuals per sample
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Coccinellidae
CLB egg masses
19 May
24 May
3 June
8 June
13 June
22 June
3 July
7 July
11 July
18 July
25 July
1 August
11 August
Date

Fig. 4.

Fig. 5.


Mean (± SE) number individuals per sample
0.00
1.00
2.00
3.00
4.00
Date
19 May
24 May
3 June
8 June
13 June
22 June
3 July
7 July
11 July
18 July
25 July
1 August
11 August
Other predators
CLB early larvae
CLB late larvae
CLB adults

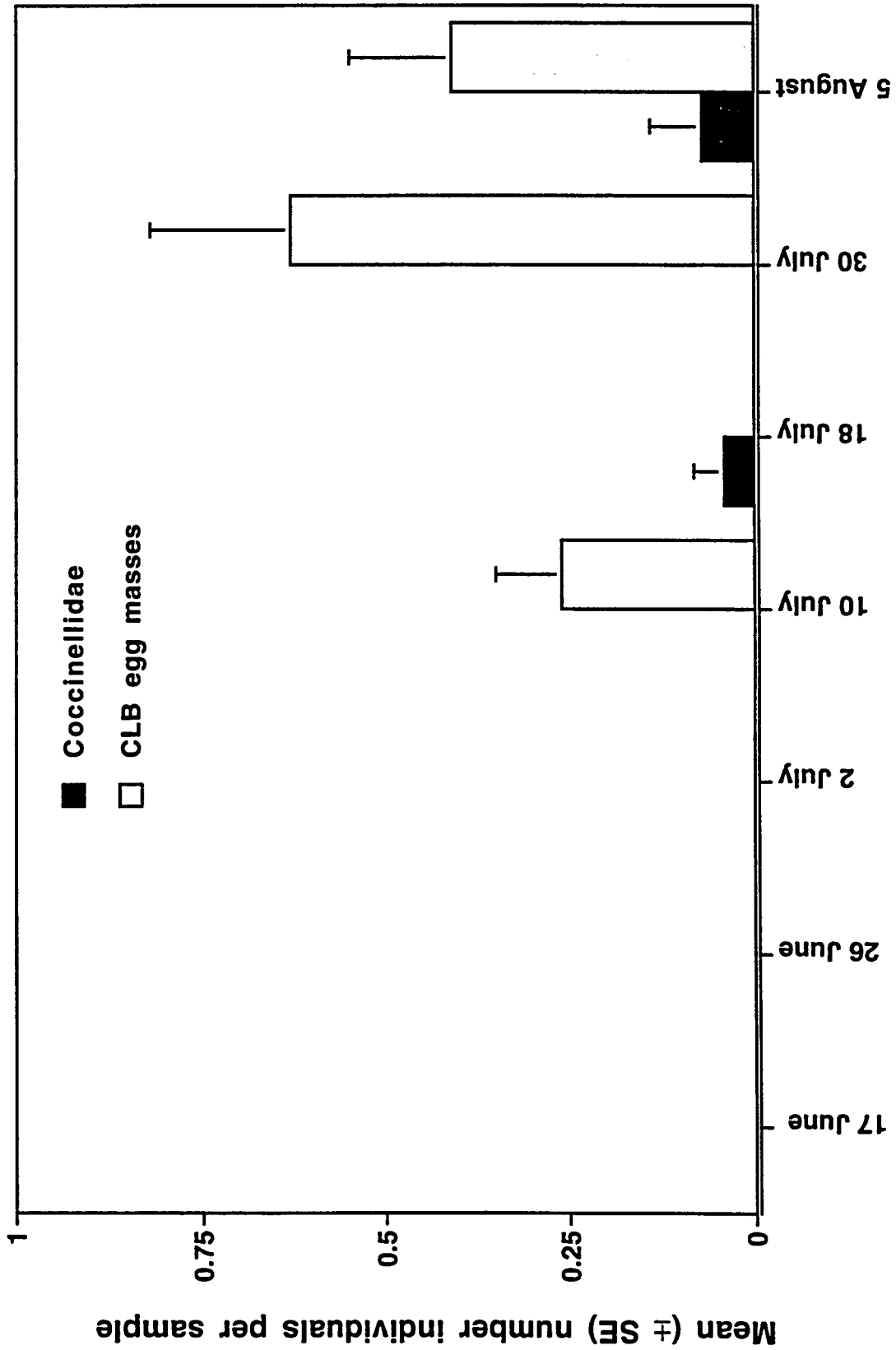

Mean (± SE) number individuals per sample
0
0.25
0.5
0.75
1
17 June
26 June
2 July
10 July
18 July
30 July
5 August
Date
Coccinellidae
CLB egg masses

Fig. 6.

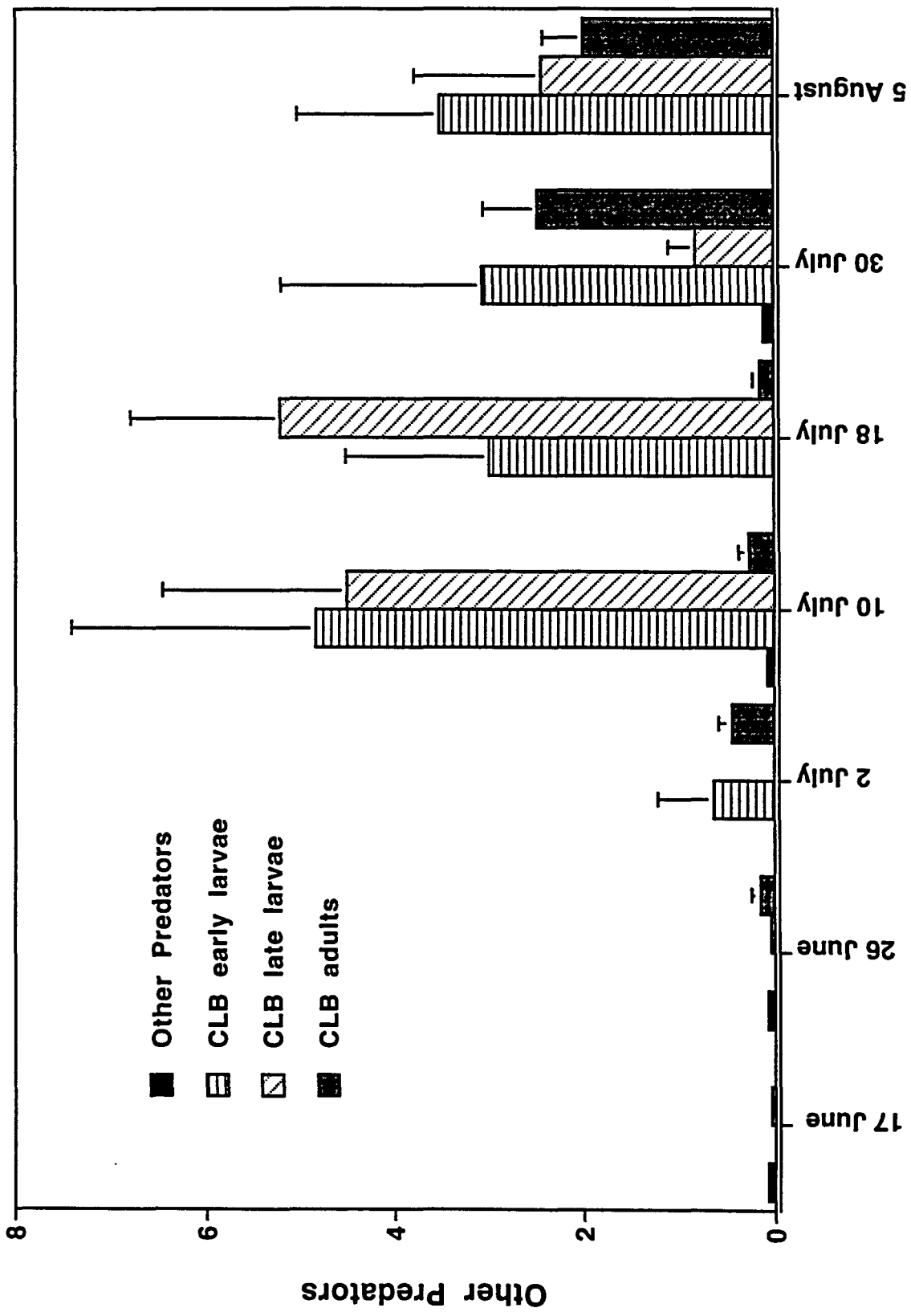

Other Predators
8
6
4
2
0
17 June
26 June
2 July
10 July
18 July
30 July
5 August
Date
Other Predators
CLB early larvae
CLB late larvae
CLB adults

Fig. 7.

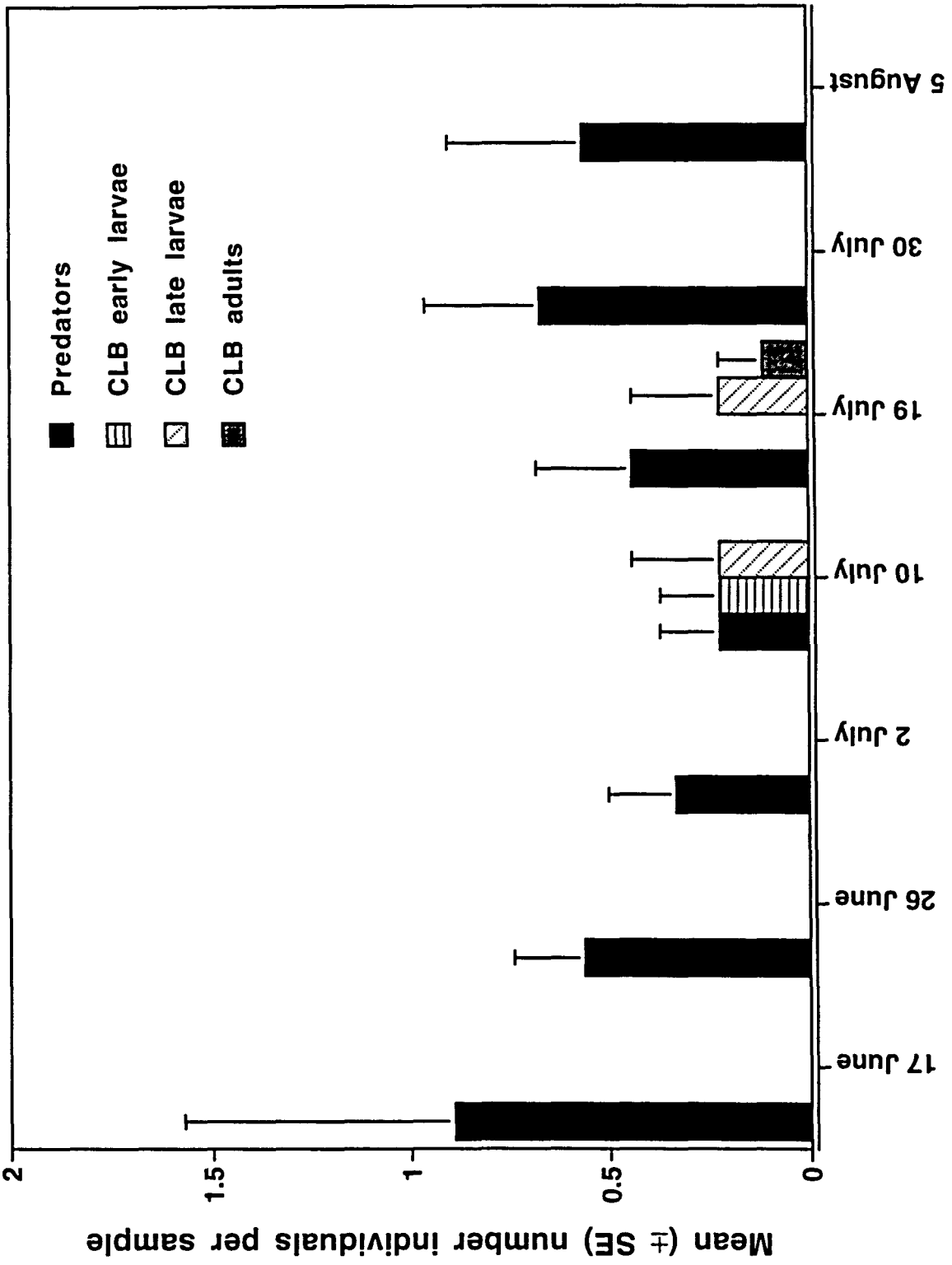

Predators
CLB early larvae
CLB late larvae
CLB adults
Mean (± SE) number individuals per sample
0
0.5
1
1.5
2
17 June
26 June
2 July
10 July
19 July
30 July
5 August
Date

Fig. 8.

