BIOLOGY AND PEST STATUS OF LESSER APPLEWORM *GRAPHOLITA PRUNIVORA* (WALSH) (LEPIDOPTERA : TORTRICIDAE) IN MICHIGAN

By

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ABSTRACT

BIOLOGY AND PEST STATUS OF LESSER APPLEWORM *GRAPHOLITA PRUNIVORA* (WALSH) (LEPIDOPTERA : TORTRICIDAE) IN MICHIGAN

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Lesser appleworm *Grapholita prunivora* (Walsh) (Lepidoptera : Tortricidae) larvae are internal fruit feeders. As a polyphagous species it feeds on numerous plant hosts, including most cultivated pome and stone fruits that are grown in northern United States.

Studies about biology, seasonality, flight bionomics, and pest status of *G. prunivora* were conducted in commercial and abandoned apple orchards located in four different fruit growing regions of west Michigan during 1991 - 1994. Larval and pupal stages characters and fruit injury characteristic were compared to three other closely related fruit feeders: codling moth *Cydia pomonella* (L), oriental fruit moth *G. molesta* (Busck), and cherry fruitworm *G. packardi* Zeller. Insects collected from injured apple fruits were used for laboratory observations.

In Michigan weather conditions, lesser appleworm typically has two generation per year, with the first generation adult flight starting in the middle of May and the second generation being present in orchards until late September. All major insect life events were described using degree day accumulations at bases of 42° F and 50° F.

Six pheromone traps designs were compared for effectiveness in insect monitoring. The Pherocon II trap design captured the highest number of lesser appleworm males. These traps captured also four other moth species.

Lesser appleworm larvae were never found in fruits collected in commercially managed orchards, even when adults moths were present in the pheromone traps in the orchards during the season. In abandoned orchards lesser appleworm injured up to 7 percent of fruits. Eighty nine percent of samples collected during the season had infestations lower than 3 percent. An oviposition preference study showed that lesser appleworm females will accept cultivated plant hosts as well as wild ones.

A comparative study of pupal length and width of mesowing and anal segment allowed for reliable pupa identification. Differences in the number of crochets on larval prolegs and the structure of anal comb allowed for separation of lesser appleworm larvae from those of oriental fruit moth, but was not reliable for separation of lesser appleworm and cherry fruitworm larvae.

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Introduction to lesser appleworm *Grapholita prunivora* (Walsh) (Lepidoptera : Tortricidae) in North America

Introduction

The lesser appleworm (LAW) *Grapholita prunivora* (Walsh) (Lepidoptera: Tortricidae) is a native North American insect. It is also a quarantined pest in Europe and Japan, and is one of the major barriers in exporting U. S. apples to Japan. Although the pest status of lesser appleworm is historically documented (Glass & Lienk 1971, Rivard & Mailloux 1974, Brown 1953), in Michigan the lesser appleworm is not presently considered as a major fruit pest (Howitt 1993). Growers who make regular insecticide applications against major fruit pests usually do not see fruit damage caused by this pest.

From the late 1800's and early 20 th century lesser appleworm was reported from different fruit growing areas as a possible pest in fruit orchards (Lugger 1898, Quaintance 1908). Early researchers compared the biology of lesser appleworm to the biology of codling moth *Cydia pomonella* (L.) (Taylor 1909). The first picture of lesser appleworm fruit injury came from a publication about codling moth and was labeled as "apples damaged by unknown caterpillar" (Simpson 1903). The same picture was later used as classical view of lesser appleworm fruit injury (Caesar 1911, Quaintance & Scott 1912). A historical Michigan orchard spray bulletin states that " lesser apple-worm, when present, requires a spray of poison" (Eustace & Pettit 1910).

After the first description of lesser appleworm (Walsh 1868) the insect gradually gained more and more attention from people working with fruit pests. Riley (1873) in Missouri, Fletcher (1898) in British Columbia, Lugger (1898) in Minnesota, and Webster & Newell (1902) in Ohio reported lesser appleworm in their reports and publications related to insects of economic importance. With the exception of research data presented by Quaintance (1908), Foster & Jones (1909), Taylor (1909), Garman (1918), and Frost (1926) most of the information about presence and importance of the lesser appleworm comes from spray calendars or reports about insects affecting orchards (Sanderson et al. 1907, Eustace & Pettit 1910, Quaintance & Scott 1912, Dean & Peairs 1913, Quaintance & Siegler 1918, Fulton 1920, Pettit & Hutson 1931).

This early interest in the importance of lesser appleworm almost disappeared from research in the 1940's, with scattered reports occurring only in extension bulletins with lesser appleworm as a pest of apples or plums (Frost 1942, Newcomer 1941). Researchers renewed research efforts on lesser appleworm in the 1950's. Brown & Jones (1953) reported the lesser appleworm as an important pest for cherries, citing four years of observations of lesser appleworm biology as a pest on this fruit in Oregon.

The last period of research interest on this pest started in the late 1960's, when Roelofs et al. (1969) discovered a sex pheromone of lesser appleworm. Trapping technique studies followed this discovery (Gentry at al. 1974, Gentry at al. 1975, Willson & Trammel 1975, Willson & Trammel 1980). Observations about the lesser appleworm's importance as a pest on apples during ten years of studies was done in orchards where insecticide use was discontinued (Glass & Lienk 1971). They found that lesser appleworm caused 72 percent and

39 percent fruit damage respectively during the last two years of the experiment. Weires et al. (1979) worked in apple orchards with reduced insecticide program in Hudson Valleys, NY, and found lesser appleworm responsible for approximately 50 % of damage done by all internal lepidopterous feeders .

Scientific and common names of *G. prunivora.*

Mr. Benjamin D. Walsh (1868) in his first description of lesser appleworm placed it first in the genus *Semasia.* Other names that exist in the literature are: *Enarmonia prunivora* (Walsh), *Laspeyresia prunivora* (Walsh), *Cydia prunivora* (Walsh), and *Grapholitha prunivora* (Walsh). The Entomological Society of America's current official name (Stoetzel 1989) is *Grapholita prunivora* (Walsh). Forbes (1923) incorrectly used Walsingham as an author name and consequently it leads to some confusion regarding the correct author. Weires & Riedl (1991) in their most recent book cited lesser appleworm as *Cydia prunivora* (Walsingham) with obviously incorrect author name.

After the first description of *G. prunivora* (Walsh 1868) there were several common names functioning for the species currently called lesser appleworm (Stoetzel 1989). The first common name given by Walsh (1868) was plum moth and was used until the late 1800's. Due to the similarity in appearance and biology to codling moth, other names that functioned during the following years placed an emphasis on a difference in size between those two insects. The other known names are: lesser apple worm (Fletcher 1898), lesser codling worm (Forbes 1923), lesser fruit worm (Anonymous, 1951). Lugger (1898) used the name apple bud moth, while Rivard & Mailloux (1974) called it "petite pyrale". Rosenfeld (1910) used the scientific name *Enarmonia prunivora* Fitch (?) for the insect that he called "pecan huskworm".

Geographical distribution

Lesser appleworm is a native insect to North America (Quaintance 1908, CIE 1975), and has been reported only once from Asia (Arakawa 1927). Although there is also a record dating back to World War I from Sweden (CIE 1975), it and the Asian record are not accepted as correct, and lesser appleworm is considered as a quarantined pest in Europe (OEPP 1979), Japan and other countries (Johnson, pers. com. 1995)

In North America the lesser appleworm occurs throughout most of the important fruit growing regions. In the US the pest is reported in the following States: Arkansas, California, Colorado, Idaho, Illinois, Indiana, Iowa, Maine, Maryland, Missouri, New York, Ohio, Oregon, Pennsylvania, Virginia, Washington, Washington DC, Wisconsin (CIE, 1975) and Michigan (Eustace & Pettit 1910). In Canada lesser appleworm is reported in: British Columbia, Ontario, Manitoba (CIE 1975) and Quebec (Rivard & Mailloux 1974).

Main hosts

Lesser appleworm was first described as a pest on plum in Illinois by Walsh in 1868, then was also reported from other plants. The native hosts include rosaceous plants: wild hawthorn (*Crataegus* spp.), wild roses (*Rosa* sp.), crabapple (*Malus* spp.), christmasberry (*Photinia* sp.), as well as cultivated plants: apple (*Malus* sp.), pear (*Pyrus* sp.), peach , cherry, plum, and prunes (*Prunus* spp.) (Walsh 1868, Howard 1900, Simpson 1903, Quaintance 1908, Wellhouse 1920, Keifer 1933, Hoerner & List 1952, Brown 1953). The lesser appleworm is also reported from fungus galls of black knot on plums and from insect galls on elm and oak (Walsh 1868, Anonymous 1922) although according to Quaintance (1908) "larvae did not infest sound plum and black-knots, but followed the injury caused by the curculio, and in the elm and oak galls the larvae are guests, and it being uncertain whether they feed upon the tissue of the gall, upon the gall insect, or in the case of the elm leaf gall, upon the sugary dust secreted by the aphids". Lesser appleworm (as pecan huskworm) is also recorded from pecan (*Carya* sp.) (Rosenfeld 1910).

Life stages

Egg: The eggs are laid singly on the young fruits or on the bottom surface of leaves. The eggs of lesser appleworm are small, 0.53 - 0.70 mm long and 0.51 - 0.55 mm wide (Foster & Jones, 1909). Fresh laid eggs are yellowish - white, and after a few days they show a pinkish ring. A day before hatching the darker anterior and posterior of the larva are visible through the egg shell as a dark spot (Taylor, 1909) which is a feature very similar to the codling moth eggs.

Larva: After hatching, larvae of lesser appleworm immediately seek a fruit. The larvae enter the fruit mainly at the calyx end, and in case of apple feed under the skin. As a shallow feeder, larva of lesser appleworm produce a blotch-type mine (Chapman & Lienk, 1971). Brown & Jones (1953) reported that in cherry, the larva bores deep into the fruit and feeds also in the area of the pit or seed. There are also reports that the lesser appleworm larva can feed on terminal shoots of young apple trees as well as infest water sprouts on older trees (Quaintance, 1908).

The full grown larva is 6 to 8 mm long (Quaintance 1908) or 7.5 to 9.5 mm (Chapman & Lienk 1971). The larval head capsule is 0.77 to 0.85 mm in width (Chapman & Lienk 1971) and is brown to dark brown in color. As it is described by Quaintance (1908, p.: 55):" the ocellar spots, a spot caudal on cheek, and tips of the well developed and strongly toothed mandibles, black; sutural lines dark brown to blackish; width 0.75 to 0.85 mm., and about as long as wide. Thoracic shield is prominent, yellowish, transparent, often with darker markings on caudal margin near median line. Anal plate brownish, with comb-like structure on caudal curvature composed of from 5 to 7 closely set dark, brown spines, the outer spine on each side considerably reduced. Spiracles small, dark brown; thoracic legs well developed, whitish, distal end dark, claw black. Abdominal prolegs well developed, each with a single circle of from 25 to 27 strongly curved, sickle-like hooks. Tubercular are as disklike, whitish, with a single, slender, light colored seta. On third abdominal segment: tubercle I central, on dorso-lateral region; tubercle II caudo - ventrad of I, on posterior annulet; tubercle III about its width above spiracle; tubercles IV and V coalesced, directly below spiracle, about twice as far from it as is tubercle III, the seta of tubercle IV being considerably reduced; tubercle VI caudo - ventrad of IV and V, and tubercle VII with three setae situated near base of proleg". Simpson (1903) when writing about an "unknown caterpillar working on outer surface of apples" reported that there are three setae on the pre-spiracular tubercule.

Despite this relatively detailed description of larvae of lesser appleworm there is a strong similarity among larvae of lesser apple worm, oriental fruit moth, *Grapholita molesta* (Busck), and cherry fruitworm, *Grapholita packardi* Zeller. Recent authors indicate the only way to recognize lesser appleworm larvae from larvae of these other two insects is that the body of lesser appleworm larvae will retain a pinkish color after boiling in hot water, and placing in 70% alcohol (Stehr 1987, Chapman & Lienk 1971). MacKay (1959) separates oriental fruit moth larvae from the other two species by the number of crochets and characteristics of spinnerets, but she also was not able to find characters for separation of lesser appleworm and cherry fruitworm larvae.

Pupa: The cocoon, is about 6 mm long, and is made of bits of surrounding bark and white silk, when larvae overwinter on tree (Quaintance, 1908). Pupae of lesser appleworm are golden brown, with a length of 4.5 to 6.0 mm (Brown 1953). During the spring, pupation in Oregon conditions takes about fourteen to eighteen days (Brown 1953). Pupae may also be found in the hollow stem of dead weeds or attached to twigs or other suitable object where the larva makes its cocoon (Brown & Jones 1953).

According to Quaintance (1908, p.: 56) who has given the most detailed description of the life stages of lesser appleworm: "pupae of lesser appleworm are about 5 mm long, uniformly brown except the thoracic region which is darker. On dorsum of abdominal segments 3 to 7, between the spiracles on each sides, are two rows of short, stout spines, projecting caudad, one row near cephalic border of segments and one near center or on caudal margin, the spines of caudal row smaller and more numerous. Remaining segments (except 1 and 2, which are spineless) with a single row. Anal segment truncate, the 7 to 8 stout spines set on caudal margin. Cremaster of from 5 to 8 slender hairs hooked at tip and arising about equally distant from each other on caudal region of anal segment. Spiracles slightly elevated, dark brown. Wing sheets and those of third pair of legs about equal in length and reaching middle of fourth abdominal segment".

Adler (1991) in her key to insect pupae in Eastern North America used size and location of terminal spines and pupal size for separation of lesser appleworm and oriental fruit moth pupae.

Adult: The lesser appleworm adult is a small moth with an overall length of 7.5 - 9.5 mm and forewing length of 4.5 - 5.5 mm. The expanse of the wings differs slightly between sexes but are within the range of 9.5 to 11 mm (Chapman & Lienk 1971). The forewing pattern contains scales of five different colors: white, blue, grayish orange, rosaceus brown, and dark brown.

Life history.

The lesser appleworm overwinters as a full grown larva in the debris on the ground (Brown & Jones 1953) or in cracks and crevices of the bark of apple trees and under bark scales on the tree trunk (Quaintance, 1908).

The spring flight of the first generation moth lasts normally three to four weeks during May and June (Brown 1953, Chapman & Lienk 1971). Eggs are deposited singly on fruit or on upper surface of leaves (Taylor 1909) and hatch in seven to twelve days in Oregon weather conditions (Brown 1953).