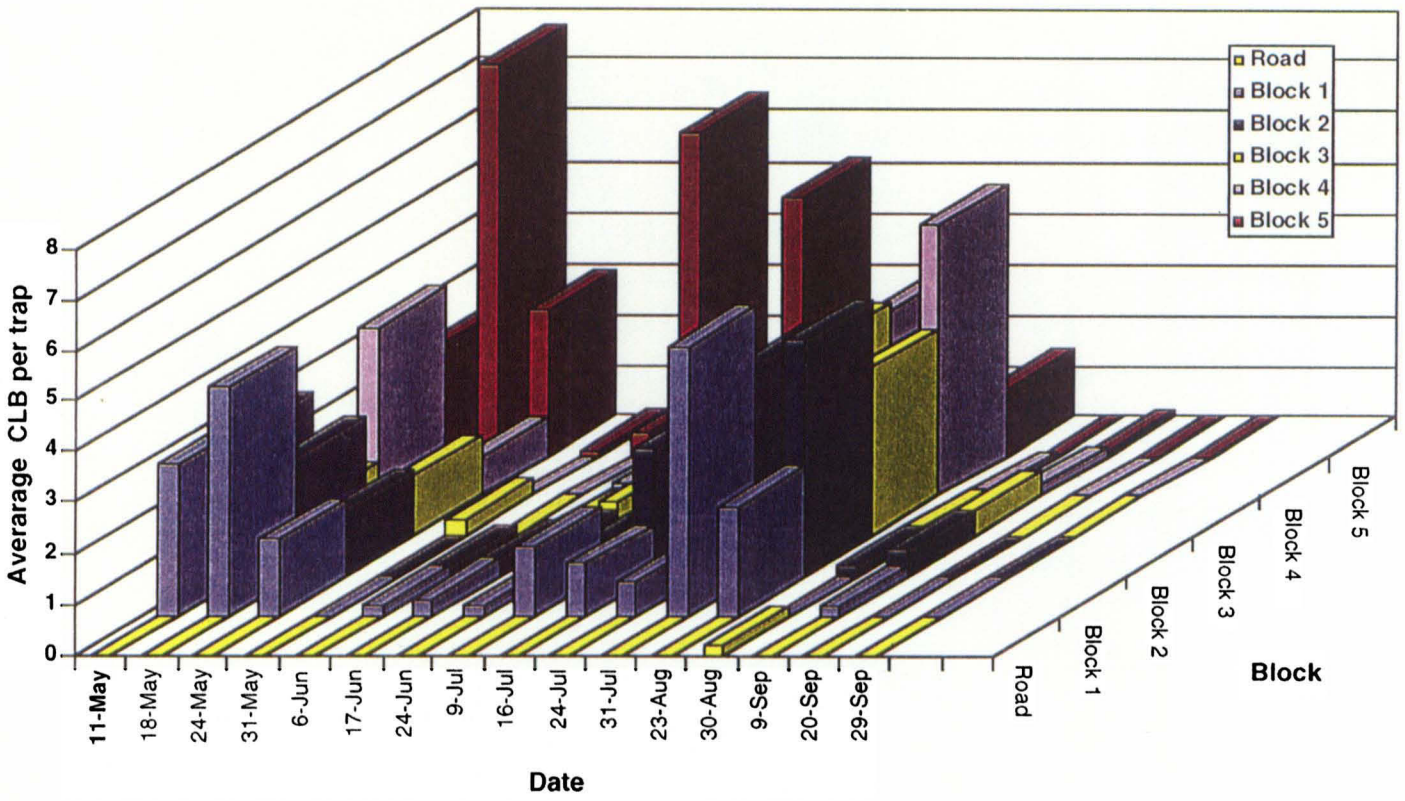

Averarage CLB per trap
0
1
2
3
4
5
6
7
8
11-May
18-May
24-May
31-May
6-Jun
17-Jun
24-Jun
9-Jul
16-Jul
24-Jul
31-Jul
23-Aug
30-Aug
9-Sep
20-Sep
29-Sep
Date
Road
Block 1
Block 2
Block 3
Block 4
Block 5
Block
Road
Block 1
Block 2
Block 3
Block 4
Block 5

Fig. 9.

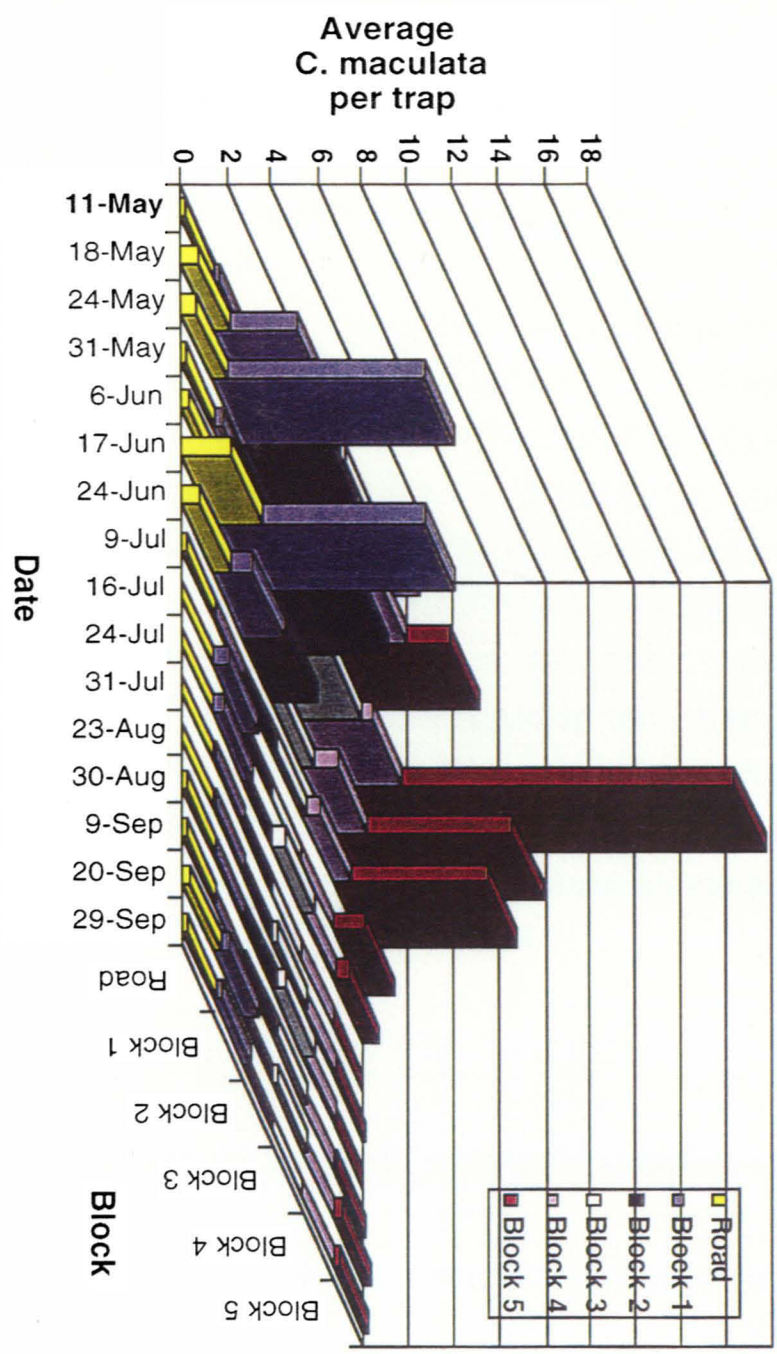

Average
C. maculata
per trap
0
2
4
6
8
10
12
14
16
18
Date
11-May
18-May
24-May
31-May
6-Jun
17-Jun
24-Jun
9-Jul
16-Jul
24-Jul
31-Jul
23-Aug
30-Aug
9-Sep
20-Sep
29-Sep
Block
Road
Block 1
Block 2
Block 3
Block 4
Block 5
Road
Block 1
Block 2
Block 3
Block 4
Block 5

Fig. 10.

CHAPTER THREE. EVALUATION OF THE NATURAL ENEMIES OF *CHRYSOMELA SCRIPTA* (COLEOPTERA: CHRYSOMELIDAE) POPULATIONS IN CENTRAL IOWA USING PARTIAL LIFE TABLE ANALYSIS

A paper to be submitted to Environmental Entomology

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Abstract

Partial life table studies of the cottonwood leaf beetle, *Chrysomela scripta* F. (Coleoptera: Chrysomelidae) that were conducted in a central Iowa plantation of the *Populus* hybrid 'Eugenei' (*P.* deltoides x *P. nigra*) in 1995 and 1996 showed that predation of first and second generation *C. scripta* eggs by Coccinellidae was the most important mortality factor. High mortality of early and late larvae (83 and 100%, respectively) was caused by abiotic factors in the second generation, 1995. An unidentified Tachinidae parasitized 4.2% of late stage larvae in the first generation 1995, and *Schizonatus latus* Walker (Hymenoptera: Pteromalidae) parasitized 8.6% of the pupae in the first generation, 1995 and 2.3 and 2.4% of the pupae in the second and third generations, 1996. Comparative life tables from the first and second generations in 1996 showed that *C. scripta* mortality caused by unknown factors was higher in the presence of natural enemies. In the first generation of 1996, 50.5% of the uncaged early larvae died compared to 34.3% of those caged, 91.4% of the uncaged late larvae died compared to 64.4% of those caged, and 25% of the uncaged pupae died compared to 18.1% of those caged. In the second generation of 1996, nearly three times the number of *C. scripta* survived to the adult stage in caged cohorts compared to uncaged cohorts.

Key words: *Chrysomela scripta*, biotic mortality, evaluation of natural enemies

Introduction

Two approaches to evaluating the impact of natural enemies on their hosts are construction of life tables to analyze mortality of host populations (Bellows et al. 1992) and comparison studies of host populations with and without natural enemies (Luck et al. 1988). Life tables provide detailed quantification of mortality, and an understanding of the ecological role of a natural enemy in a particular system. Life tables that compare mortality of populations with and without natural enemies provide a clear evaluation of a natural enemy's impact (Bellows et al. 1992). The use of cages and barriers for exclusion or inclusion have been used most frequently to compare mortality of populations with and without natural enemies (Luck et al. 1988). However, cages may change the interior microenvironment, predator and prey behavior, and plant physiology. In addition, cages are seldom predator free. Even so, the use of exclusion cages or barriers is the most appropriate approach for testing whether natural enemies have the potential to control a host population (Luck et al. 1988).