Larvae of the first summer generation may pupate either in the fruit or in the ground (Brown & Jones 1953). The pupation period of the summer generation requires twelve to twenty four days, depending on the weather conditions (Taylor 1909, Brown 1953, Chapman & Lienk 1971). Three to four days of this period is spent in the preparation of a cocoon and in the prepupal stage. In Oregon, lesser appleworm required 47 to 57 days for completion of the first generation and the second generation started in late June (Brown & Jones 1953). In eastern states the second generation started in late July or even August (Chapman & Lienk 1971). At Geneva NY, larvae of second generation were found in fruit as late as October 20 (Chapman & Lienk 1971).

Lesser appleworm as a multivoltine species completes two or three generations per year (Chapman & Lienk 1971, Rivard & Mailloux 1974, Dean 1969). In Michigan lesser appleworm has two generations during the year, with flight ending in late September or early

October (Howitt 1993).

Pest status

Lesser appleworm causes damage on the trees and fruit in several ways. Lugger (1898) reported that lesser appleworm eats the buds of apple before they expand and cause "in this way more injury than if the leaves were eaten". Foster & Jones (1909) during July and August reared lesser appleworm adults from larvae found in young vigorous growing shoots and water sprouts of apple trees. Brown & Jones (1953) reported lesser appleworm larvae feeding on sweet cherry fruit where the small hatching larva immediately searches for a fruit and mines under the outer skin, making a twisted tunnel. As the larva develops, it bores deep into the fruit and feeds entirely in the area of the pit or seed. The maturing larva tunnels to the outside of the fruit through the skin and drops to the ground.

Authors referring to the lesser appleworm larvae on apples state that larvae enter the fruit usually on the calyx end (Quaintance 1908). Presence of an inconspicuous pile of frass at the feeding site at the calyx end or on the side of fruit are the most reliable external signs of infestation. Larvae feed beneath the skin at the periphery of the central feeding site. Larvae of lesser appleworm are shallow feeders, usually not tunneling deeper than about ¼ inch and usually do not feed on the seeds (Chapman & Lienk 1971, Rivard & Mailloux 1974, Quaintance 1908).

Glass and Lienk (1971), in their 10-year-long study in apple orchards maintained without any insecticide and acaricidal sprays found the lesser appleworm a significant pest. During the first six years of study they did not observe any lesser appleworm fruit damage. In last two years lesser appleworm fed on 72% and 39% of fruit respectively. At the same time damage done by redbanded leafroller *Argyrotaenia velutinana* (Walker) and codling moth was reduced about 20% each, compared to previous years. While studying apple pests under reduced spray programs Weires et al. (1979) found lesser appleworm responsible for about 50 % of the damage caused by all lepidopteran internal feeders. In comparison of number of codling moth and lesser appleworm larvae, Foster & Jones (1909) noted the relative seasonal increase of lesser appleworm larvae over codling moth larvae. When during the beginning of the season codling moth was more abundant and reared from up to 93 % of infested fruit, during the second part of the season lesser appleworm was present in over 70 % of infested fruit.

Forsythe (1976 -1993) reports in multiple "Insecticide and Acaricide Tests" that lesser appleworm causes injury in his control apple blocks. The highest observed infestation during those years was 22.0 % and the lowest 0.4 %. During his multi - year experiments 44% of his control blocks had lower than 3 % fruit damage caused by lesser appleworm.

Control strategies. Currently, in the orchard under standard protection maintenance, lesser appleworm does not receive specific treatments for its control. Similarity of its biology to the biology of codling moth and presence of infectious stages of both species at the same time in the orchard, creates a situation where insecticide applications against codling moth appear to control lesser appleworm (Caesar 1911, Anonymous 1922). Use of codling moth mating disruption complicates that situation, and the grower may be forced to use specific lesser appleworm treatments. Carde et al. (1977) demonstrated that lesser appleworm males can also be completely disrupted in field situations by using a synthetic mixture of Z - 8: Ac with 2 % and 7% E8 - 12:Ac. In Michigan lesser appleworm is not listed in the current "Fruit Spraying Calendar" as a pest of consideration (Hull et al. 1994); however, if control is necessary standard compounds are recommended as for codling moth (Howitt 1993).

Natural enemies

The complex of natural enemies of tortricid pests of fruit is generally well known and described. Numerous predators and parasitoids are known to be very important in reducing tortricid pest populations (Mills & Carl 1991, Zimmermann & Weiser 1991). Approximately 130 species of parasitoids (mostly Hymenoptera, with a few species of Diptera) have been recorded to feed on oriental fruit moth. *Macrocentrus ancylivorus* Rohwer (Hymenoptera: Braconidae) a native North American parasitoid is able to reduce populations of oriental fruit moth by 70 % (Philips & Proctor 1970). The predator complex of oriental fruit moth includes: lady beetles, lacewings, thrips that feed on eggs, ground beetles and spiders (Rothschild and Vickers, 1991).

There are not detailed studies on natural enemies of lesser appleworm. Foster and Jones (1909) reared *Mirax grapholithae* Ashm (Hymenoptera: Braconidae) and *Phanerotoma* sp. (Hymenoptera: Braconidae) from lesser appleworm larvae. Cushman (1913) reported that larvae of *Enarmonia sp.* were parasitized by *Calliephialtes* sp. (Hymenoptera: Ichneumonidae).

Sex pheromone

In 1959, Karlson & Butenandt and Karlson & Lüscher were the first to use the term "pheromone" to refer to substances emitted by one individual and elicited a specific reaction in a second individual of the same species (Karlson & Lüscher 1959). From the beginning pheromones were regarded as an alternative means of pest control; some people almost thought of them as a " new third generation of pesticides" (Carde 1976).

Pheromones are composed of combinations of different chemicals. Primary components are chemicals emitted by an insect that elicit long-distance upwind anemotaxis in the responding insect. Secondary sex pheromone components are chemicals emitted by an insect that are not essential for eliciting upwind anemotaxis, but that in combination with the primary components evoke other aspects of the mating sequence. Generally close range responses to mating behavior are induced by secondary components (Roelofs & Carde 1977).

Primary components of lesser appleworm sex pheromone are *(Z)*- 8-dodecenyl acetate (Z8-12:Ac) and *(E)*-8-dodecenyl acetate (E8-12:Ac) (Roelofs et al. 1969). These main components are also shared by other *Grapholita* species. The most important difference appears to be the ratio of the mixture. Oriental fruit moth is best attracted to mixtures of Z8-12:Ac and E8-12:Ac in a 100 : 7 ratio (Roelofs & Carde 1974) while European plum moth, *Grapholita funebrana* (Treitschke) is attracted best at a 100 : 4 ratio (Arn et al. 1976). The redbanded leafroller *Argyrotaenia velutinana* (Walker) and European corn borer *Ostrinia nubitalis* (Hübner) sex pheromones are further examples of the importance of minor amounts of opposite geometrical isomers for sex attraction (Klun et al. 1973). For lesser appleworm the best mixture of sex pheromone chemicals contains 2,2 % of E8-12:Ac (Roelofs & Carde 1974). A higher percentage of *trans (E)* isomer plays an inhibitory role in attracting lesser appleworm males (Roelofs & Carde, 1974), although Baker & Carde (1979) found that optimum blend for capturing LAW in pheromone traps included 5.1% of the *cis* (E) isomer.

Roelofs et al. (1969) stated that males of lesser appleworm were not attracted to females of oriental fruit moth and suggested the possible role of secondary components. Oriental fruit moth sex pheromone efficacy is additionally affected by the presence of two components in the pheromone mixture: dodecanol (12:OH) and *(Z)*-8-dodecenyl alcohol (Z8-12:OH) (Baker & Carde 1979, Carde et al. 1975, Carde et al. 1979). For oriental fruit moth, the addition of dodecyl alcohol improved attractiveness of primary compounds when used for moth trapping while for lesser apple worm there were no changes in trap efficacy. The addition of Z8-12:OH to lesser appleworm pheromone drastically reduced lesser appleworm male capture. The differences in response to *cis* and *trans* isomers, and the inhibitory action of Z8-12:OH are believed to be very important in causing reproductive isolation between these sympatric, highly synchronic species (Baker & Carde 1979, Roelofs & Brown 1982).

The availability of synthetic insect sex pheromones creates an opportunity for their use as a control alternative to insecticides (Rice & Kirsch 1990). Mating disruption for control of oriental fruit moth as well as codling moth has become a more and more common practice in West Europe, USA and Australia (Carde et al. 1977, Pfeiffer & Killian 1988, Rice & Kirsch 1990, Pfeiffer et al. 1993, Carde & Minks 1995). Good results in controlling oriental fruit moth are reported by Vickers et al. (1985) where mating disruption proved to be effective and comparable in costs with traditional insecticides. In California, mating disruption for oriental fruit moth control has also become a feasible alternative to insecticide control (Rice & Kirsch 1990). By comparing abandoned blocks with insecticide, organic, and pheromone treated Virginia apple orchards Pfeiffer et al. (1993) showed that mating disruption can be effective when compared to other tactics.

Using oriental fruit moth sex pheromone for mating disruption, Pfeiffer & Killian (1988) showed its effectiveness for controlling oriental fruit moth and lesser appleworm populations. These authors achieved 100 % shutdown of trap catch and almost no fruit damage. Carde et al. (1977), used a synthetic blend of oriental fruit moth sex pheromone demonstrating 100% disruption of sexual communication in field conditions of oriental fruit moth and lesser appleworm.

Mating disruption with all its advantages may also create some problems. The pheromones are manufactured for control of one species or one group of insects (Carde & Minks 1995). By the elimination of insecticide treatments, and substituting it by species-specific pheromone control, we may create a situation where other secondary insect pests may re-establish in orchards and become significant pest problems. Rice & Kirsch (1990), after using mating disruption for control of oriental fruit moth in a peach orchard, noted the increased significance of peach twig borer, *Anarsia lineatella* (Zeller), and omnivorous leafroller, *Platynota stultana* (Walsingham), pests normally considered as secondary for peach orchards.

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CHAPTER 2

Pest status of lesser appleworm *Grapholita prunivora* (Walsh) (Lepidoptera: Tortricidae) in Michigan apple orchards

Abstract

The occurrence of lesser appleworm *Grapholita prunivora* (Walsh) in south - west Michigan apple orchards was studied during 1993 and 1994. Four commercially managed and four abandoned orchards were used for evaluating the lesser appleworm fruit infestation level. Injury caused by lesser appleworm looks similar and is often misinterpreted as damage done by other internal fruit feeders; therefore, all collected immature insects were reared to adult stage. In all commercial blocks studied, there was no fruit with injury caused by this insect. Adults of lesser appleworm, codling moth, oriental fruit moth and cherry fruit moth were reared from fruits collected in abandoned orchards. Infestation by lesser appleworm was very low, with 89 % of examined samples having less than 3 % infested fruits. Lesser appleworm although present in the Michigan apple system, is not a pest in commercial orchards and damage is controlled by standard orchard management.

Introduction

Knowing and understanding the biology and occurrence of an insect is one of the most important elements of pest management. Determining the importance of an insect as a pest for a specific crop is a crucial element in effective prevention of yield loss. In an orchard ecosystem, with many insects considered as important pests, the occurrence of a potential new pest may not always create a need for additional control.

The lesser appleworm (LAW), *Grapholita prunivora* (Walsh) (Lepidoptera: Tortricidae) is a common insect in apple orchards. It is reported throughout the northern United States and from the southern provinces of Canada (Chapman & Lienk 1971, Rivard & Mailloux 1974). As a native North American species, lesser appleworm has been collected from wild and cultivated hosts including: hawthorn (*Crategus* spp.), wild rose (*Rosa sp.*), christmasberry *(Photinia sp.)*, crabapple (*Malus* sp.), cherry and plum (*Prunus* spp.), and apple *(Malus domestica)* (Wellhouse 1920; Keifer 1933; Chapman & Lienk 1971). The lesser appleworm has also been found in black knot galls on plum and from insect galls on elm (*Ulmus sp.*) and oak (*Quercus sp.*) (Anonymous 1922).

Since the first reference about lesser appleworm (formerly known as " a plum moth") in plums (Walsh 1868) there have been three periods of research interest. The first occurred in the early 1900's. After the work of Quaintance (1908) and Foster & Jones (1909) on the biology of the moth, the lesser appleworm was found as a pest in all major fruit growing areas throughout United States and Canada (Sanderson et al. 1907, Dean and Peairs 1913, Quaintance & Scott 1912). Early in this century the Michigan orchard spray bulletin stated that " lesser apple-worm, when present, requires a spray of poison" (Eustace & Pettit 1910).

The second period of renewed research efforts on lesser appleworm began in the 1950's, with Brown and Jones (1953) reporting the lesser appleworm as an important pest for cherries, citing four years of observations on this fruit in Oregon.

The most recent period of research interest in lesser appleworm started with the discovery of the lesser appleworm female sex pheromone (Roelofs et al. 1969). Questions about its biology and pest status were raised ever since growers caught high numbers of this moth in pheromone traps, even thought no damage was found in fruit.

Lesser appleworm is currently a quarantine pest in Europe (OEPP 1979) and is considered one of the major phytosanitary barriers to exporting U. S. grown apples to Japan (J. Johnson, personal comm. 1991). Although several references indicated that lesser appleworm was a pest (Glass & Lienk 1971, Brown & Jones 1953), current fruit production systems limit its importance in commercially grown fruit (Beers et al. 1993). Growers who monitor and manage major pests using insecticides report only sporadic fruit damage caused by this insect.

The objectives of this study were to document the presence and significance of lesser appleworm in abandoned and commercial apple orchards in Michigan and to compare the level of damage that lesser appleworm does to fruit with other tortricid internal fruit feeders that are currently considered to be a major pests in orchards.

Materials and Methods

Experimental sites. This study was conducted during 1993 - 1994 in four different apple growing regions of Michigan. Within each region, one abandoned and one commercially managed apple orchard were selected for data collection. In the Kalamazoo region, the abandoned orchard was located inside the city limits, and no other apple sites were nearby. The Douglas abandoned orchard was surrounded by a commercial plantation of apples and cherries. In the Casnovia and in the Shelby regions abandoned orchards were located about 2 km from the nearest commercial apple blocks. All unmanaged orchards had received no pesticide or cultural maintenance programs for at least five years prior to the study. The size of orchards varied from about 1 ha in Casnovia, to 3-5 ha in the remaining sites. The abandoned orchards consisted of mixed varieties of apples on standard rootstocks mostly "Red Delicious", "Idared" and "Jonathan". Trees in each orchard were more than 20 years old.

Commercial orchards were separated by a distance of 6 - 10 km from abandoned blocks. The Casnovia and Kalamazoo orchards had mainly "Golden Delicious" plantings, while Douglas and Shelby orchards had "Red Delicious" and "Idared" as the main apple varieties. Each commercial orchard was managed independently by the owner according to local conditions.