A life table analysis of mortality factors of *Chrysomela scripta* F. (Coleoptera: Chrysomelidae) was conducted in Wisconsin (Burkot and Benjamin 1979). This beetle is one of the most important defoliating insect pests of *Populus* species in the north-central region of the United States (Burkot and Benjamin 1979, Harrell et al. 1982). Predators identified in the Wisconsin study are similar to those found in a natural enemy survey performed in central Iowa. Known natural enemies of *C. scripta* in the Iowa survey included *Coleomegilla maculata* DeGeer, *Hippodamia convergens* Guerin, *H. tredecimpunctata* L., and *Neoharmonia venusta* Melsheimer (Coleoptera: Coccinellidae), *Podisus maculiventris* Say (Hemiptera: Pentatomidae), Chrysopidae, Formicidae, and Neuroptera. Two parasitoids of *C. scripta* larvae were also found: *Schizonatus latus* Walker (Hymenoptera: Pteromalidae) and an unidentified Tachinidae (Diptera: Tachinidae).

This study used a combination of approaches to (1) identify causes of mortality affecting each stage of two generations of *C. scripta* in central Iowa, (2)

quantify the impact of these mortality factors on *C. scripta*, and (3) compare *C. scripta* mortality in the presence and absence of natural enemies. The first series of life tables provided detailed quantification of mortality in the presence of natural enemies; the second series contrasts *C. scripta* populations with and without natural enemies.

Materials and Methods

The study was conducted in a plantation of the *Populus* hybrid 'Eugenei' (*P. deltoides* x *P. nigra*) located 16 km southeast of Ames, Story Co., Iowa at the Ames Municipal Water Pollution Control Facility (AMWPCF). There are approximately 2785 trees per ha; spacing is 1.25 m between trees in a row and an average of 3 m between rows arranged in strips. Each strip consisted of six rows of trees with a 15.2 m planting of corn, soybeans, or switch grass separating it from the next tree-strip. Plantation replicates 1 & 2, 3 & 4, and 5 & 6 were planted in 1990, 1991, and 1992 respectively.

Study sites were located within plantation replicate 5. The average tree height in plantation replicate 5 was 2.53 m in 1995 and 2.64 m in 1996 (C. W. Mize, ISU, personal communication). Plantation study sites were chosen so as not to interfere with previously established long-term research plots. Each plantation study site consisted of 30 trees, with 5 trees in each of the 6 rows. There were nine such plantation study sites; these were arranged in three blocks with three experimental replicates in each block.

1995

A life table study of first generation *C. scripta* was started on 22 May and observations were made through 27 June. On 22 May, one fresh, undisturbed *C. scripta* egg mass was flagged on each of three trees in each of nine plantation sites. Thus, a total of 27 egg masses, or cohorts, were studied. All other egg masses present on each tree, or which were laid after study egg masses were

flagged, were removed. The number of eggs in each cohort was recorded. Cohorts were monitored every second day until hatching occurred. The number of undamaged eggs in each egg mass was recorded, as was the number of successfully hatched eggs. In addition, damaged egg masses were examined to determine the cause of mortality. In some cases eggs disappeared; in others, whole eggs were consumed except for a small amount of the chorion attached to the leaf surface. This latter type of damage is typical of egg predation by Coccinellidae (Shade et al. 1970). Eggs also collapsed with no visible cause. Such eggs may have been fed upon by predators with sucking mouthparts (e.g., Pentatomidae, Neuroptera, Anthocoridae, or Araneae), or may have been infertile. The number of early and late larvae were recorded twice weekly. Early stage larvae (first and early second instars) generally feed on the same leaf on which the egg mass is found, or on adjacent leaves. Late larvae (late second and third instars) are more mobile, and are distinguished by their clearly visible eversible glands. Any observed cause of mortality was noted (e.g. predation or attack by parasitoids).

Most pre-pupae move down the tree to pupate on low branches and in the vegetation near the base of the tree. Prior to the pre-pupal stage, debris was cleared in a 91.4 cm diameter circle around the base of the tree and a 15.2 cm tall aluminum barrier was placed around the cleared area. Petroleum jelly spread along the top interior edge of the barrier prevented larvae from moving out of the area. The number of pupae present on the tree and in the vegetation at the base of the tree was recorded.

Late larvae are mobile, and frequently move from branch to branch. This movement made it difficult to follow flagged cohorts. Therefore, the following changes in methods were made for the second generation study in 1995. A lateral branch or basal sprout with several actively growing terminals was selected on three trees in each of the nine plantation plots. Selected branches were isolated from the rest of the tree: a ring of masking tape (to protect the tree) was put around the tree trunk above the selected branch and Pest Barrier Tree Tanglefoot® (The

Tanglefoot Company, Grand Rapids, MI) was applied to the tape. Any lateral branches or basal sprouts that might come into contact with the isolated branch were removed or tied back. A pair of *C. scripta* was placed on a growing terminal in a polyester organdy sleeve cage (38 x 13 cm) which was sealed with Velcro® and tied at each end. Once an egg mass was observed, it was flagged and the sleeve cage was removed. Flagged egg masses (cohorts) were observed in the same manner as was the first generation. The petroleum jelly used on the aluminum barriers in the first generation study melted and covered the entire inner surface. In the second generation, it was replaced with Shell Alvania® EP Grease 2 Multipurpose extreme pressure grease NLGI Grade 2 (Shell Oil Company, Houston, TX).

Twenty-seven cohorts were studied for the second generation study, which started 9 July and continued through 27 July. This period was 1 - 2 wk after second generation *C. scripta* activity began in the field because M-trac® (Mycogen, San Diego, CA) was applied to the plantation on 4 July to reduce the *C. scripta* population.

1996

Data for life table construction were collected for cohorts that developed on open or caged branches. In or near each of the nine plantation sites, one branch on each of three trees was isolated as in the 1995 second generation study. Uncaged cohorts were placed on these branches. Caged cohorts were placed on a second branch on each tree, or on adjacent trees.

The first generation study was started on 6 June and continued until 25 June. Adult *C. scripta* were difficult to collect when the study started because they were only present in large numbers in the older trees in plantation replicates 1 & 2. Therefore, egg masses were collected and returned to the laboratory until egg hatch. The number of larvae in each clutch ranged from 10 to 70. These larval clutches were paired so that approximately the same number of larvae were placed

on the caged and uncaged branches of a given tree. Within 24 hours of hatching, larval clutches were placed in the field by pinning the leaf on which the larvae were located to a leaf on the study branch. After placing the larvae, branches in the caged treatment were inspected and natural enemies were removed. The branches were then covered with sleeve cages (38 x 13 cm). These cages did not enclose sufficient leaf material for complete development, so larger cages were constructed for the second generation study. A total of 14 caged and 14 uncaged cohorts were studied. After larvae were placed in the field, observations were made as in the first generation, 1995.

The second generation study started on 8 July and continued until 4 August 1996. Mating pairs of *C. scripta* were caged on each branch and checked daily. After oviposition, eggs in each mass were counted, mating pairs were removed, and each branch was left caged or uncaged. Larger sleeve cages (1.22 x 0.46 m) were used for the second generation. These were placed on the caged branches before or immediately after egg hatch. Study branches were carefully inspected and natural enemies were removed before the cages were put in place. A small gauge cotton rope was used to secure the cages to the tree. The base of the cage was tied closed around the branch, each of five attachments were tied either to other branches in the tree or to 2.54 x 5.08 cm wooden stakes driven into the ground. The top of the cage was secured with a slip knot so that it could be opened. Both caged and uncaged cohorts were observed twice weekly. Caged cohorts completed development within the cages: late larvae tended to wander on the inside surface of the cage; pupae attached to the cage or branch at the base of the cage.

Data analysis

Life tables were constructed from field data according to Pedigo and Zeiss (1996). The number mean number of *C. scripta* entering each life per cohort was calculated. This was the lx term used to construct the life tables. The mean number of *C. scripta* dying during each life stage stage (dx) per cohort was

calculated for each mortality factor. The mean number dying per cohort was calculated as a percentage of the mean number entering each stage (100qx) for each mortality factor. Survival as a percentage of the mean number entering each stage was also calculated.

Weather data for the study time periods in 1995 and 1996 were collected at the Ames Municipal Water Pollution Control Facility. Voucher specimens have been deposited in the Department of Entomology Insect Collection, Iowa State University, Ames, Iowa.

Results

Identification of mortality factors

Several mortality factors affecting *C. scripta* during this study were easily identified and quantified (i.e., coccinellid feeding on eggs and attack by the tachinid parasitoid or *S. latus*). Many *C. scripta* larvae disappeared from the study branches, indicating that there were other, less easily identified mortality factors affecting *C. scripta.* These may have included movement off study branches, predation by predators that left little or no evidence (i.e., Pentatomidae, Neuroptera, Anthocoridae, or Araneae), and abiotic factors such as wind, precipitation, and temperature.

The cages used for the second generation study in 1996 did not eliminate all predators. One adult *C. maculata* was found feeding on eggs in one cage on 12 July 1996, an adult Chrysopidae was found in another cage at the end of the study. Two adult Pentatomidae were observed feeding on late larvae, pupae, and adults through the cages during the week of 22 July 1996. Data from these cages were not used in life table construction.

1995

In the first generation, 40.7% of the eggs were preyed on by Coccinellidae and 6.7% were killed by unknown factors (Table 1). Unknown factors killed 58.9%

of the early larvae. An unidentified tachinid parasitoid killed 4.2% of the late larvae and 40.0% were killed by unknown factors. Unknown factors and parasitism by *S. latus* killed 17.9 and 8.6% of the pupae, respectively. Because of sampling errors and problems with the methods, survival of pupae was 100.6% (Table 1). On each observation date, larvae not in the study cohort on each tree were removed. However, a number of non-study larvae were missed. Thus, there were trees on which more pupae were found compared to larvae that started in the cohort.

There was higher egg mortality in the second generation. Eggs were preyed upon by Coccinellidae and died from unknown factors (73 and 11.7%, respectively). Unknown factors killed 83 and 100% of the early and late larvae, respectively. None of the larvae in the study survived to pupate (Table 2). This was likely caused by high temperatures and rainfall. Early and late larvae were present on study trees beginning on 11 July. Maximum daily temperatures from 10 - 15 July were above 30°C (Table 3). While the high temperature threshold for *C. scripta* is not known, these high temperatures may have caused larval mortality. In addition, on 15 July, 0.28 cm of rain fell in two rain events that lasted approximately 5 hours, and another 3.1 cm of rain fell on 16 July. Brief, violent thunderstorms lasting less than 1 hour moved through the area on that date (J. A. Jarrard, personal observation). No larvae were present on study trees on the next sampling date, 19 July.

1996

In three of the cages used for the first generation study, insufficient numbers of leaves were enclosed. Starvation caused high mortality in these cages; data from the three cages were excluded from life table calculations. In the remaining first generation caged cohorts, 34.3% of the early larvae, 47.8% of the late larvae, and 18.1% of the pupae experienced mortality from unknown factors. In the uncaged cohorts, unknown mortality killed 50.5% of the early larvae, 91.4% of the late larvae, and 25.0% of the pupae (Table 4). While the early and late larvae were

in the field, maximum temperatures were above 30°C on 5 days and rain fell on 8 days (Table 3).

In the second generation caged cohorts, abiotic factors killed 20.5% of the eggs, 15.1% of the early larvae, 28.8% of the late larvae, and 19% of the pupae. In the uncaged cohorts, 1.9% of the eggs were killed by unknown mortality, and 57.3% of the eggs were preyed on by Coccinellidae. Unknown factors killed 66.2% of the early larvae, and 33.9% of the late larvae. Survival of uncaged late larvae was 102.4% (Table 5). This is an artifact of the methods used; larvae from non-study branches pupated within the barrier. Thus, in several trees, there were more pupae than there were late larvae. Unknown factors killed 31.5% of the pupae, with an additional 0.8 and 2.4% being killed by predation and *S. latus* parasitism (Table 5). Maximum temperatures were above 30°C on two days while eggs, and early and late larvae were in the field, and one rain event occurred while pupae were in the field (Table 3).