Sample collection. During 1993 and 1994 fruit samples from each orchard were collected every week for 15 weeks starting two weeks after the beginning of flight of first generation lesser appleworm. The final samples were collected during commercial harvest time for each variety of apple. In each commercial and abandoned orchard, four trees were randomly chosen every week for fruit collection. In abandoned orchards 100 randomly chosen fruits per tree from different sides of the tree were collected. In commercial blocks, 100 apples were evaluated on each tree and only fruits with visible damage were collected for further observation. In 1993 in the Douglas abandoned orchards, a sample of 100 fruit was collected from more than one tree, because few fruits were available due to early season frost.

Pheromone traps were used for monitoring flight of lesser appleworm adults. Pherocon II traps (Trece Inc.) with lesser appleworm lure (Scentry Inc.) were placed in the center part of each block at 1.5 m height in the beginning of the season. Traps were checked weekly for adult moth capture. Traps and pheromones were replaced every four weeks.

Fruit evaluation. Collected fruit were placed in plastic boxes (40.3 cm L x 27.7 cm W x 15.1 cm H) with mesh tops. A thin layer (0.5 cm) of fine vermiculite on the bottom of each box and 20 strips of corrugated paper (~ 1.5 x 4 cm) provided space for larval pupation. Boxes were put in growth chambers with a 16 : 8 (L : D) h photoperiod and 25 : 15^{0} C temperature. After two weeks in the growth chamber, the fruit, corrugated paper, and vermiculite were examined for the presence of larvae and pupae. All collected larvae were placed in plastic Petri dishes (100 mm D x 15 mm H) with cotton and small pieces of fruit for further rearing. Pupae were placed in plastic vials (75 mm H x 15 mm D) with vented tops. Larvae and pupae were held in a growth chambers under the same conditions as the fruits. Adults were identified to species.

Statistical analysis. For comparison of fruit infestation by different internal fruit feeders, mean numbers of reared insects were compared. To avoid repeated measurements oriental fruit moth, codling moth and lesser appleworm fruit infestation in relation to location and year were analyzed separately by two way analysis of variance (ANOVA) and means from n = 15 samples per year were compared using HSD Tukey's comparison of mean at P =0.05 (CoStat 1990). The four orchards used for each growing season were considered as replicate blocks. Kolmogorow - Smirnow test was used for testing normality of frequency distributions of lesser appleworm fruit infestation (CoStat 1990, Sokal & Rohlf 1981).

Results and Discussion

Under Michigan weather conditions lesser appleworm had two generations per year. The first generation flight started in the beginning of May (DD_{50} : 200-225) with maximum moth flight during middle of June. The flight of second generation started in early August (DD_{50} : 1500-1700) and continued through the end of September with peak flight activity in late August. The typical flight seasonality of lesser appleworm in abandoned and commercial orchards in Casnovia are shown on Figure 1. The four studied regions showed a difference in moth activity by calendar days but similar timing when plotted against degree-days base 50 F accumulation.

The pheromone trap catch of the spring population of lesser appleworm in commercial orchards during spring of 1993 and 1994 were twice of that in abandoned orchards; however, during flight of the second generation lesser appleworm the pheromone trap catch of adults in commercial blocks was minimal (Figure 1). In all four regions, no lesser appleworm injury was found on fruit during study in commercially protected orchards where pesticides were applied according to current needs, even when moths were caught in pheromone traps in orchards. This indicates that the insecticidal control targeted for the major apple pests also protected fruit against potential injury from lesser appleworm. The presence of adults in orchards during the spring might be due to migration from wild hosts, especially since each commercially protected block that was sampled was located near the border of the orchard. The lack of detectable

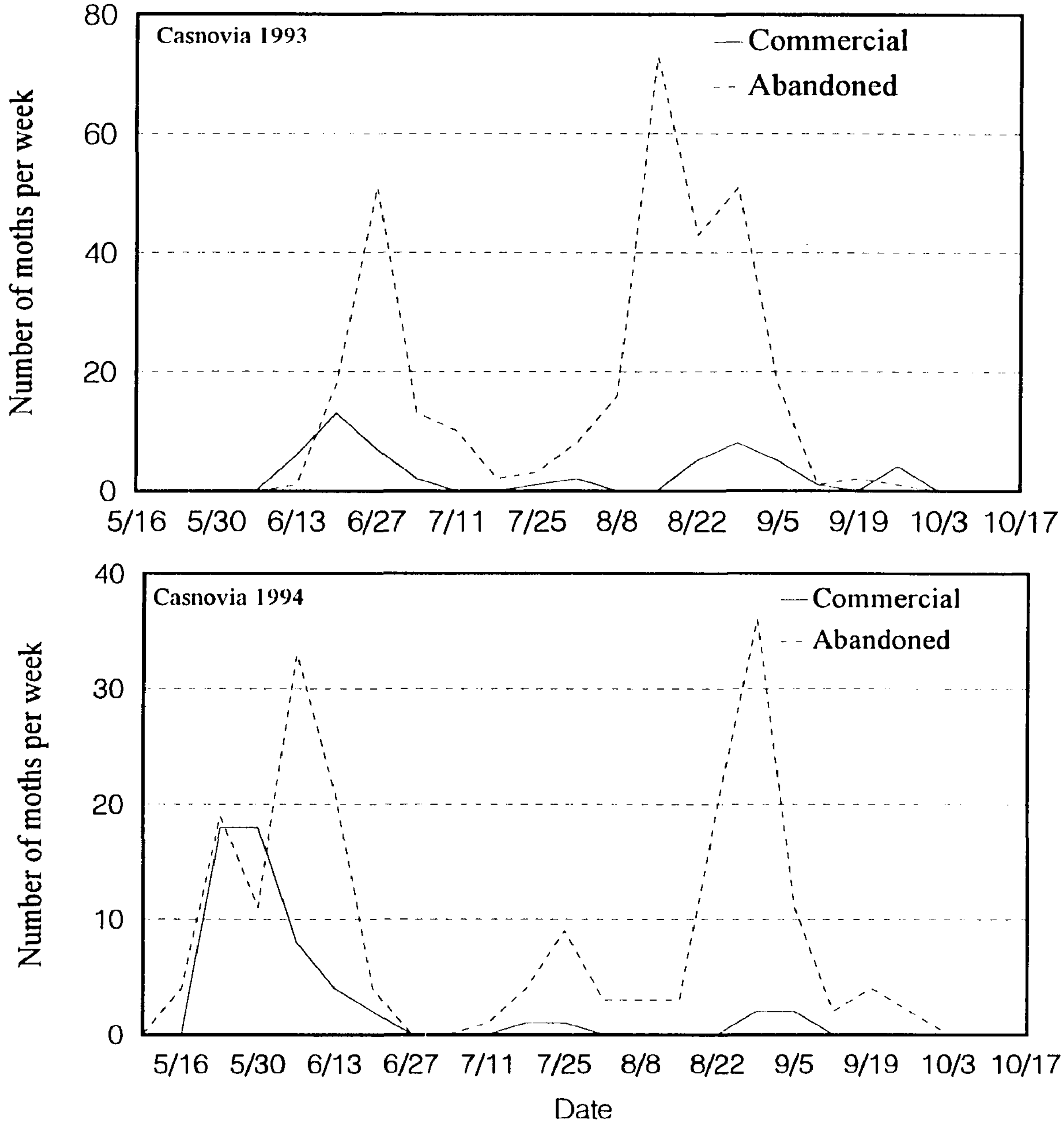

Figure 1. Lesser appleworm flight seasonality during 1993 and 1994 in abandoned and commercial apple orchards Casnovia MI.

injury on fruit was probably related to the protectant activity of insecticides used for control of other pests in the commercially protected orchards.

Lesser appleworm was successfully reared from fruit collected from all abandoned orchards. There were no significant differences in fruit infestation by lesser appleworm between 1993 and 1994 ($F = 2.903$; $df = 1, 3$; $P = 0.091$ ns) (Table 1). Fruits in Douglas and Casnovia abandoned orchards ($P = 0.05$) had significantly higher lesser appleworm fruit infestation than fruits from Shelby and Kalamazoo orchards ($F = 7.61$; $df = 3, 1$; $P \leq 0.001$).

In case of oriental fruit moth there was a significant difference among mean fruit infestations during 1993 and 1994 ($F = 6.598$; $df = 1, 3$; $P = 0.0115$), while for codling moth no significant differences were detected ($F = 3.14$; $df = 1, 3$; $P = 0.079$). Oriental fruit moth fruit infestation was significantly higher in the Douglas abandoned orchard than in others ($F = 32.47$; $df = 3,1$; $P = .000$). For codling moth mean fruit infestation were significantly lower in Casnovia orchard than in Douglas, Kalamazoo, and Shelby abandoned blocks ($F = 4.606$; $df = 3,1$; $P = 0.0044$). Mean fruit infestation for studied pests during 1993 and 1994 in abandoned orchards of all regions are presented in Table 2.

Frequency analysis on samples collected from abandoned blocks showed that in 89.2 % of samples lesser appleworm infestation ranged from 0 to 3 % (Table. 3). Out of n = 120 samples collected during two years we found infestation higher than 3 % only in 13 samples, all of them from the Douglas and Casnovia abandoned orchards.

In addition to lesser appleworm, three other internal fruit feeding tortricids were reared from fruit collected from abandoned orchards: codling moth *Cydia pomonella*

Table 1. Two way analysis of variance (ANOVA) of lesser appleworm fruit infestation in four abandoned apple orchards in Michigan.

Source	df	MS	F	P
	Lesser appleworm			
Year	1	4.7	2.9	.0912 ns
Location	3	12.32	7.61	.0001 ***
Year x loc	3	6.31	3.89	.0108 *
Error	112	1.61		
	Oriental fruit moth			
Year	1	296.88	6.59	.0115 *
Location	3	1461.07	32.47	.0000 ***
Year x loc	3	302.38	6.72	.0003 ***
Error	112	44.99		
	Codling moth			
Year	1	47.81	3.14	.0790 ns
Location	3	70.11	4.6	.0044 **
Year x loc	3	13.65	0.89	.4451 ns
Error	112	15.22		

* = $0.01 \leq P \leq 0.05$
** = $0.001 < P \leq 0.01$
*** = $P \leq 0.001$

Table 2. Mean fruit infestation by four internal fruit feeders: lesser appleworm (LAW), oriental fruit moth (OFM), codling moth (CM), and cherry fruitworm (CFW) in four abandoned Michigan apple orchards during 1993 and 1994.

Location	Number of samples n	Mean fruit infestation ± SD			
		1993			
		LAW	OFM	CM	CFW
Douglas	15	1.95 ± 2.39	22.58 ± 17.42	7.78 ± 8.43	0.00
Casnovia	15	1.13 ± 1.91	2.38 ± 3.25	2.47 ± 1.97	0.00
Shelby	15	0.23 ± 0.62	1.68 ± 2.55	2.47 ± 1.97	0.00
Kalamazoo	15	1.20 ± 1.96	2.88 ± 2.81	4.70 ± 5.51	0.02 ± 0.13
		1994			
Douglas	15	2.00 ± 2.13	10.00 ± 8.89	4.47 ± 3.33	0.38 ± 0.71
Casnovia	15	2.48 ± 2.04	3.13 ± 3.26	2.56 ± 2.99	0.15 ± 0.40
Shelby	15	1.20 ± 1.51	2.63 ± 2.93	4.51 ± 3.32	0.03 ± 0.18
Kalamazoo	15	0.58 ± 0.94	1.08 ± 1.38	3.43 ± 3.22	0.12 ± 0.45

Table 3. Frequency analysis for fruit infestation by lesser appleworm (LAW) in four Michigan abandoned apple orchards analyzed for normality of distribution by Kolmogorow - Smirnow test.

Percent of fruit infested by LAW	Number of samples n	Percentage of samples	Expected frequency	Deviation
0. 00 - 0.99	56	46.67	42.23	13.77
1.00 - 1.99	33	27.50	33.92	-0.92
2.00 - 2.99	18	15.00	26.72	-8.72
3.00 - 3.99	6	5.00	12.74	-6.74
4.00 - 4.99	3	2.50	3.67	-0.67
5.00 - 5.99	1	0.83	0.63	0.36
6.00 - 6.99	2	1.67	0.06	1.93
7.00 - up	1	0.83	0.00	0.99

(L.), oriental fruit moth *Grapholita molesta* (Busck), and cherry fruit worm *Grapholita packardi* Zeller (Figure 2 and Figure 3). The codling moth is a one of the most important pests in Michigan apple orchards (Howitt, 1993). The oriental fruit moth is considered as a pest on peach, but can also cause economic injury on apples, mainly during the later part of the season, when peaches have been harvested. The cherry fruit worm is found in apples sporadically, with cherries and blueberries as its main hosts. Although cherry fruit worm was found in every abandoned orchard, the number of reared adults was very low.

The abundance of lesser appleworm in four Michigan abandoned orchards (Table 2 and Table 3) was much lower than that reported by Glass and Lienk (1971) in New York. These authors found that lesser appleworm injured 72 and 39 % of fruit during the last two years of their study respectively, even through in previous years lesser appleworm was not found in the orchard. This may indicate much higher population in NY, but also some of the differences might by explained by the differences in methods used in each experiment for detecting the source of injury.

Confusion of characters of lesser appleworm injury dates back to the earliest references. Misidentification of lesser appleworm has been common since it was first found. The most commonly reproduced picture with injured fruit comes from the work of Simpson (1903), when this author presented it as " injury caused by an unknown caterpillar". Using of the number of reared adults as an indicator of fruit infestation, removed possible errors from larvae or injury misidentification.

The absence of lesser appleworm fruit infestation in commercially managed orchards suggests that the current control techniques applied in apple system effectively control lesser appleworm. However, the use of autocidal methods such as mating

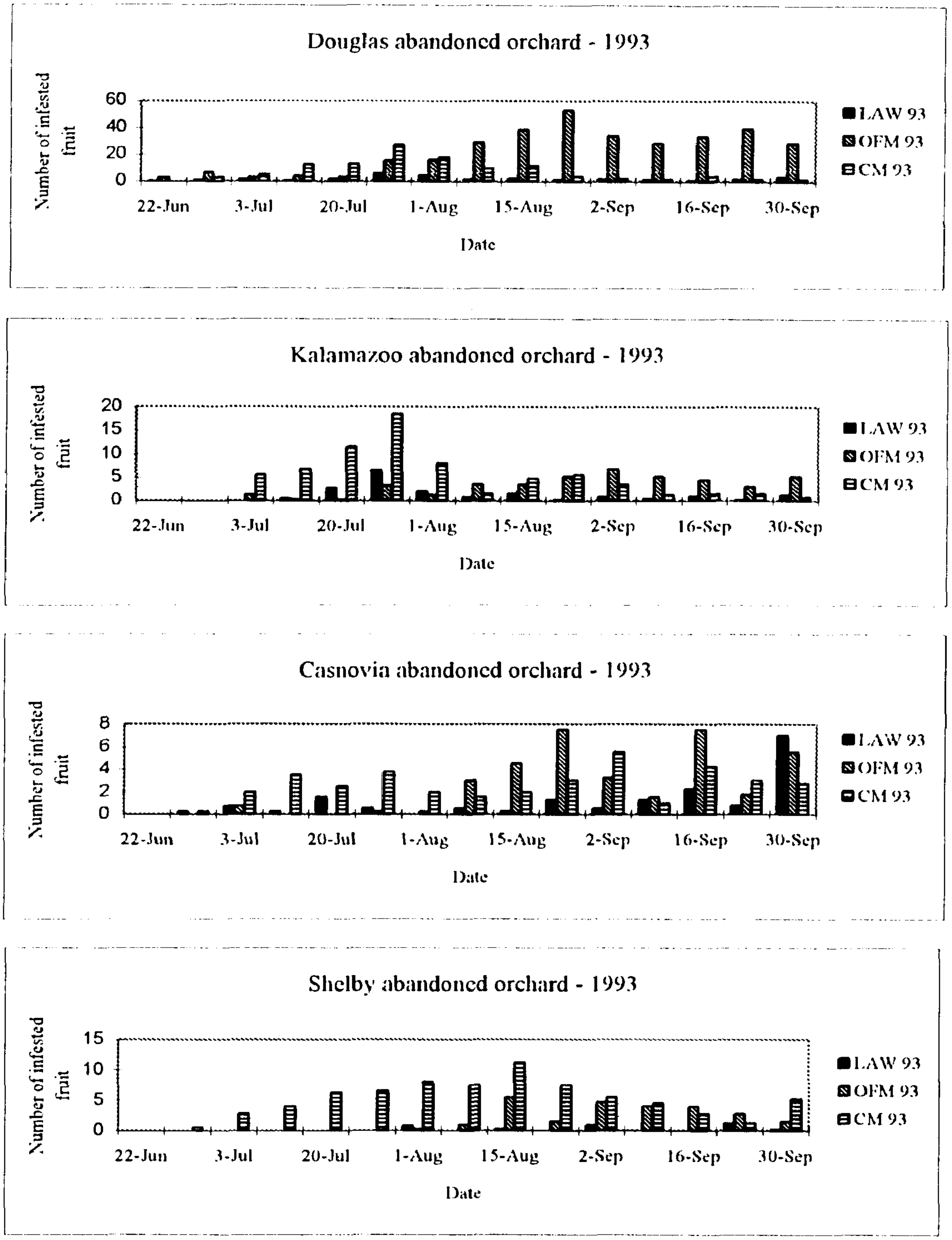

Figure 2. Lesser appleworm (LAW), oriental fruit moth (OFM), and codling moth (CM) fruit infestation in Douglas, Kalamazoo, Casnovia, and Shelby abandoned apple orchards during 1993 season.

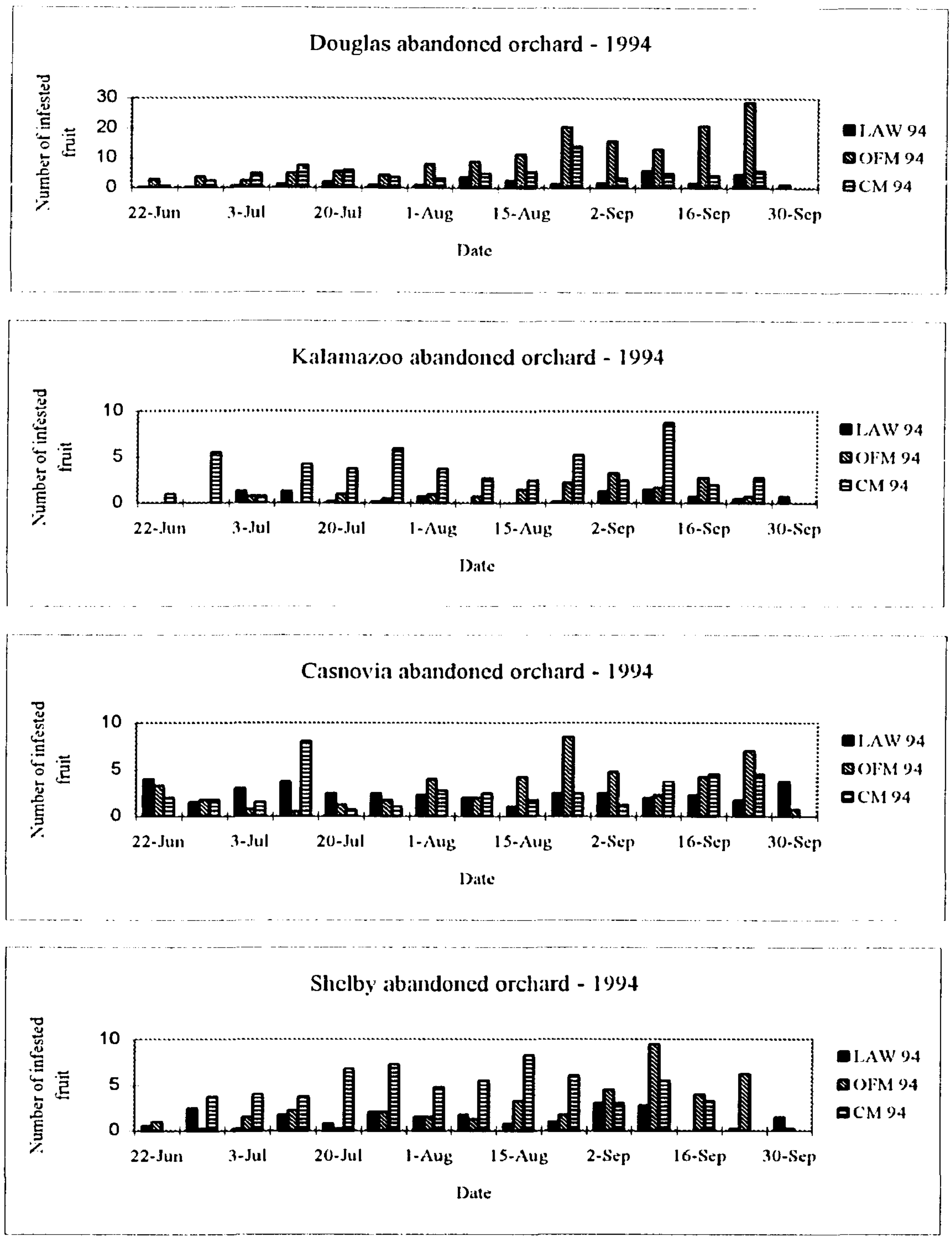

Figure 3. Lesser appleworm (LAW), oriental fruit moth (OFM), and codling moth (CM) fruit infestation in Douglas, Kalamazoo, Casnovia, and Shelby abandoned apple orchards during 1994 season.

disruption or release of sterile males to control codling moth, oriental fruit moth or various leafrollers may significantly reduce the number of insecticide sprays made in orchards (Pfeiffer et al. 1993, Carde and Minks 1995). This new situation along with positive changes in the ecosystem, might enhance the development of other insects such as lesser appleworm to become a pest.

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CHAPTER 3

Seasonality of lesser appleworm *Grapholita prunivora* (Walsh) (Lepidoptera : Tortricidae) in Michigan apple orchards.

Introduction

Lesser appleworm *Grapholita prunivora* (Walsh) is noted from all major fruit growing areas in morthern U. S. and southern Canada. Together with three other tortricid species: codling moth, *Cydia pomonella* (L.), oriental fruit moth, *Grapholita molesta* (Busck), and cherry fruitworm, *Grapholita packardi* Zeller, the lesser appleworm is considered as an important internal fruit feeder (Chapman & Lienk 1971, Weires et al. 1979). Lesser appleworm, as a polyphagous species, can feed on numerous plant hosts, including most cultivated pome and stone fruit (Rivard & Mailloux 1974). In Michigan and the Pacific Northwest (Washington, Oregon) lesser appleworm is not considered as an important pest, however the validity of accurate detection and monitoring is very important (Howitt 1993, Beers et al. 1993).

Monitoring of pest insects in the field is one of most important elements of integrated pest management. Different methods and tools are necessary to observe changes that happen to pest populations. Direct observation in the field is the best way to monitor insect occurrence, but it is often difficult or almost impossible to accomplish due to problems associated with the identification of immature stages of pests or characters of injury. A good example of the above involves description of larval stages and fruit injury caused by lesser appleworm and oriental fruit moth (Chapman & Lienk 1971). The use of sex pheromone trap for detecting insect flight and concept of day-degree methods may be very useful in monitoring and understanding insect occurrence in the field.

The concept that temperature has a direct effect on growth of plants and arthropods was formulated as long ago as in the middle of the 18th century (after Wilson & Barnett 1983). Below a certain given temperature an insect cannot conduct its physiological and biochemical reactions at a high enough rate to support growth. Different insects have different developmental thresholds, but for practical purposes standardized thresholds are used for important pests. Pruess (1983) suggested that calculation of heat unit accumulation above thresholds of 40, 50, and 60° F (or 5, 10, 15° C) should be used for degree day accumulations. In exothermic arthropods development rate changes with temperature of the environment (Logan et al. 1976). Based on assumptions that the developmental rate is a nonlinear function, with a similar form for most insects and that the temperature in a given area fluctuates predictably from season to season, Taylor (1981) investigated the properties of the physiological time scale for various insects in different temperatures and discussed its evolutionary significance.

The use of a computer-operated Predictive Extension Timing Estimator (PETE) (Welch et al. 1978) was very closely related to the practical use of a degree day concept. Use of this interactive modeling system fed by biological observations together with heat accumulation for specific areas and pests was the basis for predicting the occurrence and importance of many orchard pests (Welch et al. 1981). In Michigan, the Fruit Spray Calendar currently uses the average degree day accumulation together with average calendar date for defining correct timing of insects' control treatments (Hull et al. 1994).

Using pheromone traps for monitoring insect pest populations has proved to be another excellent tool in pest management. Together with the concept of degree-day accumulation, it can significantly improve the timing and effectiveness of protective techniques. Pheromone traps designed to monitor one species are sometimes useful for monitoring other insects. With the high diversity of apple insect communities (Szentkirályi & Kozár 1991, Strickler & Whalon 1985) it may be very helpful if we can monitor and control more than one insect species by using one blend of pheromone. Attempts to control the whole leafroller complex by mating disruption in European apple orchards by using a single disruptant component proved to be successful and economically comparable with pesticide treated control (Carde & Minks 1995). On the other hand, the use of single component disruptant for mating disruption of the leafroller pest complex in Virginia orchards did not work satisfactionary, mainly due to lower effectiveness against one of leafroller species (Pfeiffer et al. 1993).

For accurate prediction of spring insect emergence from its overwintering sites, it is important to know where we can actually find the diapausing stage. Different overwintering sites of one species and related with it various times of emergence from winter diapause due to sunshine (heat) exposure may resulted in prolonged first generation emergence. The use of correctly placed traps with sex pheromone and the correct biofix point can significantly improve pest control (Riedl et al. 1976).

In my study I tried to determine the importance of the degree-day concept and pheromone trap catches for predicting lesser appleworm occurrence and seasonality in orchards. To determine an additional factor that may play an important role in timing moth occurrence in the field, particularly in the spring, I attempted to identify overwintering sites.

Material and Methods

Experimental site. During the 1992,1993, and 1994 seasons abandoned apple orchards in four fruit growing regions of Michigan were used for observation of lesser appleworm seasonality. Sites were located in Douglas, Kalamazoo, Shelby, and Casnovia, MI. (Figure 1). Each of the orchards was more than 25 years old, with mixed apple varieties. All study orchards were abandoned, with no maintenance activity at least five years prior to the study. There were no pesticides applied in the orchards, except for the Douglas block where fungicides were applied during 1991.

Degree day observation. In the Douglas and Shelby locations degree-days (DD) at base 50° F and 42° F were calculated during the entire season on computerized weather stations. A continuous integration method for calculating degree-days accumulation was provided automatically by EnviroCaster (Neogen Corporation, Lansing MI). In Shelby degree-day during 1993 and 1994 started to be accumulated on April 13 and 21 respectively. In Douglas during 1992, 1993, and 1994 the beginning dates were respectively: March 04, 06, and 25.

Pheromone trap efficacy. During 1993 and 1994, sticky traps (Pherocon II, Trece, Inc.) with sex pheromones for codling moth, oriental fruit moth and lesser appleworm were placed 1.5 m high in the orchards and monitored weekly. Three replicates of each sex pheromone trap for each species were used every year. Groups of traps for three different insects were separated by approximately 100 m. All collected moths were

Figure 1. Location of experimental sites in Michigan

identified and removed from traps. Traps and pheromone lures were replaced every 4 weeks.

Data were analyzed using one-way analysis of variance (ANOVA) and HSD Tukey's test for mean separation ($P = 0.05$). The total number of moths collected annually by traps was used as one replication.

Overwintering sites: Five methods were used to look for overwintering sites of lesser appleworm.

a) During late fall 1992 four metal cone cages per one tree were placed under four randomly chosen trees in the Douglas abandoned orchard. Each cage was 1 m diameter at the base and 90 cm high. At the top of each cage a plastic jar was placed for collecting emerging moths. Traps were checked the following spring, during the first generation flight of lesser appleworm moths.

b) In August of 1992, 1993, and 1994 corrugated paper was placed on four randomly chosen trees in each abandoned orchard around the trunk and on four scaffold branches. Approximately 10 cm wide paper bands were placed 1 m high on the trunk and on the branches approximately 1.5 m from the trunk. During the first year of the study bands were removed the following April, but because of bird damage to this sample, during the following years of observations they were removed in November.

c) In April 1994, in the Douglas orchard soil samples were collected from under each of four trees fitted with the paper bands. Four samples of soil 25 x 50 cm in area and 5 cm deep were collected from under each tree without plants and debris and were placed in plastic boxes with a mesh top for air circulation. Samples of weeds and other debris from under the tree were collected in separate containers. The vegetation from 1 m diameter around the trunk was also collected. Containers with soil (n =16), debris and vegetation (n =16), and vegetation from around the trunk (n = 4) were held at ambient weather conditions and emerging insects were noted.

d) During early April 1994 in the Douglas orchard using the same trees that were sampled with corrugated paper were also sampled by collecting bark. From one tree, a 25 cm wide strip of bark was excised from the trunk and inspected for overwintering insect stages. Additionally, a 20 cm strip of bark from four lower branches of each tree was inspected for the presence of lesser appleworm stages.

e) On November 15, 1993 at the Kalamazoo abandoned orchard four samples of apples remaining on trees and from under the trees were collected. Two hundred apples (4 x 50) were collected per sample from trees in close proximity. A total of four samples were collected. Fruit were placed in a growth chamber at 16 : 8 h photoperiod and 24: 16 ° C temperature. Emerging larvae were collected and reared to adults.