Discussion

1995

Chrysomela scripta egg predation by Coccinellidae was higher in the second generation (73% of the second generation eggs vs. 40.7% of the first generation eggs). This was unexpected, since the natural enemy survey (Chapter Two) showed that the number of Coccinellidae was similar when *C. scripta* eggs were found in both generations (0.04 ± 0.04 Coccinellidae per branch on 24 May and 7 July). Comparison of total mortality and specific mortality factors shows that egg predation was the most important factor affecting the egg stage, while unknown factors were responsible for most of the mortality of early and late larvae, and pupae (Fig. 1). Second generation mortality from unknown factors was higher for all *C. scripta* stages than for the first generation. Egg predation was the most important mortality factor for eggs, but unknown factors caused all mortality for later stages (Fig. 2). The combination of rain and wind have been shown to cause

mortality in *C. scripta* larvae (B. R. Bingaman, Dept. Horticulture, ISU, unpublished data). Maximum daily temperatures were above 30°C while early and late larvae were in the field (10 - 15 July), and heavy rain fell on 16 July (Table 3). Thus, it is likely that the high mortality of early and late larvae (72 and 100%, respectively) was caused by these environmental conditions.

1996

Comparison of life table data from the caged and the uncaged cohorts provides an estimate of how much mortality is caused by abiotic factors (rain, wind, temperature) and how much is due to natural enemies. In the first generation, uncaged larvae and pupae experienced higher mortality than did caged cohorts; 50.5% of the uncaged early larvae died compared to 34.3% of those caged, 91.4% of the late larvae died compared to 47.8% of those caged, and 25% of the uncaged pupae died compared to 18.1% of those caged (Table 4, Fig. 3). Much of this mortality may have been caused by high temperatures and rainfall; maximum temperatures were above 30°C on 5 days and rain fell on 8 days while early and late larvae were in the field. Natural enemies probably caused the additional mortality in the uncaged cohorts.

The second generation uncaged larvae and pupae also experienced higher unknown mortality (Table 5, Fig. 4); 66.2% of the uncaged early larvae died compared to 15.1% of those caged, 33.9% of the uncaged late larvae died compared to 28.8% of those caged, and 31.5% of the uncaged pupae died compared to 19% of those caged. Nearly three times the number of *C. scripta* survived to the adult stage in caged cohorts compared to uncaged cohorts (Table 5).

Role of natural enemies

The presence of natural enemies increased mortality for all stages of *C. scripta*. Predation by Coccinellidae causes high egg mortality. First and second generation 1995 and uncaged second generation 1996 cohorts experienced 40.7,

73, and 57.3% egg mortality. An unidentified Tachinidae parasitized 0.8 ± 1.4% of the late larvae in the first generation 1995, but was not observed again in the life table study. *Schizonatus latus* parasitized first generation pupae in both 1995 and 1996. The pathogen *Nosema scripta* (Microsporida: Nosematidae) has been described from *C. scripta* (Bauer and Pankratz 1993). This pathogen causes chronic infections that reduce fecundity and longevity in adults after several generations. *Nosema scripta* may have contributed to the unknown mortality of both the caged and the uncaged cohorts. No other pathogens are reported to infect *C. scripta*.

The mortality caused by unknown factors was higher in the uncaged cohorts than the caged cohorts for most stages in both generations. Although much of the added mortality was probably caused by natural enemies, some may have been caused by abiotic factors. Environmental conditions in the cages may have been modified compared to the uncaged study branches. The cages may have provided protection from wind and rain, thus rainfall would cause higher mortality in the uncaged cohorts. In contrast, reduced air movement in the cages may increase the temperatures, thus causing higher mortality in the caged cohorts. In addition, cages may have rubbed against egg masses and caused mortality. Dead early and late larvae were found in cages on several observation dates, but the cause of death was not determined.

These results are similar to the life table study conducted in Wisconsin (Burkot and Benjamin 1979) (Table 6). In the Wisconsin study, 12.4% of the second generation eggs were killed by predators, principally *C. maculata*. This is lower than the mortality from predation by Coccinellidae in any generation of our study. Other important causes of egg mortality were identified as infertility, and "collapsed eggs" (an unidentified term). Burkot and Benjamin (1979) also noted several predators of larvae, including the pentatomids *Podisus maculiventris* Say and *Perillus biocularus* Fab., as well as Chrysopidae and Syrphidae larvae. In addition, *S. latus* was identified as a parasitoid of pupae. Predators of adult *C.*

scripta included Pentatomidae and spiders. Overall survival ranged from 7 - 10% in the Wisconsin study and from 0 - 26% in the current study.

Egg predation by Coccinellidae caused between 40 and 73% mortality and was the most important mortality factor of *C. scripta* eggs in both the first and second generations in both years. High mortality of early and late larvae was caused by abiotic factors in the second generation, 1995. But, no other important mortality factors were identified for larvae or pupae. Comparison of data from the caged and uncaged studies shows that *C. scripta* mortality was higher in the presence of natural enemies. From this, we can say that the natural enemy complex in central Iowa plays a role in reducing the *C. scripta* population.

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Table 1. Life table of first generation *C. scripta* cohorts at the AMWPCF hybrid *Populus* plantation 16 km southeast of Ames, Iowa, June 1995

Life Stage (x)	No. alive at beginning of x (lx)	Factors responsible for dx (dxF)	No. dying during x (dx)	dx as a percent of lx (100qx)	Survival as a percent of lx (Sx)
Egg	62.9 ± 6.8 (n = 27)	Predation	25.6 ± 23.1	40.7	
		Unknown	4.2 ± 12.6	6.7	
		Total	29.8 ± 21.6	47.4	60.7
Early	38.2 ± 16.7 (n = 23)	Unknown	22.5 ± 16.1	58.9	49.7
Late	19.0 ± 18.8 (n = 22)	Tachinid parasitoid	0.8 ± 1.4	4.2	
		Unknown	7.6 ± 12.9	40.0	
		Total	8.4 ± 12.9	44.2	85.3

Table 1 continued

Pupa	16.2 ± 11.4 (n = 21)	Pteromalid parasitoid	1.4 ± 2.1	8.6	
		Unknown	2.9 ± 5.0	17.9	
		Total	4.3 ± 4.7	26.5	100.6
Adult	16.3 ± 15.1 (n = 18)				

n, number of cohorts used to calculate mean

Table 2. Life table of second generation *C. scripta* cohorts at the AMWPCF hybrid *Populus* plantation 16 km southeast of Ames, Iowa, July 1995

Life Stage (x)	No. alive at beginning of x (lx)	Factors responsible for dx (dxF)	No. dying during x (dx)	dx as a percent of lx (100qx)	Survival as a percent of lx (Sx)
Egg	62.6 ± 11.9 (n = 22)	Predation	45.7 ± 30.9	73.0	
		Unknown -high temp	7.3 ± 19.2	11.7	
		Total	53.0 ± 25.0	84.7	42.2
Early	26.4 ± 22.6 (n = 8)	Unknown - high temp - rainfall	21.9 ± 21.5	83.0	72.0
Late	19.0 ± 22.5 (n = 3)	Unknown - high temp - rainfall	19.0 ± 22.5	100	0
Pupa	0.0				

Table 2 continued

Adult	---

n, number of cohorts used to calculate mean

Table 3. Weather data collected during 1995 and 1996 life table studies at the AMWPCF southeast of Ames, Iowa

Date	T_{max} (°C)	T_{min} (°C)	Precipitation (cm)	hours of precipitation
Generation 1, 1995				
7 June	30.0	16.7	0.0	
Generation 2, 1995				
9 July	28.3	15.0	Trace	0.5
10 July	32.2	16.7	0.0	
11 July	32.8	17.7	0.0	
12 July	37.8	21.7	0.0	
13 July	36.1	22.2	0.0	
14 July	35.0	25.0	0.0	
15 July	30.6	21.1	0.28	5
16 July	27.8	20.6	3.10	
17 July	27.2	16.7	0.0	
18 July	28.3	15.6	0.0	
19 July	31.1	15.6	0.05	3.5
Generation 1, 1996				
12 June	31.1	15	0.20	
13 June	33.3	15.6	0.0	
14 June	32.8	16.1	0.0	
15 June	31.7	15.6	0.0	
16 June	28.3	16.7	0.89	7
17 June	24.4	20.6	1.73	8
18 June	21.7	16.7	0.08	4.5
19 June	28.3	16.7	Trace	

Table 3 continued

20 June	26.1	18.3	0.15	5.5
21 June	30.6	20	1.75	7.5
22 June	25	18.3	0.48	
23 June	26.1	17.7	0.08	3.5
24 June	26.7	17.7	0.84	
Generation 2, 1996				
17 July	32.2	14.4	5.0	10.5
18 July	33.3	20	1.62	
28 July	26.1	16.7	1.90	1.5
29 July	26.1	16.7	Trace	0.5
30 July	24.4	13.9	0.03	

Table 4. Life table of first generation *C. scripta* cohorts at the AMWPCF hybrid *Populus* plantation 16 km southeast of Ames, Iowa, June 1996

Life Stage (x)	No. alive at beginning of x (lx)	Factors responsible for dx (dxF)	No. dying during x (dx)	dx as a percent of lx (100qx)	Survival as a percent of lx (Sx)
a Caged cohorts					
Early	27.1 ± 20.4 (n = 11)	Abiotic - high temp	9.3 ± 16.9	34.3	66.4
Late	18.0 ± 10.5 (n = 11)	Abiotic - high temp - rainfall	8.6 ± 14.5	47.8	64.4
Pupa	11.6 ± 6.3 (n = 9)	Abiotic - high temp - rainfall	2.1 ± 3.4	18.1	91.4
Adult	10.6 ± 5.8 (n = 8)				

Table 4 continued

b Uncaged	**cohorts**				
Early	27.9 ± 16.8 (n = 14)	Unknown - high temp	14.1 ± 15.2	50.5	62.7
Late	17.5 ± 15.2 (n = 11)	Unknown - high temp - rainfall	16.0 ± 13.5	91.4	25.1
Pupa	4.4 ± 2.9 (n = 8)	Pteromalid parasitoid	0.1 ± 0.4	2.3	
		Unknown - high temp - rainfall	1.1 ± 1.1	25.0	
			1.3 ± 1.3	29.5	81.8
Adult	3.6 ± 2.4 (n = 7)				

n, number of cohorts used to calculate mean

Table 5. Life table of second generation *C. scripta* cohorts at the AMWPCF hybrid *Populus* plantation 16 km southeast of Ames, Iowa, July 1996

Life Stage (x)	No. alive at beginning of x (lx)	Factors responsible for dx (dxF)	No. dying during x (dx)	dx as a percent of lx (100qx)	Survival as a percent of lx (Sx)
a Caged cohorts					
Egg	61.9 ± 7.8 (n = 21)	Abiotic - high temp	12.7 ± 14.4	20.5	80.1
Early	49.6 ± 13.5 (n = 21)	Abiotic - high temp	7.5 ± 8.8	15.1	83.9
Late	41.6 ± 16.8 (n = 21)	Abiotic	12.0 ± 9.2	28.8	74.5
Pupa	31.0 ± 13.5 (n = 20)	Abiotic - high temp - rainfall	5.9 ± 6.8	19.0	81.0
Adult	25.1 ± 15.7 (n = 20)				

Table 5 continued

b Uncaged	**cohorts**				
Egg	56.9 ± 15.8 (n = 26)	Predation	32.6 ± 21.5	57.3	
		Unknown - high temp	1.1 ± 20.5	1.9	
			33.7 ± 20.5	59.2	53.1
Early	30.2 ± 14.6 (n = 20)	Unknown - high temp	20 ± 17.4	66.2	41.1
Late	12.4 ± 10.3 (n = 17)	Unknown - high temp	4.2 ± 7.1	33.9	102.4
Pupa	12.7 ± 8.8 (n = 16)	Pteromalid parasitoid	0.3 ± 0.6	2.4	
		Predation	0.1 ± 0.3	0.8	
		Unknown - high temp - rainfall	4.0 ± 5.6	31.5	
			4.4 ± 5.4	34.6	65.4

Table 5 continued

Adult	8.3 ± 7.5
	(n = 16)

n, number of cohorts used to calculate mean

Table 6. Life table for second generation of *C. scripta* at Boscobel, Wisconsin, 1977 (Burkot and Benjamin 1979)

Life Stage (x)	No. alive at beginning of x (lx)	Factors responsible for dx (dxF)	No. dying during x (dx)	dx as a percent of lx (100qx)	Survival as a percent of lx (Sx)
Egg	510	Predators	63	12.4	
		Infertility	31	6.1	
		Collapsed	12	2.3	
		Unknown mortality	175	34.4	
			281	55.2	45.0
Instars I & II	229	Unknown mortality	155	67.7	32.3
Instar III	74	Pentatomids	1	1.4	
		Unknown mortality	25	33.3	
			26	34.7	64.9
Pupae	48	Parasites	2	3.2	
		Predators	1	2.1	

Table 6 continued

		Died while emerging	2	4.3
		Failed to emerge	6	11.7
			11	21.3	77.1
Adults	37			

Fig. 1. Mortality of cohorts from a life table study of the first generation of *C. scripta*, 1995. % mortality is calculated as a percentage of organisms entering each life stage.