In winter of 1993/94 additional observations were made on 2-3 year old and 5-6 year old branches collected from different parts of the tree canopy. Branches were removed and inspected in laboratory for presence of overwintering insects.

Results and Discussion.

Degree - day observations. In these experiments I used two developmental thresholds to describe lesser appleworm activity using the degree day concept. There is not an established developmental threshold for lesser appleworm. Because of biology similarities of this insect with biology of the codling moth and oriental fruit moth for my observations I choose DD base 42° F (as for oriental fruit moth) and DD base 50° F (as for codling moth) for observations.

Over three years of study in Douglas MI, the lesser appleworm started its first generation flight in the middle of May (Figure 2) with DD_{50} accumulation of 203 - 268. Peak first generation flight occurred at 500 - 636 DD_{50}. Maximum flight of the second generation occurred at 1535 - 2128 DD_{50} (Table 1). In Shelby MI. abandoned orchard during 1993 and 1994 lesser appleworm started its first generation flight at 111 - 241 DD_{50} accumulation (Figure 3). The peak of first generation flight occurred at 259 - 527 DD_{50}, while the peak of second generation flight occurred at 1423 - 1787 DD_{50}.

During observations at the two locations considerable differences were observed over the three year period. Despite different starting dates for heat accumulation actual DD values between major events also showed a wide range of differences (Table 2). Three years of data from the Douglas orchard showed a smaller difference in total DD accumulation between different years than two years data from Shelby. In New York Agnello et al. (1993) calculated degree day accumulations for numerous orchard pests using a sine wave method. During 5 years of observation on lesser appleworm they similarly observed a wide gap between major events during different years. Without citing the starting point of degree days accumulation, these authors found first lesser appleworm moths in pheromone traps at an accumulation of 82-254 DD_{50}. Similar differences existed among other major events in lesser appleworm life (Agnello et al. 1993). In my study the big difference in sum of accumulated degree-days may be related to timing of starting point for heat accumulation. Data from Shelby orchard, specially from 1993, with the lowest DD_{50} accumulation, may be biased by the date when calculating of degree-days started. It is possible that some heat accumulation in the field

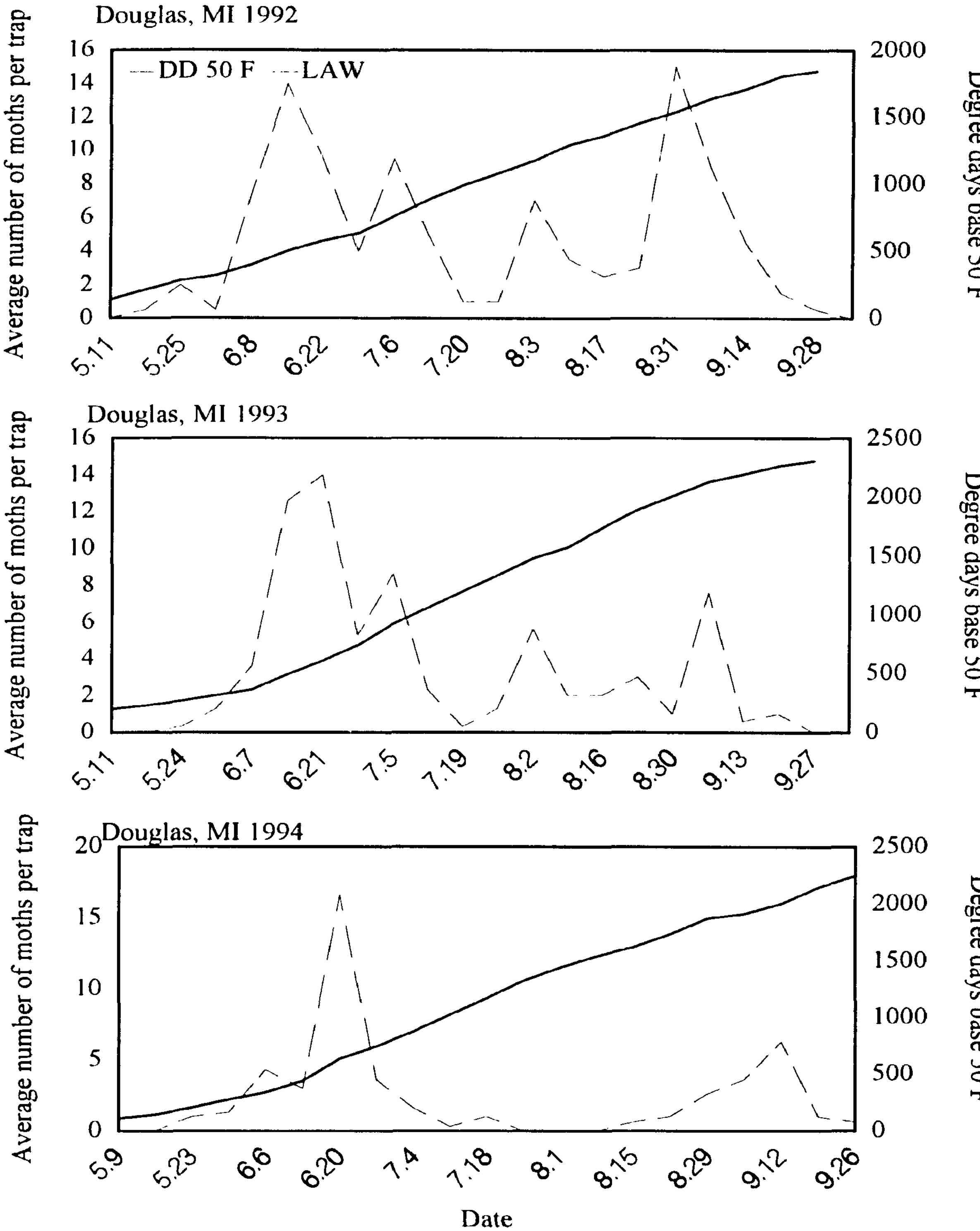
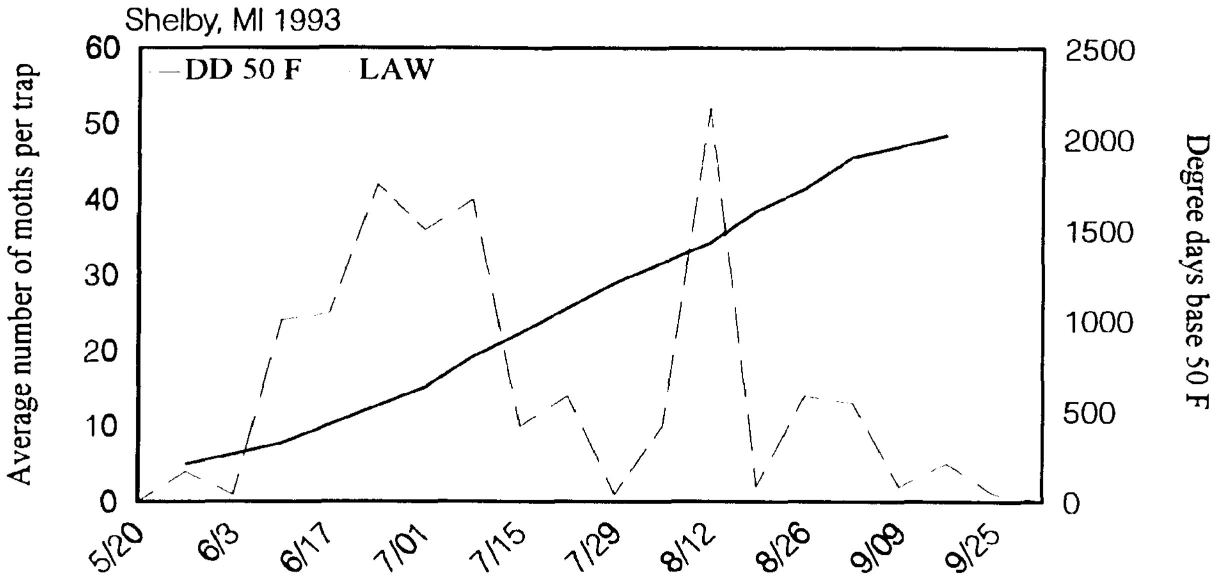


Figure 2. Degree days base 50 F accumulation and lesser appleworm flight seasonality in Douglas, MI abandoned orchard during 1992 - 1994.

Table 1. Lesser appleworm life events in comparison with accumulation of degree days base 42° F and 50° F

		Degree days accumulations						
		Douglas				*Shelby*		
		1992	*1993*	*1994*	*Range*	*1993*	*1994*	*Range*
1st catch	date	*18-May*	*24-May*	*23-May*	*5/18 -5/24*	*27-May*	*18-May*	*5/18-5/27*
	DD_{42}	430	533	414	414 -533	521	296	296 - 521
	DD_{50}	212	268	203	203 -268	241	111	111 - 241
Peak of 1st gen.	date	*15-Jun*	*21-Jun*	*20-Jun*	*6/15 -6/21*	*24-Jun*	*1-Jun*	*6/01 -6/24*
	DD_{42}	904	1069	1037	904 -1069	1010	541	541 -1010
	DD_{50}	500	606	636	500 - 636	527	259	259 - 527
End of 1st gen.	date	*20-Jul*	*19-Jul*	*25-Jul*	*7/19 -7/25*	*29-Jul*	*29-Jun*	*6/29-7/29*
	DD_{42}	1656	1889	2007	1656-2007	1967	1153	1153-1967
	DD_{50}	989	1201	1325	989 -1325	1205	679	679 -1205
Peak of 2nd gen.	date	*31-Aug*	*6-Sep*	*12-Sep*	*8/31-9/12*	*12-Aug*	*7-Sep*	*8/12-9/07*
	DD_{42}	2517	3189	3047	2517-3189	2297	2843	2297-2843
	DD_{50}	1535	2128	2002	1535-2128	1423	1787	1423-1787
End of 2nd gen.	date	*28-Sep*	*20-Sep*	*26-Sep*	*9/20-9/28*	*18-Sep*	*28-Sep*	*9/18-9/28*
	DD_{42}	3026	3427	3417	3026-3427	3176	3282	3176-3282
	DD_{50}	1843	2264	2244	1843-2264	2016	2069	2016-2069



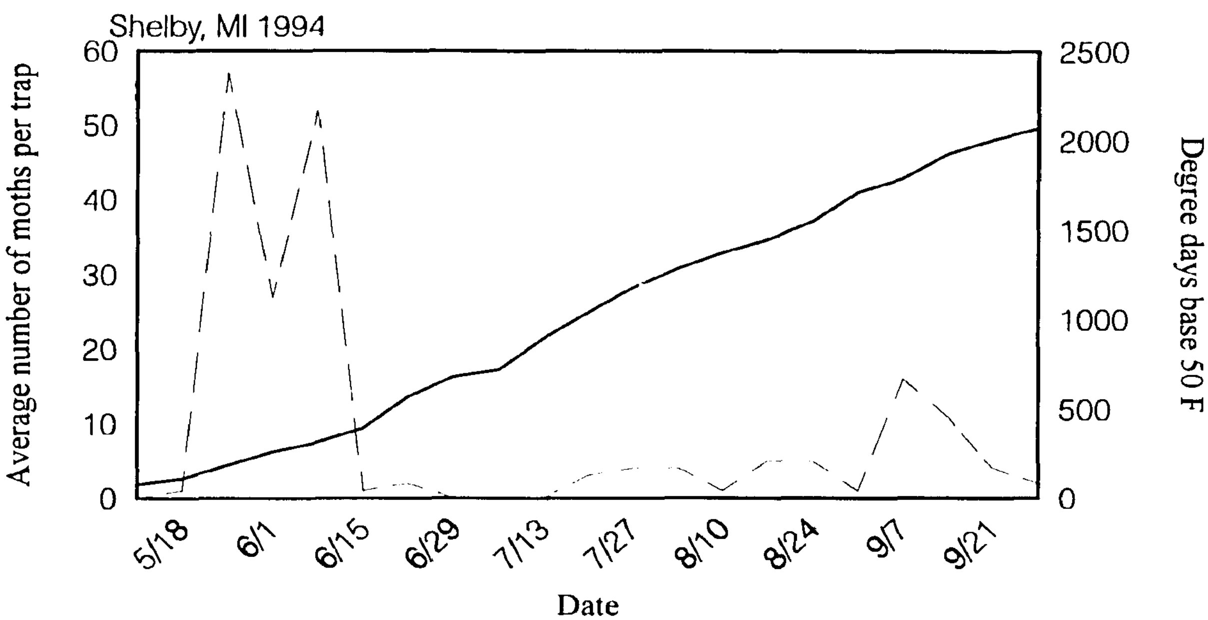


Figure 3. Degree days base 50 F accumulation and lesser appleworm LAW flight seasonality in abandoned orchard during 1993 -1994, Shelby, MI.

Table 2. Lesser appleworm degree days base 42 F and 50 F accumulation in relation to previous event

Insect event		Degree days accumulation starting from previous event						
		Douglas				Shelby		
		1992	1993	1994	Range	1993	1994	Range
1st catch	DD_{42}	430	533	414	414 - 533	521	296	296 - 521
	DD_{50}	212	268	203	203 - 268	241	111	111 - 241
Peak of	DD_{42}	474	536	623	474 - 623	489	245	245 - 489
1st gen.	DD_{50}	288	338	433	288 - 433	286	148	148 -286
End of	DD_{42}	752	820	970	752 - 970	957	612	612 - 957
1st gen.	DD_{50}	489	595	689	489 - 689	678	420	420 - 678
Peak of	DD_{42}	861	1300	1040	861 -1300	330	1690	330 -1690
2nd gen.	DD_{50}	546	927	677	546 - 927	218	1108	218 -1108
End of	DD_{42}	509	238	370	238 - 509	879	439	439 - 879
2nd gen.	DD_{50}	308	136	242	136 - 308	593	282	282 - 593

may already occurred, but was not included.