Fig. 2. Mortality of cohorts from a life table study of the second generation of *C. scripta*, 1995. % mortality is calculated as a percentage of organisms entering each life stage.

Fig. 3. Mortality of the caged and the uncaged cohorts from a life table study of the first generation of *C. scripta*, 1996. % mortality is calculated as a percentage of organisms entering each life stage.

Fig. 3. Mortality of the caged and the uncaged cohorts from a life table study of the second generation of *C. scripta*, 1996. % mortality is calculated as a percentage of organisms entering each life stage.

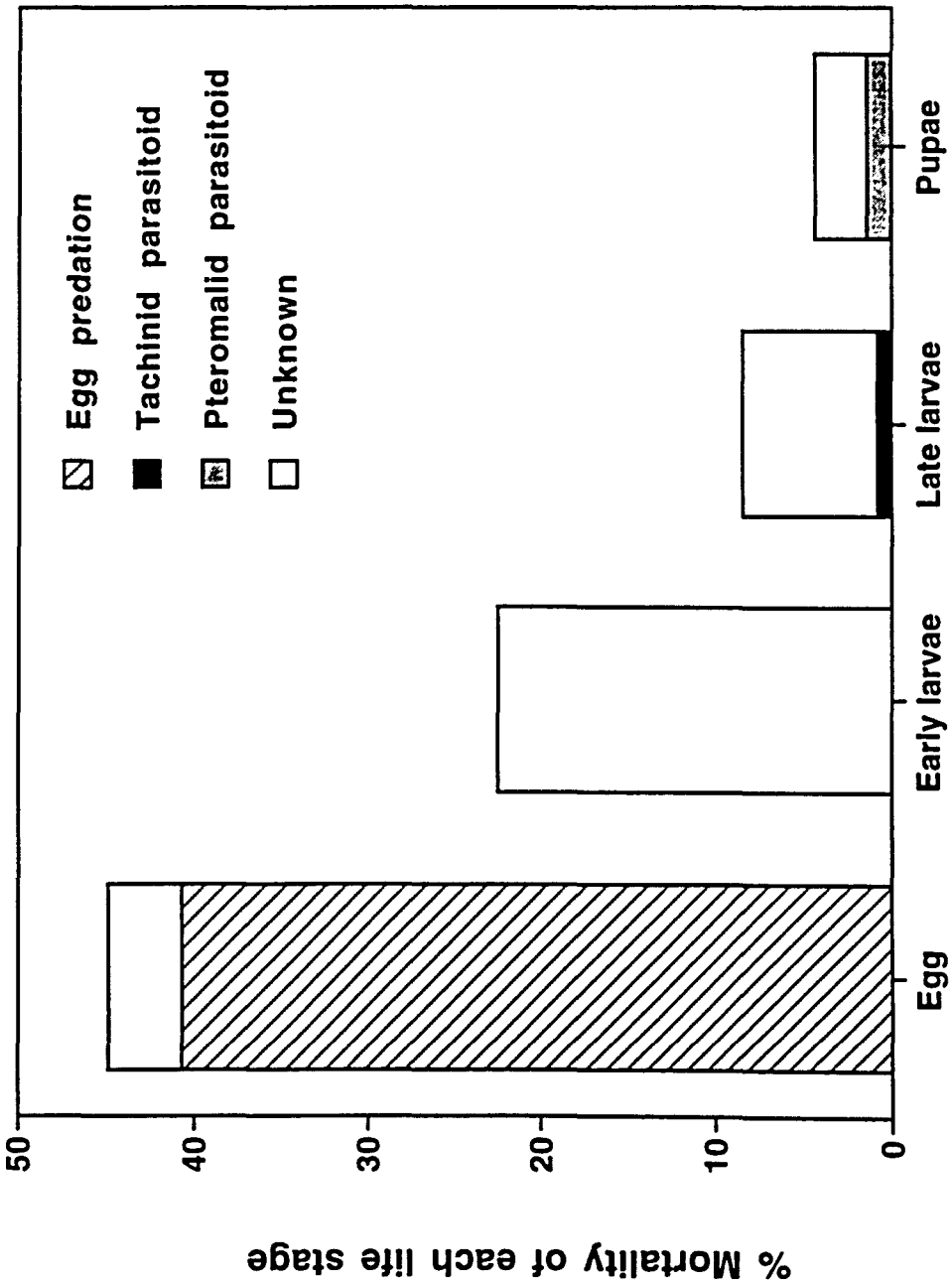
% Mortality of each life stage
50
40
30
20
10
0
Egg predation
Tachinid parasitoid
Pteromalid parasitoid
Unknown
Egg
Early larvae
Late larvae
Pupae
Life stage

Fig. 1.

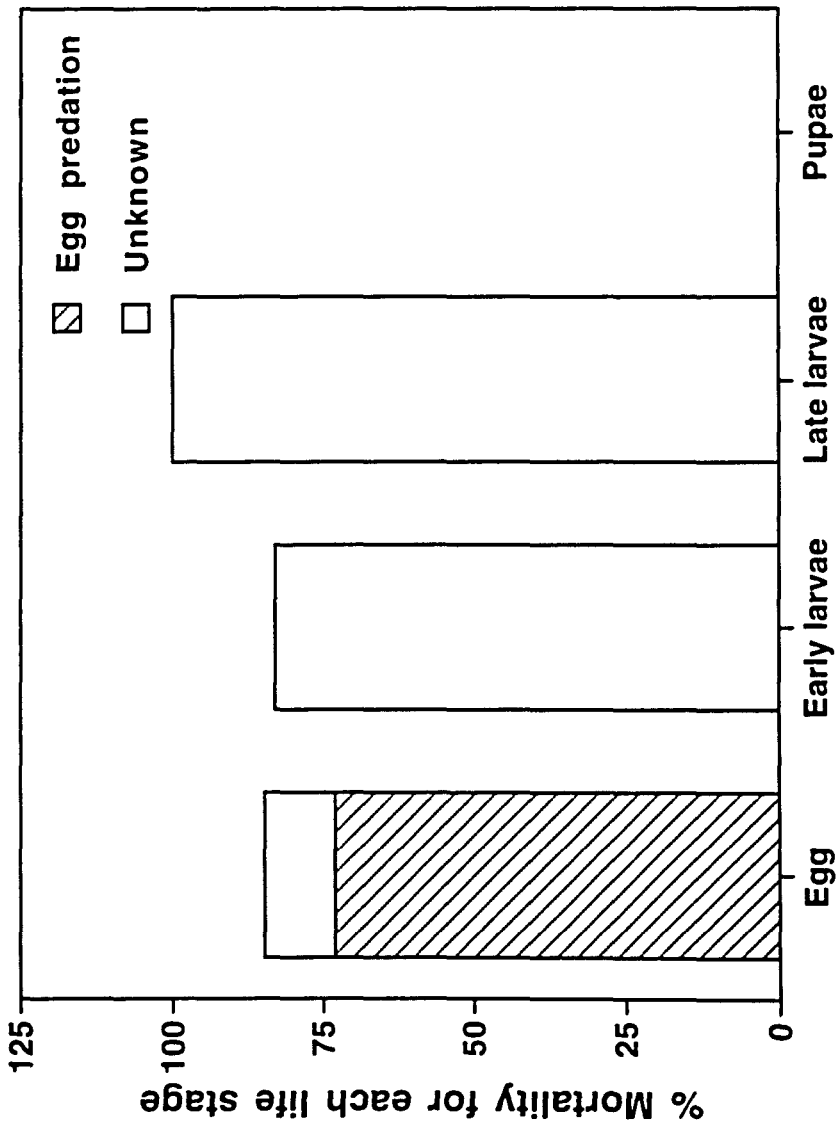
Egg predation
Unknown
% Mortality for each life stage
125
100
75
50
25
0
Egg
Early larvae
Late larvae
Pupae
Life stage

Fig. 2

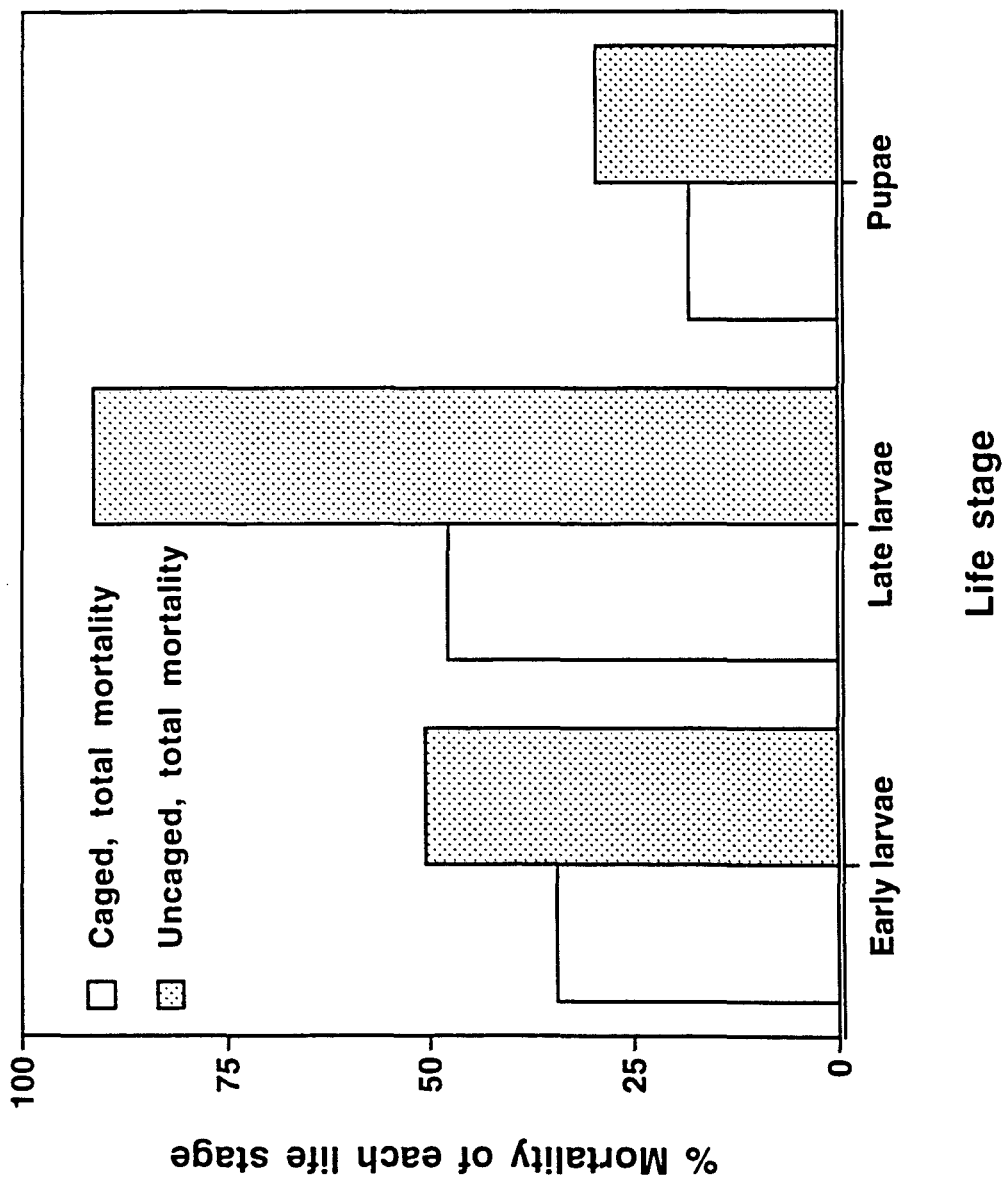
% Mortality of each life stage
100
75
50
25
0
Caged, total mortality
Uncaged, total mortality
Early larvae
Late larvae
Pupae
Life stage