Pheromone trap efficacy. Lesser appleworm sex pheromone traps were able to capture four other moth species: oriental fruit moth, codling moth, obliquebanded leafroller, *Choristoneura rosaceana* (Harris), and eyespotted bud moth, *Spilonota ocellana* (Denis and Schiffermüller) (Table 3). The numbers of captured oriental fruit moth and lesser appleworm males differ significantly (P = 0.05) from the numbers of three other species. While it was not a surprise that this trap was very effective in attracting the oriental fruit moth, the codling moth and obliquebanded leafroller were probably captured accidentally. Lesser appleworm pheromone traps can be used reliably as a monitoring tool for oriental fruit moth (Figure 4). The correlation coefficient between number of oriental fruit moths captured in oriental fruit moth traps and number of oriental fruit moths captured in lesser appleworm traps during 1993 and 1994 were r = 0.60 and r = 0.69 respectively. The eyespotted bud moth capture was higher than capture of obliquebanded leafroller and cherry fruitworm, but these differences were not significant (P = 0.05). Oriental fruit moth sex pheromone traps captured five other species of moths, all of them in significantly lower numbers (P = 0.05). Traps for collecting codling moth collected only one other species, the oriental fruit moth.

Lesser appleworm and oriental fruit moth share the same component: *cis*-8-dodecenyl acetate (Z8-12:Ac) as their main ingredients of sex pheromone (Roelofs et al. 1969). The addition of different optical isomer *trans* E8-12:Ac is responsible for wider activity spectrum. Optimum attractant of oriental fruit moth to Z8-12:Ac requires the addition of 6 - 7 % of the *trans* isomer, while for lesser appleworm it worked best when 2.2 % of *trans* isomer was present (Roelofs & Carde 1974). Gentry et al. (1975) by using

Table 3. Capture of differerent moths by sex pheromone traps of three internal fruit feeding tortricids

Captured moths	Number of captured moths Traps[1] /localization/year							
	OFM Fen. 94	OFM Fen. 93	OFM Doug. 94	LAW Fen. 94	LAW Fen. 93	LAW Doug.94	CM Fen. 94	CM Fen. 93
OFM[2]	1138	964	993	198	110	107	10	8
LAW[2]	9	6	17	92	162	142	-	-
CM[2]	-	19	1	1	-	-	552	452
OBLR[2]	1	-	-	1	-	-	-	1
ESBM[2]	6	5	10	1	5	13	-	-
CFW[2]	-	-	3	-	-	-	-	-

[1] - Total number of captured moths during the season. Three traps per localization,
[2] - OFM- oriental fruit moth *G. molesta*, LAW - lesser appleworm *G. prunivora*, CM - codling moth *C. pomonella*, OBLR- obliquebanded leafroller *Ch. rosaceana*, ESBM - eyespotted bud moth *S. ocellana*, and CFW - cherry fruitworm *G. packardi* .

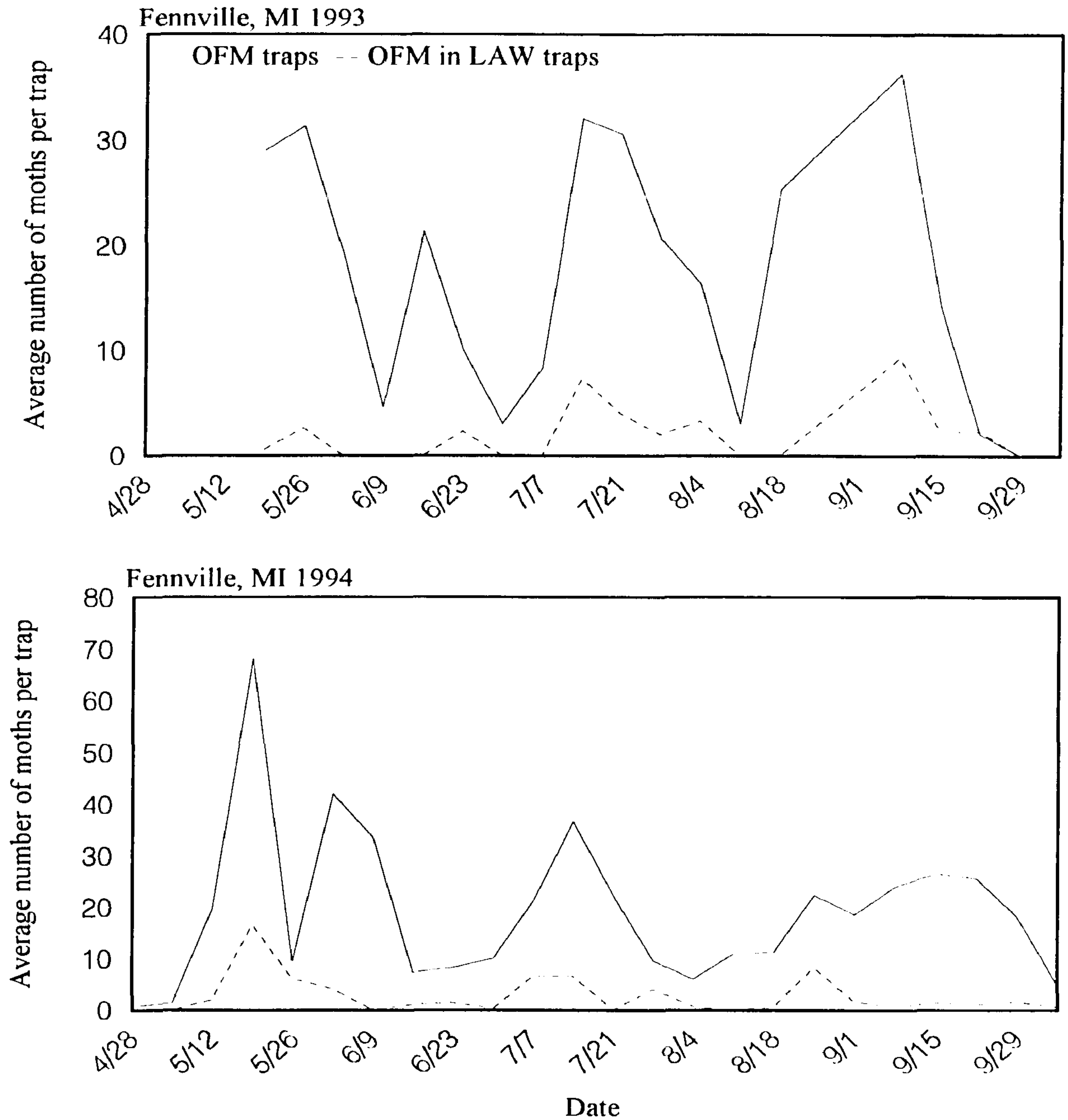


Figure 4. Collection of oriental fruit moths OFM in lesser appleworm LAW pheromone traps in comparison with regular oriental fruit moth traps. Fennville, MI 1993 -1994

isomer blends of 8-dodecenyl acetate were able to capture oriental fruit moth, lesser appleworm, and pecan bud moth, *Gretchena bolíana* (Slingerland), males in the same trap. Lesser appleworm was also collected in oriental fruit moth traps by Willson & Trammel (1980) during their study in New York. Attraction of lesser appleworm males to pheromones of other species with different components: eyespotted bud moth with Z8-14:OAc, obliquebanded leafroller with complex of Z11-14:OAc, E11-14:OAc, and Z11-14OH, and codling moth with E,E-8,10-12OH (Roelofs & Brown 1982, Arn 1990) was incidental, and capture was not significant.

A similar situation was observed by Klun et al. (1973) for European corn borer, *Ostrinia nubilalis* (Hübner) and redbanded leafroller moth, *Argyrotaenia velutinana* (Walker), where the minor quantity of opposite geometrical isomers in the pheromone lures played an important role in attraction of different moth species.

Overwintering sites. During the winters of 1991/1992, 1992/93, 1993/94 the search for overwintering lesser appleworm larvae included: top layer of soil under the tree, weeds and debris, bark on trunk and branches, leftover fruit and artificial overwintering sites provided by corrugated paper bands on tree trunk. After three years of observations on multiple sites, no single overwintering lesser appleworm larvae was found.

According to old references, lesser appleworm overwinter as a full grown larva in cocoons formed in cracks and crevices of the bark, under bark scales and "wherever suitable protection may be found" (Quaintance 1908). Cocoons are made of bits of surrounding bark and therefore are very "difficult to detect" (Quaintance 1908). Larvae were also found in paper band around tree trunks, as used for codling moth (Quaintance

1908). Brown (1953) found lesser appleworm overwintering sites in cherry orchards in debris on the ground, where them spun cocoon during the fall. Due probably to the life history and length of stages, lesser appleworm larvae were found also in harvested fruit, where they were able to be active during storage time (Foster & Jones 1909). Larvae were found in partly devoured fruit of hawthorn on the tree and on the soil (Foster & Jones 1909). Overwintering larvae of lesser appleworm were also found on the ground in the rubbish around the apple bin of a vinegar factory at the rate of 4 lesser appleworm larvae to 135 larvae of codling moth (Foster and Jones 1909).

The larvae of a closely related species, oriental fruit moth, overwinter as full grown larvae on the tree or in the ground beneath the tree. The actual distribution of overwintering larvae is dependent on many factors such as: tree variety, ripening date, amount of rough bark on the tree, or amount of weeds and debris on the ground (Steiner 1932).

During my observations practically all possible overwintering sites in the orchard were sampled extensively. The most intensive search effort was in the Douglas abandoned orchard, where pheromone trap data indicated a high number of lesser appleworm males. All observations on soil and on trees revealed no stages of lesser appleworm.

The use of degree-day concept as the only element of predicting lesser appleworm occurrence is not precise enough. The high variability of accumulated heat units for the same insects life events within the same or between different locations suggests that additional monitoring factors must be used for accurate prediction of lesser appleworm life events. Riedl et al. (1976) compared four insect forecasting methods for codling moth and found that degree day and developmental unit summations together with the use of biofix are the most reliable method in predicting the beginning of first generation egg hatch. In my study, I suspect that at least two additional factors may be involved in the low accuracy of forecasting the lesser appleworm presence in orchards: a) lack of data for lesser appleworm physiological thresholds used in predicting lesser appleworm life events and b) the possible variable effects of overwintering sites affecting lesser appleworm appearance in the spring.

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CHAPTER 4

Trap efficacy and flight bionomics of lesser appleworm *Grapholita prunivora* (Walsh) (Lepidoptera : Tortricidae) in Michigan

Introduction

The traditional use of broad spectrum pesticides for fruit orchard pest control creates a situation where minor or occasional pests are not normally encountered in the orchard system. The lack of visible damage provides the illusion that those insects are "nonexistent". Recent ecological, biological, and sociological issues associated with wide use of broad spectrum pesticides in food production has led to the reevaluation of our dominant paradigm of pest management. The development of semiochemical and other non - chemical alternative control strategies has given hope to what McNeil (1991) calls "intelligent management" programs. This may calm many of the ecological and sociological fears that are present, but we are still at the beginning of a long road for common, practical use of those new technologies.

Lesser appleworm *Grapholita prunivora* (Walsh) is one of four important tortricid species that feed internally on apple fruit. As a native North American species, it is a quarantined insect in Europe and Asia (OEPP 1979). The known host are rosaceous plants mainly cultivated apple, plum, cherry, wild hawthorn and crabapple (Chapman &

Lienk 1971, Quaintance 1908, Wellhouse 1920). Lesser appleworm is reported from all main fruit growing areas in US and Canada, but it is considered as a pest of minor importance.

In the past the lesser appleworm did not gain much attention as a pest from researchers and practitioners. Due to similarities in seasonal phenology in most fruit growing regions and characters of fruit injury similar to injury caused by codling moth, *Cydia pomonella* (L). (Chapman & Lienk 1971, Rivard & Mailloux 1974, Howitt 1993) or oriental fruit moth, *Grapholita molesta* (Busck)(Weires et al. 1979), the lesser appleworm was not considered as an economic pest in commercial orchards setting. However in situations where insecticide use was for some reasons significantly reduced or eliminated, lesser appleworm demonstrated its capacity as a serious pest (Glass & Lienk 1971, Weires et al. 1979). In observations with reduced spray schedules in the Hudson Valley the lesser appleworm was responsible for approximately 50 % of injuries caused by all internal lepidopterus feeders (Weires et al. 1979). During a 10 year study of discontinued insecticide use, Glass & Lienk (1971) found lesser appleworm responsible respectively for 72 and 39 percent of the fruit damage during the last two years of the study.

Recognition of the potential pest status of the lesser appleworm began after discovery of its sex pheromone (Roelofs et al. 1969, Roelofs & Carde 1974). The use of pheromone traps for detection and monitoring flight helped in understanding the general flight seasonality of this insect as well as in better understanding its pest status (Howitt 1993, Beers et al. 1993).

For effective monitoring and recommendations for fruit protection we need to understand the life system of a pest species. The behavioral - ecological approach to the study can be very profitable for pest management (McNeil 1991). The behavior of the insect when in contact with a pheromone plume, and the way an insect enters and escapes the trap or plume can be different in different species and therefore affect the accuracy of monitoring (David & Birch 1985). The availability of different trap designs forces the question of which one to use for a specific pest. The analysis of the effects of environmental factors such as temperature and light intensity on daily activity patterns can help in understanding moth behavior in the field (Rothschild & Minks 1974), what in consequence will improve fruit protection.

My studies started in 1991 to investigate the biology of lesser appleworm due to its quarantine status in Europe and Japan. With the possibility of quarantine restrictions, methods for monitoring adult presence in orchards and its practical use were necessary to determine. During my studies I explored the male lesser appleworm daily activity pattern in apple orchards in relation to sunset and the temperature under the tree canopies. Additionally, the efficiency of different trap designs for monitoring lesser appleworm flight were evaluated in field conditions.

Materials and Methods

Location. During the summers of 1991- 1993 observations on lesser appleworm flight biology and the efficacy of six different traps designs were conducted in a 20+ year old abandoned apple orchard in Douglas, MI. There were three dominant apple varieties : "Idared", "Jonathan", and "Red Delicious". The orchard where observations took place was located near the border of the orchard property, surrounded by apple, cherry, pear orchards, and a woodlot. During the study fungicide sprays were applied only in 1991; thereafter, there were no pesticide sprays or maintenance activity in the test orchard.

Trap efficacy. During 1991, 1992, and 1993 six different trap designs were assigned for testing: Pherocon I PC (wing) (Zoecon Co.), Pherocon II (diamond) (Trece Inc.), Pherocon III (delta), Multipher I (Bio-Controle), tube trap, and Gypsy moth trap. Evaluated trap designs were baited with LAW sex pheromone lures (Scentry, Inc.) and placed in the orchard. In 1991 traps were placed in orchard in the middle of July, after the flight of first generation of lesser appleworm. During 1992 and 1993 traps were placed in the orchard at the beginning of the May and remained until the end of flight of second generation. During 1992 and 1993, the tube trap design which caught the lowest number of lesser appleworm during the previous years was removed from the study, and the remaining trap designs tested as in 1991.

Traps were placed in a randomized complete block design, with plots 100 m apart. During 1991 and 1992 each trap design was replicated twice, and during 1993 each trap design was replicated three times. Within the block each of the six different trap designs was located randomly at equal distances around a tree. The position of the traps within each group was rotated clockwise every week. Traps were placed at height 1.5-1.8 m high (Gentry et al. 1974). Sex pheromone lures and traps were changed every four weeks. Traps were checked daily and captured moths removed.

Flight biology observation. Using traps from the trap design efficacy studies, observations on lesser appleworm flight biology timing and temperature were done during the first and second generations flights. Each trap was checked for the presence of male lesser appleworm adults every half hour during late afternoon /early evening from four hours before the sunset to one hour after sunset. The exact time for sunset was obtained from Nautical Almanac Office, United States Naval Observatory, Washington, DC. After evening observation traps were checked again during the next day morning for detection of any moth flight during the night hours. During each sampling interval the number of captured moths and temperature were noted. The presence of other species captured in lesser appleworm pheromone traps was also noted. Captured moths were removed from traps and identified.

Statistical analysis. Data collected during trap efficacy studies were analyzed using one way analysis of variance (ANOVA) with randomized complete block design for total trap catch efficacy during each year. Mean trap efficacy was compared using Duncan's Multiple range Test ($P = 0.05$). Due to the different number of replicates during years for comparison of three year data, two way ANOVA for completely randomized block and Duncan Multiple Range Test ($P = 0.01$) was used for data comparison. Tube trap catch was not included for three-year comparisons.

Results and Discussion

Under the weather conditions of Southwest Michigan lesser appleworm has two generations per year. Flight of the first generation in 1992 and 1993 started in the beginning of May with maximum flight during the middle of June. Flight of the second generation started in early August, with peak activity during the beginning of September. The last moths were collected in late September (Figure 1). Data from the 1991 season are not available because of misidentifications of moth captured during the flight of the first generation.

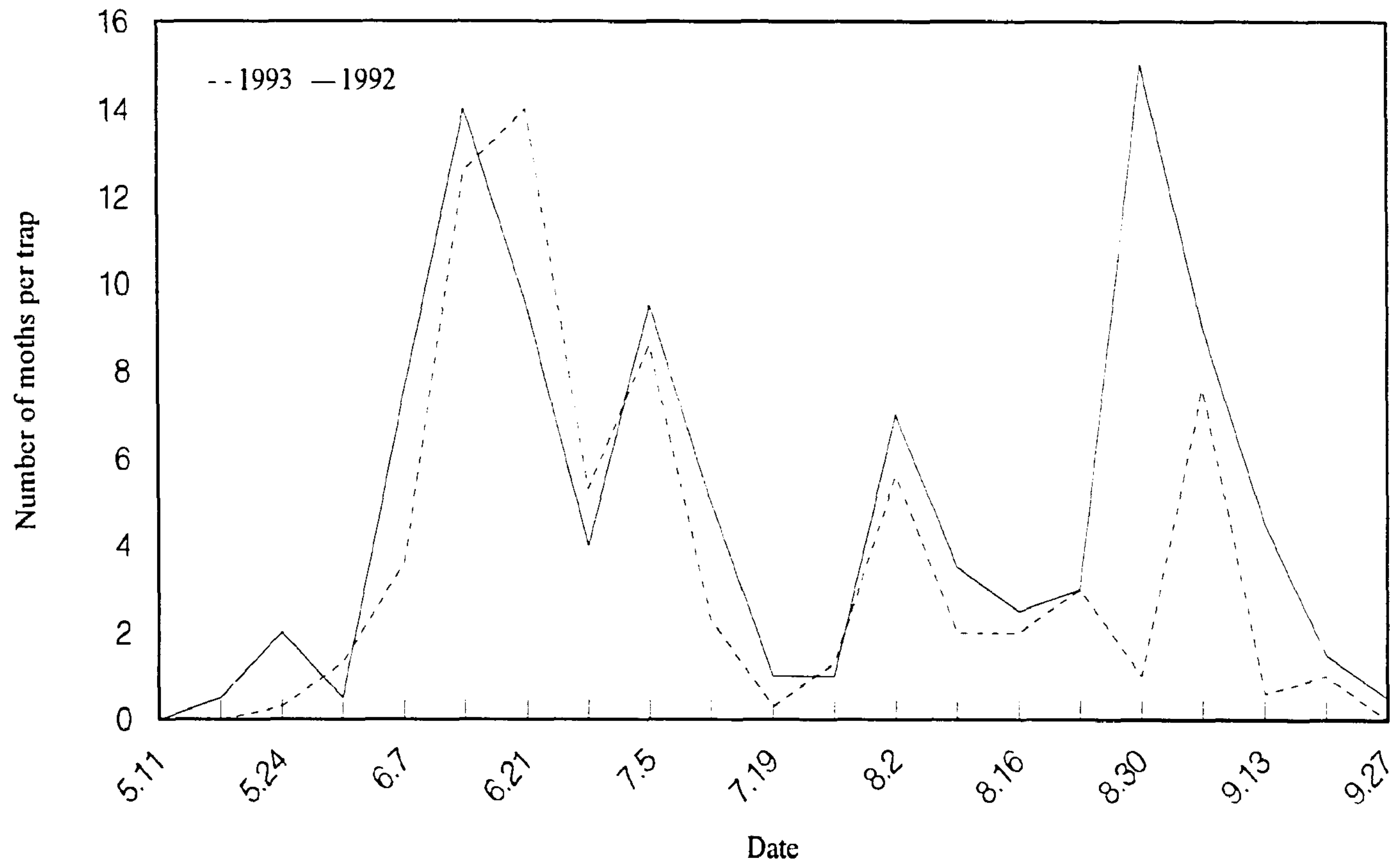

Figure 1. Lesser appleworm flight seasonality during 1992 and 1993 in the abandoned apple orchard, Douglas, MI .

During the study significantly different numbers of adult moth were observed in pheromone traps each year ($F = 4.43$; $df = 2, 4$; $P = 0.0255$) (Figure 2). The highest number of lesser appleworm (499 males) was observed in 1991. During 1992 and 1993 fewer lesser appleworm adults were caught: 169 and 216 respectively. The difference in numbers of collected moths among years is difficult to explain. Some of possible reasons may include different weather patterns during those summers, with summers of 1992 and 1993 colder than of 1991. In 1993 trees had additionally a very light crop, compared to the previous two years, due to early season frost damage. During all three years of study trap x year interaction was not significant ($P = 0.0822$).

Effects of diurnal light periods and temperature on flight. Lesser appleworm moth flight activity was observed at temperatures from 18 °C to 25 °C (64 - 76 °F) (Figure 3). In temperatures below and above those thresholds lesser appleworm males were observed very sporadically. There was no observable lesser appleworm moth flight at temperatures below 17 °C (62 °F) (Figure 3). Flight threshold temperatures for the lesser appleworm are higher than for codling moth or oriental fruit moths. Batiste et al. (1973) reported that adults codling moth were not attracted to pheromone when the temperature was below 13 °C or above 27 °C. During my observations, no lesser appleworm moths were caught at temperature above 29 °C (82 F).

Observations of crepuscular flight showed that lesser appleworm flight activity was limited to only late afternoon and early evening hours, starting 3 hours before sunset and ending no later than 30 minutes after sunset (Figure 4). I found no lesser appleworm flying activity during the night or during full day light. During 1992 - 1994 lesser appleworm was never collected by using UV light traps (R. Kriegel, per com. 1994).

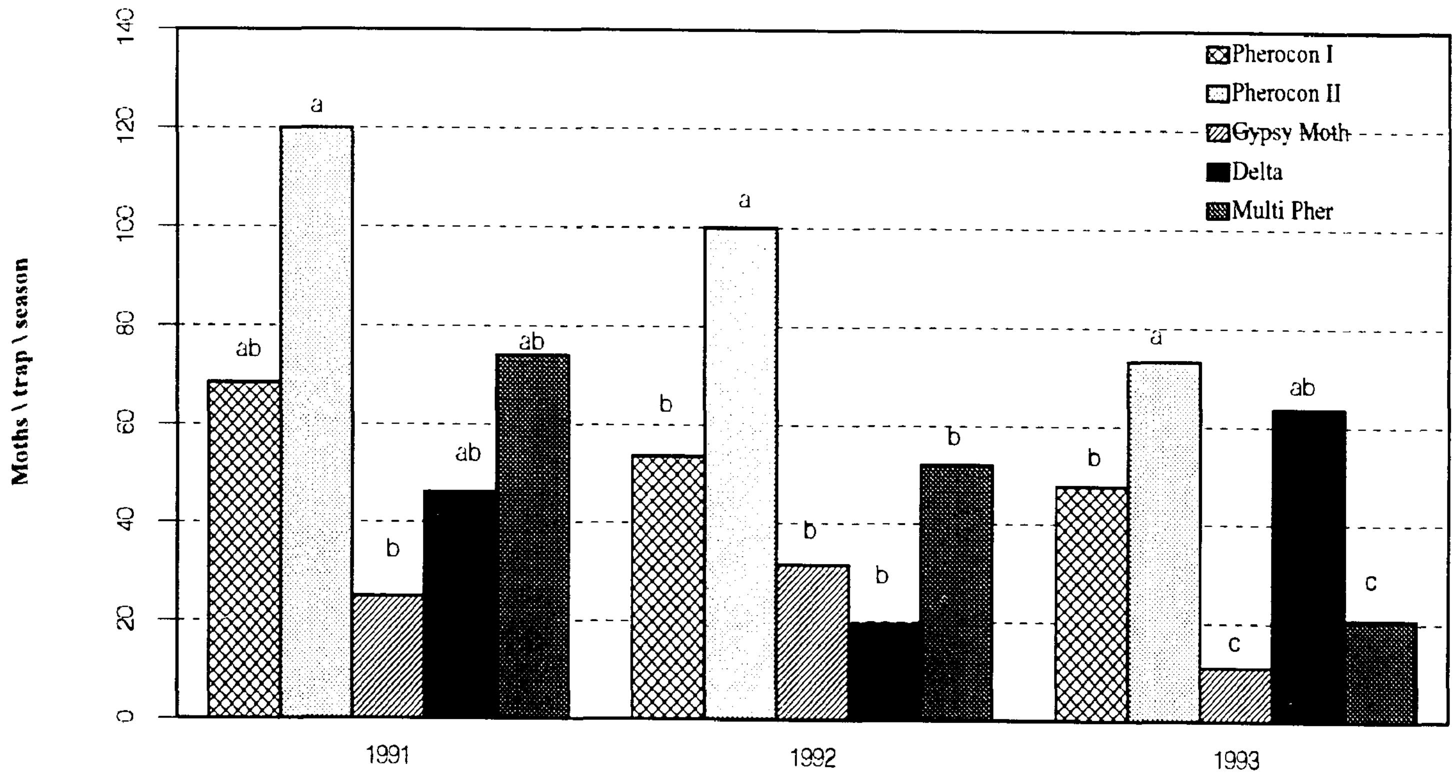

Figure 2. Comparison of average number of lesser appleworm moths captured per trap during the season. In 1991 data were collected only during the flight of second generation of lesser appleworm (after July 15).

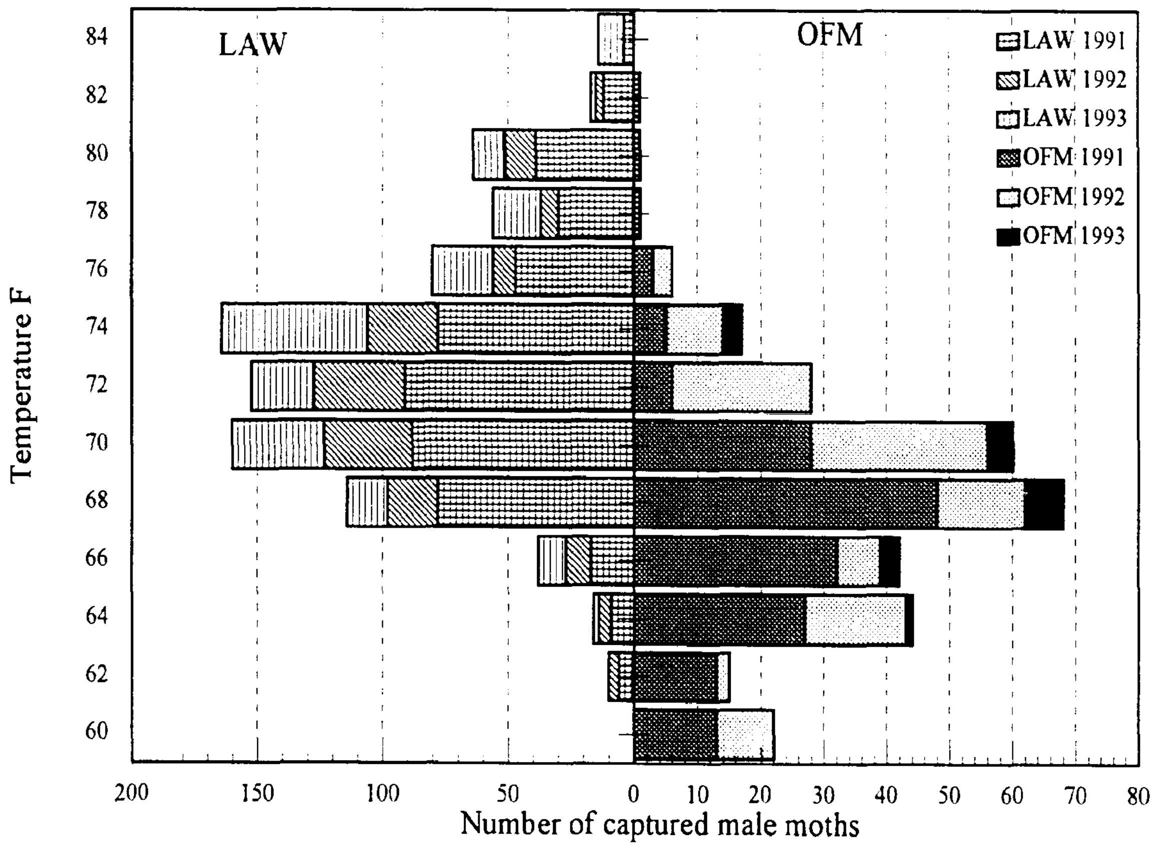

Figure 3. Lesser appleworm (LAW) and oriental fruit moth (OFM) flight pattern in relation to ambient temperature. Data collected in abandoned apple orchard, using of Pherocone II traps and LAW sex pheromone during 1991-1993, Douglas, MI.