Fig. 3.

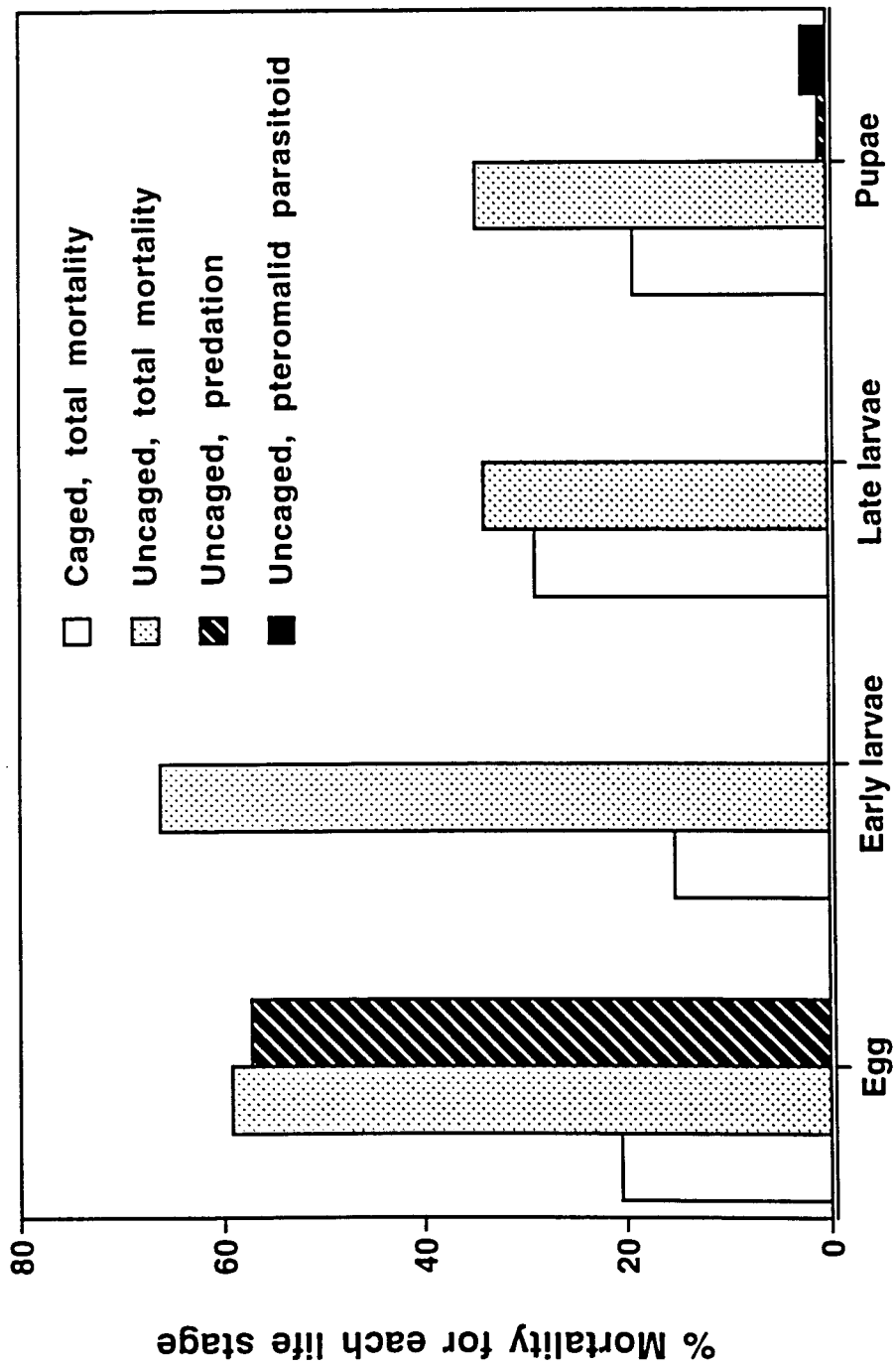
Caged, total mortality
Uncaged, total mortality
Uncaged, predation
Uncaged, pteromalid parasitoid
% Mortality for each life stage
0
20
40
60
80
Egg
Early larvae
Late larvae
Pupae
Life stage

Fig. 4.

CHAPTER FOUR. TEMPERATURE DEPENDENT DEVELOPMENT OF A *CHRYSOMELA SCRIPTA* (COLEOPTERA: CHRYSOMELIDAE) POPULATION IN CENTRAL IOWA

A paper to be submitted to Environmental Entomology

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Abstract

A laboratory study of the temperature dependent development of the cottonwood leaf beetle, *Chrysomela scripta* F. (Coleoptera: Chrysomelidae) at 14, 18, 22, 26, and 30°C was conducted in central Iowa. Thermal requirements for development were calculated: 62.4 DD above a minimal temperature threshold of 8.5°C for eggs, 39.8 DD above 6.0°C for instar I, 40.2 DD above 9.2°C for instar II, 95.9 DD above 7.3°C for instar III, 46.4 DD above 10.5°C for pupae, and 173.8 DD above 7.9°C for larval development (instars I to III). Total preimaginal development requires 282 ± 16.9 degree-days and a minimal temperature threshold of 8.6 ± 1.6°C. The pre-oviposition period required 95.4 DD above 11.1°C. Survival ranged from 76 to 89.3% for temperatures between 22 and 30°C, but tended to be lower at 14°C (48.0 ± 33.9%). Development was fastest at 30°C (13.7 ± 0.6 d). Development times predicted from the degree-day model were within 2 calendar day of development observed in the field for the first and second generations in 1995 and 1996.

Key words: *Chrysomela scripta*, temperature dependent development, degree-day model

Introduction

Temperature has an important influence on the rate of development and metamorphosis of poikilotherms (Laudien, H. 1973, Rockstein 1964, Tauber and Tauber 1987, Wigglesworth 1972). In multivoltine species, developmental response to temperature affects the rate of increase and the number of generations per year. This becomes especially important when managing insect pest species. Effective chemical, biological, and cultural control of many crop pests is dependent upon accurate timing of control measures relative to pest life stages.

Temperature-dependent growth models have been developed for many major crop pests. Such models are developed from laboratory studies of development at a series of constant (and sometimes variable) temperatures. From these data, the developmental threshold, *t*, of a species, and the degree-days required for development, *K*, can be calculated. These are then used to predict developmental rate in the field from daily temperature measurements using the thermal unit, or degree-day approach. The calculations and assumptions behind the degree-day approach were reviewed by Higley et al. (1986). Degree-day models can be used to predict the occurrence of generations or of specific life stages in the field.

One of the most important defoliating pests of *Populus* in the north central United States is the cottonwood leaf beetle, *Chrysomela scripta* Fabricius (Coleoptera: Chrysomelidae) (Burkot and Benjamin 1979, Harrell et al. 1982). It feeds on the leaves of poplar trees throughout the United States and Canada (Burkot and Benjamin 1979, Drooz 1985). It has three to five generations each season in the north-central region and up to seven in the southern states (Burkot and Benjamin 1979, Caldbeck et al. 1978).

Although several studies have examined development times of *C. scripta* under field and laboratory conditions, there is no generally accepted temperature dependent growth model for *C. scripta* (Burkot & Benjamin 1979, Head & Neel 1973). Burkot and Benjamin (1979) carried out a laboratory study that determined

the effects of temperature on developmental times and survival at six temperatures. However, this study gave very little detail on rearing techniques or statistical design. Thus, interpretation and application of their results is difficult. In addition, the length of individual stages was not calculated, making it difficult to predict the occurrence of specific life stages for a given generation. To develop a detailed thermal unit model that will facilitate timing of chemical and biological pesticide applications, scouting for damage and population estimates, and other research/management activities, a study of the effects of temperature on *C. scripta* is needed. The objectives of this study were to (1) determine development rates and thresholds of each life stage of *C. scripta* under constant temperatures, and (2) develop a predictive degree-day model of *C. scripta* development.

Materials and Methods

Experimental Procedures

Five growth chambers were calibrated, and each was set at constant temperature (14, 18, 22, 26, and 30 ± 2 °C) and 16:8 (L:D). A container of water was placed in each growth chamber to maintain humidity above 50%. Temperatures were assigned randomly among the cabinets. The study was replicated three times. Replicate 1 began 1 February and ended on 31 March 1996, replicate 2 ran from 13 July to 24 October 1996, and replicate 3 ran from 6 October to 21 December 1996. Thermometers measuring maximum and minimum temperatures were placed in each cabinet and checked daily during the third replicate.

Chrysomela scripta used in the study were from laboratory-reared colonies established from field collected adults, larvae, and/or egg masses from central Iowa. The colonies were maintained at 22 °C and 16:8 (L:D). Mating pairs used for replicate 1 were approximately five generations from the field. For replicate 2, mating pairs were taken from the field. Mating pairs used for replicate 3 were raised from larvae and eggs collected in the field.

Beetles were fed freshly cut apical tips of greenhouse grown 'Eugenei' (*Populus deltoides* x *Populus nigra*). *Chrysomela scripta* adults display preferential selection among *Populus* clones, and among leaf age classes (Caldbeck et al. 1978). The leaf plastochron index (LPI), which is used to distinguish leaf age classes in *Populus* (LPI estimates physiological and morphological development of young leaves), was used to select food material (Erickson & Michelini 1957, Larson & Isebrands 1971). Bingaman and Hart (1992) have shown that the most-preferred leaf age class for *C. scripta* feeding was LPI 3, although all leaves younger than LPI 7 are acceptable. This study used leaves of LPI 0 through 5. When food material was collected, the apical tip of a tree was cut at least 4 inches below LPI 5 using pruning sheers. Apical tips were transported to the laboratory in a cooler. Within 30 minutes, stems were trimmed with a razor blade and placed directly into an 'Aquapic' filled with warm tap water.

For each replicate, ten mating pairs of *C. scripta* were isolated from the laboratory-reared colony and maintained at 22°C. Each pair was placed in a 0.24 liter cardboard cage (Neptune Paper Products, New Jersey) with an organdy cover and a plastic lid resting over the top to prevent desiccation. Cages were checked daily for oviposition. Fresh leaves and damp paper toweling were provided daily.

Within 24 h of oviposition, egg masses were placed on damp toweling in small (6.5 x 9.5 x 2.0 cm) plastic boxes (Tristate Plastics, Dixon, KY) and moved to a temperature treatment. An attempt was made to have one egg mass per female per temperature; however, not all females laid five egg masses during the course of the experiment. Egg masses were monitored daily for hatching. Within 24 h of hatching, a small paint brush was used to transfer five first instars from each egg mass to new food material in a snap-box cage (13.0 x 18.0 x 3.5 cm plastic box with a circular hole cut in one end). An 'Aquapic' containing water and food material was inserted through the hole and cages were set on end so that the apical tip was held upright. Five cages were prepared for each temperature treatment. Thus, there were 25 *C. scripta* larvae per temperature for each replication.

Larvae were checked daily for molting and mortality. Foliage was changed before desiccation, wilting, yellowing, or depletion of food material occurred. The interval at which foliage was changed varied with temperature: every 3 d at 26 and 30 °C, 3 to 5 d at 18 and 22 °C, and every 5 d at 14 °C.

Newly emerged adults were weighed 24 h after eclosion. To determine the pre-oviposition period, new adults were maintained in the snap-box cages until at least one egg mass was deposited. There were 1 - 4 females in each cage.

Statistical Analysis

Survival data was analyzed using analysis of variance (SAS 1987). Developmental data were analyzed using a SAS program written by B. Gollands (Cornell University) and followed the procedures used by Gabriel and Obrycki (1990). The data analysis only included individuals that completed development. A linear regression was performed on the mean developmental rate (1/d) using general linear models (SAS 1987) for all temperatures for egg, instars I, II , and III (including pre-pupa), and pupa, as well as total larva (instar I to III), total preimaginal development (egg to adult), and pre-oviposition period. Temperature-developmental rate equations were calculated for each stage. The lower thermal threshold, *t*, for each stage was calculated by extrapolating the regression line through the x-axis (temperature). The thermal constant, *K* (degree-day requirement for development), was calculated as 1/slope of the temperature-developmental rate equation. Standard errors for *t* and *K* were calculated as in Gabriel and Obrycki (1990). The equation for the standard error of *t* was

$$SE_t = \frac{\sqrt{MS\ LACK\ FIT}}{b} \times \sqrt{1 + \frac{1}{N} + \frac{\left(\overline{Y}/b\right)^2}{SSX}}$$

where MS Lack Fit is the mean square error for the lack of fit of the temperature-development equation, *b* = slope of the temperature-development equation, N =

total number of observations for all treatments, $\bar{Y}$ = mean developmental rate for all observations, and SSX = sum of squares for treatments. The standard error of K was calculated as

$$SE_K = \frac{\sqrt{\frac{MS\ LACK\ FIT}{SSX}}}{b^2}$$

Model Validation

This study was validated using data collected in a life table study conducted in a *Populus* plantation 16 km south-east of Ames, IA at the Ames Municipal Water Pollution Control Facility in 1995 and 1996 (Chapter Three). Degree-days (DD) were accumulated using the equation $DD = [(m_1 + m_2)/2] - t$. Where DD = degree-days for a given 24 h period, m_1 = the maximum temperature during the period, m_2 = the minimum temperature during the period (if $m_2 < t$, set equal to t), and t = the minimum threshold temperature for the stage (Pedigo & Zeiss 1996). Degree-day accumulations were based on daily minimum and maximum temperatures recorded at the Ames Municipal Water Pollution Control Facility, 16 km southeast of Ames, Iowa in Story county.

Results

In replicate three, the total preimaginal development at 14 °C was >10 d shorter. Equipment malfunction was suspected, so the third replicate at 14 °C was excluded from all analyses. As temperature increased, developmental time for all stages decreased (Table 1). The range of developmental time was: from 13.5 d at 14°C to 3.0 d at 30 °C for eggs, 4.6 d at 14 °C to 1.7 d at 30 °C for instar I, 9.6 d at 14 °C to 2.1 d at 30 °C for instar II, 15.9 d at 14 °C to 4.6 d at 30 °C for instar III (including pre-pupa), and 10.4 d at 14 °C to 2.4 d at 30 °C for pupa. Total development (egg to adult) ranged from 53.8 d at 14 °C to 13.7 d at 30 °C.

There was no difference in total survival among the temperatures tested

($F = 2.77$; d.f. = 4; $p = 0.0942$). Survival tended to be lower at 14 °C (48%). The range of survival for each stage was 64 - 97.3% for instar I, 81.3 - 97.1% for instar II, 93.3 - 98.6% for instar III (including pre-pupa), and 97.4 - 100% for pupa (Table 2).

A total of 282 ± 16.9 degree-days above *t* (8.6 °C) is required for total preimaginal development of *C. scripta* (Table 3, Fig. 1). Degree-day requirements above *t* for each life stage are 62.4 ± 4.0 DD above 8.5 °C for eggs, 39.8 ± 3.6 DD above 6.0 °C for instar I, 40.2 ± 3.8 DD above 9.2 °C for instar II, 95.9 ± 13.2 DD above 7.3°C for instar III, and 46.4 ± 2.8 DD above 10.5 °C for pupae. The *t* value for pupae was higher than for any other life stage.

Degree-day model predictions from the laboratory study were compared with observed development data from life table studies performed in the field on the first and second generations in 1995 and 1996 (Chapter Three). The degree-day model predicts a minimum of 282 DD above 8.6 °C for complete preimaginal (egg to adult) development. For first generation 1995, *C. scripta* adults were first observed 317.7 DD after egg masses were selected (Table 4). No *C. scripta* survived to the adult stage in second generation 1995, so no comparison was possible. For the first generation of 1996, first instars were placed in the field for the life table study. Because of this, degree-day requirements for development were estimated by the requirements for larval development (instar I to pre-pupa) and pupae. This estimate predicted complete development (from instar I to adult emergence) in 220 DD above 8.6 °C (Table 4). Complete development in the field was observed 236.3 DD after larvae were placed in the field. Second generation 1996 development (egg to adult emergence) in the field was observed 276.1 DD after egg masses were selected (Table 4).

Discussion

Burkot and Benjamin (1979) determined the optimal rearing temperature for *C. scripta* in Wisconsin to be 23 °C. This was the temperature at which they observed the highest survival. They calculated a minimal developmental threshold

of 10.8 °C and a total of 257 ± 26 degree-days required for development from egg to adult. Both of these values are similar to our *t* and *K* values. The egg to adult developmental times at temperatures above 21 °C from the Wisconsin study were comparable with those found in the current study; 19.8 d at 21 °C and 18.1 d at 23 °C versus 20.9 d at 22°C, 14.2 d at 27 °C versus 15.4 d at 26 °C, and 13.8 d at 28 °C versus 13.7 d at 30°C. However, there was an 11 d difference between the Wisconsin egg to adult developmental time at 17 °C (42.5 d) compared to our time at 18 °C (31.1 d).

Field data from the life table study compared well with development times predicted by the degree-day model. Predictions were within 2 calendar days of observed developmental time for first generation 1995 and within 1 calendar day for first and second generation 1996 (Table 4). Degree-day model predictions from the Burkot and Benjamin study were also compared with data from the life table studies (Table 5). Their model predicts a minimum of 257 DD for complete development (egg to adult). For the first generation of 1995, *C. scripta* adults were first observed 260.2 DD after egg masses were selected. This was the same as the predicted degree-days and occurred on the same calendar day. For the first generation of 1996, adults were first observed 229.9 DD after egg masses were selected. This was 4 calendar days before the date predicted by the Wisconsin model. Both the Wisconsin and Iowa models can be used to predict the occurrence of adult emergence in field populations within 1 - 4 calendar days. The average temperature during June and July was similar in 1995 (21.3 °C and 23.1 °C) and 1996 (20.7 °C and 21.4 °C) (Iowa Climatological Data 1995, 1996).

The degree-day model presented here, combined with sampling of *C. scripta* populations within *Populus* plantations will provide critical information on the occurrence of life stages important to management strategies such as application of chemical and biological pesticides, damage scoring, population estimates, and other research/management activities. Some of these actions, such as use of *Bacillus thuringiensis* var. san diego, require stage-specific timing to be effective. Further study of the impact of fluctuating temperatures, or temperatures

higher than 30 °C may be necessary to achieve a model to predict occurrence of immature stages of *C. scripta*.

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Table 1. Preimaginal development time and pre-oviposition period (d; $\bar{x}$ ± SD) of *C. scripta* from central Iowa under constant temperatures (±2 °C; 16:8 [L:D])

	Temperature (°C)				
Stage	14	18	22	26	30
Egg	13.5 ± 3.6	6.5 ± 0.4	4.5 ± 0.5	3.5 ± 0.6	3.0 ± 0.1
Instar I	4.6 ± 1.8	3.8 ± 0.7	2.5 ± 0.5	2.1 ± 0.3	1.7 ± 0.2
Instar II	9.6 ± 0.8	5.0 ± 1.0	3.0 ± 0.5	2.2 ± 0.1	2.1 ± 0.3
Instar III	15.9 ± 0.2	9.5 ± 0.8	6.4 ± 0.8	4.6 ± 0.2	4.6 ± 0.2
(including pre-pupa)					
Pupa	10.4 ± 0.2	6.4 ± 0.1	4.4 ± 0.5	3.1 ± 0.3	2.4 ± 0.5
Total larva	26.1 ± 2.0	15.7 ± 0.4	10.3 ± 0.8	7.7 ± 0.4	7.0 ± 0.3
Total development	53.8 ± 0.6	31.1 ± 0.3	20.9 ± 1.5	15.4 ± 0.6	13.7 ± 0.6
Pre-oviposition	31.8 ± 0.0	13.7 ± 1.3	9.3 ± 0.8	6.1 ± 0.2	5.2 ± 0.5

Table 2. Stage specific survival (mean ± SD) of *C. scripta* at constant temperatures (16:8 photoperiod) from three replicates.

	Temperature (°C)				
Stage	14	18	22	26	30
Instar I	64.0 ± 45.3	94.7 ± 6.1	97.3 ± 4.6	92.0 ± 9.6	96.0 ± 6.9
	(8, 24)[1]	(22, 24, 25)[2]	(23, 25, 25)	(21, 24, 24)	(22, 25, 25)
Instar II	87.5 ± 0.0	97.1 ± 2.5	85.2 ± 9.9	95.2 ± 8.3	81.3 ± 8.3
	(7, 21)	(21, 23, 25)	(22, 19, 21)	(18, 24, 24)	(21, 22, 18)
Instar III	95.3 ± 6.7	98.4 ± 2.8	93.3 ± 5.8	98.6 ± 2.4	98.4 ± 2.8
(including pre-pupa)	(6, 19)	(20, 23, 25)	(22, 17, 19)	(18, 24, 23)	(20, 22, 18)
Pupa	97.4 ± 3.7	100.0 ± 0.0	98.0 ± 3.4	100.0 ± 0.0	98.5 ± 2.6
	(6, 18)	(20, 23, 25)	(22, 16, 19)	(18, 24, 23)	(20, 21, 18)
Total development	48.0 ± 33.9	89.3 ± 12.2	76.0 ± 12.0	86.7 ± 12.9	78.7 ± 6.1
	(6, 18)	(20, 23, 25)	(22, 16, 19)	(18, 24, 23)	(20, 21, 18)

[1] n for replicates 1 and 2

[2] n for replicates 1, 2, and 3

Table 3. Thermal requirements for development of *C. scripta* in central Iowa

Stage	t (°C)	K (DD)
Egg	8.5 ± 1.5	62.4 ± 4.0
Instar I	6.0 ± 2.1	39.8 ± 3.6
Instar II	9.2 ± 2.3	40.2 ± 3.8
Instar III (including Pre-pupa)	7.3 ± 3.3	95.9 ± 13.2
Pupa	10.5 ± 1.5	46.4 ± 2.8
Total Larva (Instar I - III)	7.9 ± 2.3	173.8 ± 16.3
Total development (Egg - adult)	8.6 ± 1.6	282.0 ± 16.9
Pre-oviposition	11.1 ± 0.6	95.4 ± 4.2

Table 4. Comparison of *C. scripta* development times predicted by a laboratory based degree-day model and those observed in life table studies performed in the field in 1995 and 1996

Generation	Dates	DD Accumulated until stage observed	DD Predicted	Difference in DD
Generation 1, 1995	22 May - 20 June (first egg mass to first adult appearance)	317.7	282 (egg - adult)	+ 32.3 DD, + 2 calendar day
Generation 2, 1995	no survival to adult			
Generation 1, 1996	6 June - 24 June (early larvae to first adult appearance)	236.3	220.2 (early - adult[1])	+ 16.1 DD, + 1 calendar day
Generation 2, 1996	8 July - 29 July (first egg mass to first adult appearance)	276.1	282 (egg - adult)	- 10.6 DD, - 1 calendar day

[1] estimated by adding DD required for total larva and pupa

Table 5. Comparison of *C. scripta* development times predicted from a model developed by Burkot and Benjamin (1979) and observed in life table studies performed in the field in 1995 and 1996

Generation	Dates	DD Accumulated until stage observed	DD Predicted	Difference in DD
Generation 1, 1995	22 May - 20 June (first egg mass to first adult appearance)	260.2	257 (egg - adult)	+ 0 DD, + 0 calendar day
Generation 2, 1995	no survival to adult			
Generation 1, 1996	No comparison			
Generation 2, 1996	8 July - 29 July (first egg mass to first adult appearance)	229.9	257 (egg - adult)	- 35.1 DD, - 4 calendar day

[1] estimated by adding DD required for total larva and pupa

Fig. 1. Meanrate (1/days) of total preimaginal development of *C. scripta* determined from a laboratory study conducted under constant temperatures (14, 18, 22, 26, and 30 ± 2 °C; 16:8 [L:D]) in central Iowa. The equation of the line is y = 0.003x - 0.028, where x is temperature and y is the meanrate (1/d) of development.

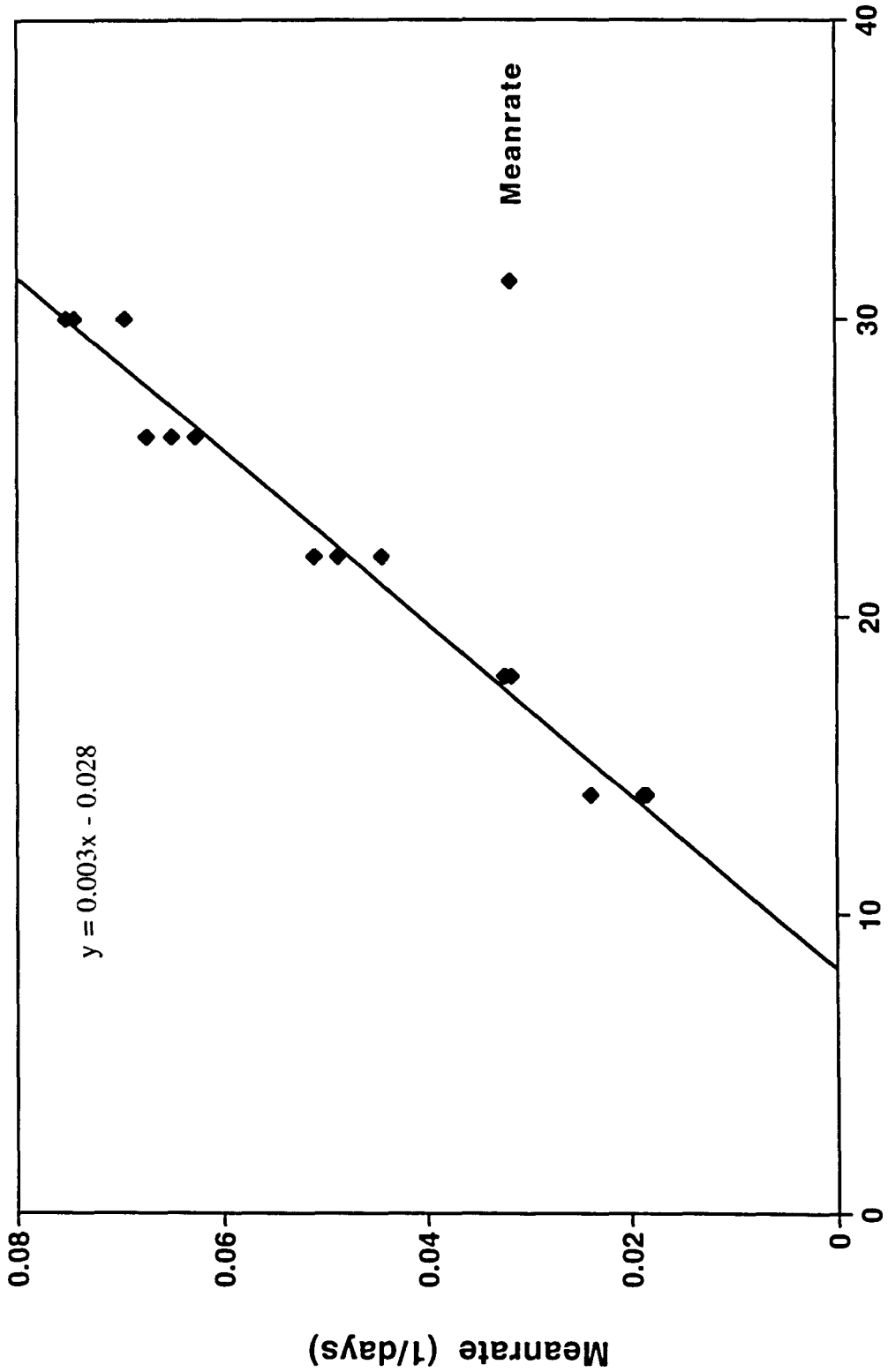
Meanrate (1/days)
0.08
0.06
0.04
0.02
0
0
10
20
30
40
Temperature (°C)
Meanrate
y = 0.003x - 0.028

Fig. 1

CHAPTER FIVE: GENERAL SUMMARY

The three studies discussed in this thesis were conducted in order to gain a better understanding of the biology and ecology of the cottonwood leaf beetle, *Chrysomela scripta* F. (Coleoptera: Chrysomelidae) in central Iowa. This work is important because of the growing interest in the use of *Populus* hybrids, in short rotation forest plantations for production of biomass as an alternative fuel source. As more acres are put into production, problems with pests, such as *C. scripta*, will increase. The application of insecticides may be impractical within the economics of the alternative fuels industry. Also, many plantations will be placed on marginal agricultural land where the environmental costs of insecticide applications are unacceptable.

The first study was a survey of the natural enemy complex of *C. scripta* in selected plantation and natural sites. The objectives were to (1) identify what natural enemies are present, and when they occur, (2) determine whether different families/species of natural enemies occur in natural versus plantation sites, and (3) determine if densities of *C. scripta* and natural enemies differed within the plantation.

Absolute sampling was conducted weekly in 1995 and 1996, and visual observations were taken in conjunction with absolute sampling in 1996. Natural enemies found in plantation sites included *Coleomegilla maculata* DeGeer, *Hippodamia convergens* Guerin, *H. tredecimpunctata* L, and *Neoharmonia venusta* Melsheimer (Coleoptera: Coccinellidae), *Podisus maculiventris* Say (Heteroptera: Pentatomidae), Chrysopidae (Neuroptera), Cantharidae, Formicidae, Lampyridae, Nabidae, Araneae and Opiliones. Natural enemies found in natural sites included *C. maculata*, *P. maculiventris*, Cantharidae, Formicidae, Lampyridae, Reduviidae, Araneae, and Opiliones. Yellow sticky traps were also used in both 1995 and 1996. These traps captured *Coccinella septumpunctata*, *Adalia bipunctata*, *H.*

parenthesis, *Cycloneda munda*, *Harmonia axyridis*, and three undetermined, predatory species (Coleoptera: Chrysomelidae), Carabidae, and Neuroptera.

Representatives of many of the same families of natural enemies were found in both plantation and natural sites. This was surprising, since monocultural agriculture environments generally tend to have a depauperate fauna. However, weeds are allowed to grow in the plantation used in this study, and trees are interspersed with corn, soybeans, and switch grass. This diversity in the environment may provide resources for natural enemies normally lacking in a monocultural setting. Thus, the natural enemy complex in the plantation more resembles that in natural sites.

There was no evidence that *C. scripta* or natural enemy densities differed as to location within the plantation. Sticky traps captured *C. scripta* and *C. maculata* throughout the plantation on the first sampling date in 1996. This may indicate that *C. scripta* overwinter within the plantation, and not along a wooded streambed west of the plantation, as was previously hypothesized.

The second study used partial life table analysis of *C. scripta* from the first and second generations in 1995 and 1996. The objectives of this study were to (1) identify causes of mortality affecting each stage of two generations of *C. scripta* in central Iowa, (2) quantify the impact of these mortality factors on *C. scripta*, and (3) compare *C. scripta* mortality in the presence and absence of natural enemies.

Predation by Coccinellidae was the most important mortality factor for *C. scripta* eggs in the first and second generations in both years. Early and late larvae of the second generation 1995 experienced high mortality (83 and 100%, respectively). This may have been caused by a combination of high temperatures and rain. An unidentified Tachinidae parasitized 4.2% of the late stage larvae in the first generation 1995, and *Schizonatus latus* Walker (Hymenoptera: Pteromalidae) parasitized 8.6% of the pupae in the first generation 1995 and 2.3 and 2.4% of the pupae in the second and third generations, 1996. Comparative life tables from the first and second generations in 1996 show that *C. scripta* mortality was higher in the presence of natural enemies (i.e., uncaged cohorts).

The third study examined the effect of temperature on preimaginal development of *C. scripta*. The objectives of the study were to (1) determine development rates and thresholds of each life stage of *C. scripta* under constant temperatures, and (2) develop a predictive degree-day model of *C. scripta* development.

The degree-day requirement for development (K), and temperature threshold of development (t) values were calculated for each life stage: eggs required 62.4 ± 4.0 degree-days (DD) above 8.5 °C, instar I requires 39.8 ± 3.6 DD above 6.0 °C, instar I needs 40.2 ± 3.8 DD above 9.2°C, instar III (including the pre-pupal stage) needs 95.9 ± 13.2 DD above 7.3 °C, and pupae require 46.4 ± 2.8 DD above 10.5 °C. A total of 173.8 DD above 7.9 °C are required for larval development (instar I to pre-pupa), and 282 ± 16.9 degree-days above 8.6°C are required for total preimaginal development (egg to pupa). The pre-oviposition period for *C. scripta* lasts 95.4 DD above 11.1 °C.

These degree-day predictions were compared with field data from the life table studies of the first generation in 1995 and the first and second generations in 1996. Degree-day predictions for total preimaginal development were within 2 calendar d of observed development.

The degree-day model developed will be useful for the timing of management practices and research. Further laboratory and field based life table and feeding studies are needed to evaluate the potential of *P. maculiventris*, the Neuroptera, and Araneae as naturally occurring biological control agents for *C. scripta*.

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