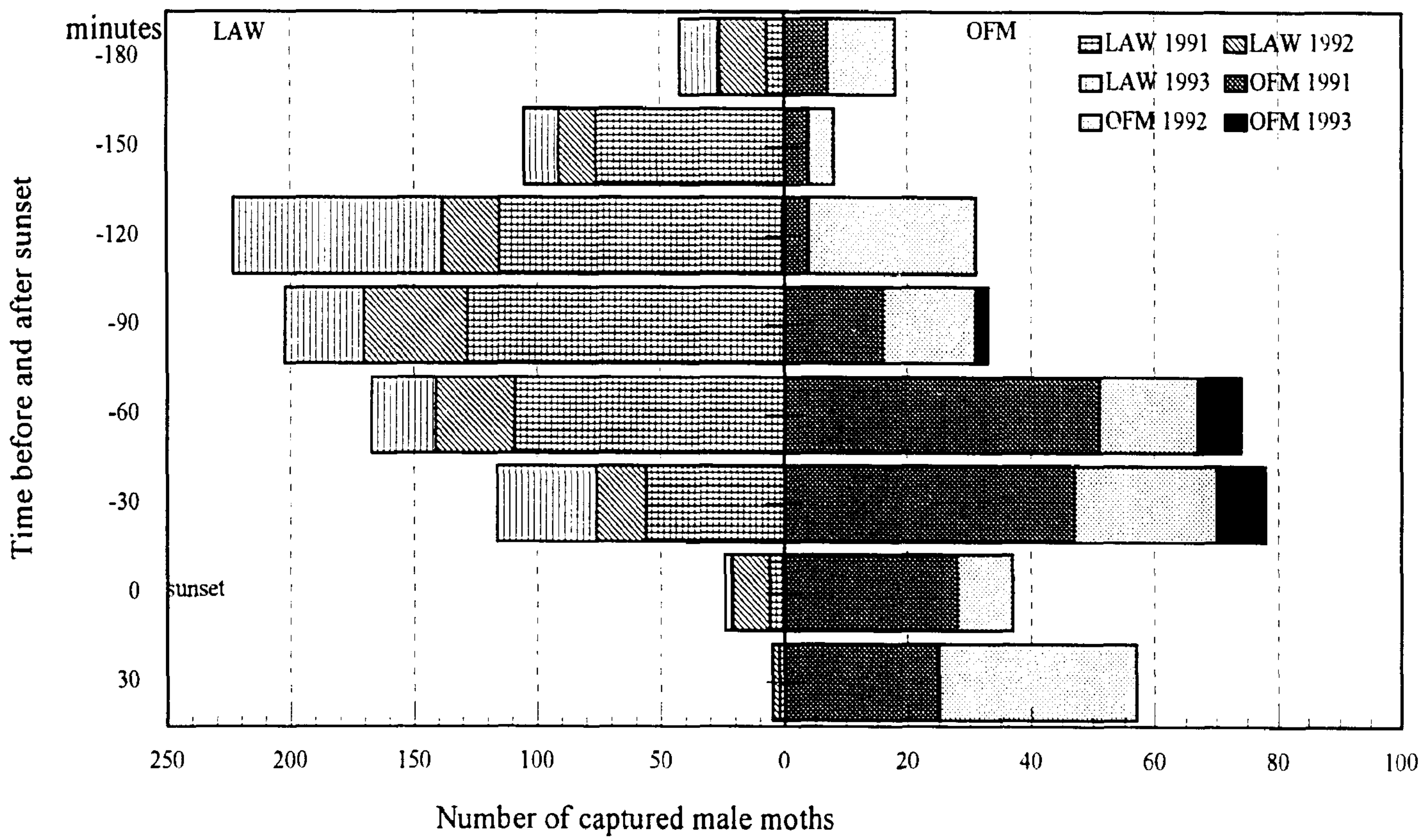

Figure 4. Crepuscular flight of lesser appleworm (LAW) and oriental fruit moth (OFM). Data collected in abandoned apple orchard, using Pherocone II traps and the lesser appleworm sex pheromone during 1991 - 1993, Douglas, MI.

The highest number of moths was observed 120 min to 60 min before sunset. Lesser appleworm moths observed by Gentry et al. (1975) in Georgia also terminated flight activity always before dark (9 p.m.). My observations showed that during the days with higher temperatures lesser appleworm males tended to start flying later in the day, but earlier on days with cooler temperatures. A similar trend was observed in codling moth flight (Castrovillo and Carde 1979), cabbage looper, *Trichoplusia ni* (Hbn.)(Sower at al. 1971), lesser peach tree borer, *Synanthedon pictipes* (Grote & Robinson) (Gorsuch et al. 1975), and spruce budworm, *Choristoneura fumiferana* (Clem.) (Sanders & Lucuik 1972). For *Argyrotaenia velutiana* (Walker) and five other Lepidoptera species Comeau et al. (1976) found that the diel periodicity was modified by ambient temperature.

Comparison of different trap designs for capturing males of lesser appleworm. Comparing the six trap designs for monitoring flight, the Pherocon II trap captured the highest number of adult lesser appleworm males (Table 1). Although Pherocon I CP, Multipher and Delta trap designs caught significantly lower number of moths (P =0.01) they are also useful for monitoring lesser appleworm flight. The Gypsy moth trap every year showed the lowest number of captured males of lesser appleworm except in 1991, when tube trap was included in observations. The tube trap was removed from observation after 1991 due to very low moth capture.

Trap choice plays an important role in monitoring insect activity in the field. Beroza et al. (1973) , Lewis & Macaulay (1976) and many other authors (see references in Carde 1979) discuss the importance of the right trap design for accurate monitoring. During a pheromone trap efficacy study on oriental fruit moth and lesser appleworm, Gentry et al. (1975) found that the Pherocon 1CP trap design caught the highest number

Table 1. Comparison of six pheromone trap designs for capturing males of lesser appleworm. Data collected in Douglas, MI abandoned orchard during 1991 -1993

Trap design	Number of collected lesser appleworm moths per trap design / season			
	1991	1992	1993	1991 - 1993
Pherocon II	120 a	100 a	73.7 a	94.42 a
Pherocon I	68.5 ab	53.5 b	47 b	55.28 b
Delta trap	46 a	19.5 b	63.7 ab	46.00 bc
Multi pher trap	74 ab	52 b	13.3 c	41.71 bc
Gypsy moth trap	25 b	31.5 b	11 c	20.85 c
Tube trap	21 b	n/a	n/a	n/a

Means in the same column followed by the same letter are not significantly different at P = 0.05 Duncan Multiple Range Test of oriental fruit moth. They did not have the Pherocon II trap in the observations. Lewis & Macaulay (1976) during a study on traps for pea moth, *Cydia nigricana* (Steph.) examined the effect of trap design on the pheromone plume shape emitted and its resulting effects on trap catch. The triangular shaped trap, that resembles the Pherocon II traps, caught the most moths. Because of this and the ease in servicing this trap, it is now the most commonly used trap in apple IPM programs (Johnson & Herr 1995).

During this study additional data were also collected on the flight biology of oriental fruit moth. Using the same traps and pheromone for lesser appleworm I collected specimens of both species. This is not surprising since both species share the same main component of their sex pheromone: *cis*-8-dodecenyl acetate (c8-12:Ac) (Roelofs et al. 1969). The optimum mixture for capturing lesser appleworm male contains 2.2 percent of *trans* isomer, while for oriental fruit moth the optimum percentage of *trans* isomer is 6.4 percent (Roelofs & Carde 1974). Linn and Roelofs (1989) using oriental fruit moth as an example, discussed the importance of each component in the multicomponent blend of pheromones. Minimal changes in the *cis* / *trans* ratio of isomers changed significantly the level of male response.

The similarity between the sex pheromone of those two species allowed the collection of additional data about oriental fruit moth flight pattern by using only lesser appleworm pheromones. During 1991, 1992, and 1993 I caught 182, 137, and 17 males of oriental fruit moth. Gentry et al. (1974) used different blends of oriental fruit moth pheromone and captured numerous males of lesser appleworm when the concentration of E (*trans*) isomer was 2.5 -7.5%. In other study Gentry et al. (1975) also collected pecan bud moth, *Gretchena bolliana* (Slingerland), using the oriental fruit moth pheromone. In my tests using commercially available oriental fruit moth pheromone (Trece, Inc.) I did not capture males of lesser appleworm, which suggests a slight difference in the mixture of the lure used in this study.

Oriental fruit moth males started flight at a lower temperature than lesser appleworm (Figure 3). A temperature below 17 °C was not a barrier for moth activity. Rothschild & Minks (1974) in their study in Australia did not capture oriental fruit moth when temperature was below 15 °C. In Europe the lower flight activity threshold is 11 °C (Roehrich, 1961). Under my study conditions the highest trap catch occurred in temperatures from 18 °C to 22 °C. In temperatures above and below this range, I observed a significantly lower number of oriental fruit moth. Rothschild & Minks (1974) did not observe temperature dependency in their work, and suggested rather that flight appeared to be an "all" or "nothing" activity above or below the threshold. Further investigation are necessary to determine the reason that oriental fruit moth males were captured in this study. One of possibilities may include suggestion, that the release of the pheromone is mediated by temperature, and different ratios of *cis/trans* isomers are released at different temperatures.

The oriental fruit moth started flight at similar intervals before sunset as the lesser appleworm, but 69.6 percent of the males were captured during the 90 minutes before sunset (Figure 3 and Figure 4). Out of all captured oriental fruit moth males 17.8 percent were captured 30 min. and more after sunset. Rothschild and Minks analysis (1974) showed that flight is primarily related to sunset time, but may also be modified by temperature. Under their conditions, activity began at 150 min before sunset and peaked at 60 min before sunset to shortly after sunset. Gentry et al. (1975) did not relate their data to sunset but showed that oriental fruit moth activity started before that of lesser appleworm and lasted longer into the night, with some captures between 3 - 5 am.

Among available varieties of pheromone trap designs Pherocon II and Pherocon I proved to be the most useful and accurate for lesser appleworm flight monitoring. Other types of trap were not as useful for accurate moth observations. By using the daily pattern of lesser appleworm flight activity in relation to light intensity and temperature more accurate monitoring programs can be developed. During the length of study, observation on flight pattern also proved to be very useful for successful maintaining of moths colony.

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CHAPTER 5

Lesser appleworm *Grapholita prunivora* (Walsh) (Lepidoptera : Tortricidae) oviposition preference and female fecundity when reared in laboratory

Introduction

Lesser appleworm, *Grapholita prunivora* (Walsh) (Lepidoptera : Tortricidae) belongs to a group of pests whose larvae are internal fruit feeders. This species, an indigenous North American insect, is reported from all of the most important pome fruit growing areas of United States and Canada. Larvae of lesser appleworm can be commonly found in abandoned apple orchards, but rarely in commercially protected blocks (Beers et al. 1993, Howitt 1993). Larvae feed mainly on fruit of rosaceous plants including: cultivated apples, pears, peaches, plums and on wild hawthorns, crabapples, and roses (Chapman & Lienk 1971, Quaintance 1908).

Due to its low economic significance lesser appleworm tends to be classified as a secondary or even accidental pest in orchards. According to Chapman's (1973) classification of host categories, apples appears to be the primary host for this pest. Lesser appleworm adults use apple fruit as an oviposition site and emerged larvae are able to complete their development by using only this food source (Chapman 1973).

The current host range of lesser appleworm suggests that before the introduction of cultivated apple into North America, hawthorn and crabapple were probably used as the primary hosts. After introduction of apples to North America , about twenty native tortricid species switched to apples and used it as a main host (Chapman 1973). Within the Rosaceae family, apples are the most closely related cultivated fruit to native crabapple and hawthorns (Chapman & Lienk 1971). The close botanical relationship, similarity in habitat, and abundance of cultivated trees were probably the main factors for exploiting them as a feeding and oviposition site (Wellhouse 1920).

Ovipositional preference of different female insects species has been studied extensively during last years. Behavioral, genetic, and ecological factors are important determinants in insect oviposition behavior (Thompson & Pellmyr 1991). Overviews of the most recent research developments can be found in Browne (1993), Thompson & Pellmyr (1991), Jaenike (1990), Renwick (1989), and Courtney et al. (1989).

The objective of this study was to determine the oviposition preference of lesser appleworm, using two commercially grown and one natural host plant in a simultaneous choice experiment. I also evaluated the number of the offspring per single female when limited choices of ovipositional sites were available.

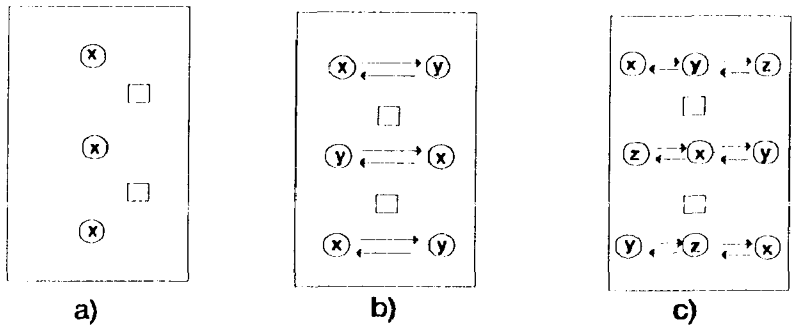
Materials and Methods

During the summer of 1994 a lesser appleworm colonies was established from adults reared out of apple fruit collected from four different abandoned orchards. Fresh "Red Delicious" and "Golden Delicious" apples varieties were used as the main larval food for maintaining the lesser appleworm colony in the laboratory.

For testing lesser appleworm oviposition preference between two cultivated and one wild hosts, a simultaneous choice experiment was designed as referred by Singer (1986). During July and August fruits from apples (var. "McIntosh'), plums (var. "Stanley") and wild hawthorn were collected and used for testing lesser appleworm oviposition preference. During the experiment seven different combination of fruits were tested: 1) apples, 2) plums, 3) hawthorns, 4) apples plus plums, 5) apples plus hawthorns, 6) plums plus hawthorns, and 7) apples plus plums plus hawthorns. Fruits were placed in clear 40.3 cm L x 27.7 cm W x 15.1 cm H plastic boxes (Sterilite Corp. USA) covered with mesh top lids. In various treatment combinations three fruits of apples, plums or/and hawthorn clusters (8 - 12 fruit per cluster) were attached to box bottom with double - stick magic tape (Scotch Corp. USA). The design of the assays is shown in Figure 1. All combinations were designed to be replicated four times and performed twice: in July and in August. Due to female mortality after release into cages, the second series of experiments (in August) contained only three replications of each combination.

At the beginning of the experiment one male and one female adult lesser appleworm were released into each box. Water and food were provided in two small plastic containers with cotton dipped in a) water and b) honey diluted by water and placed in every box. Water was added every day to both food containers. Adult moths released into the boxes were a maximum of two days old, and were obtained from a laboratory colony.

Boxes with fruit and adult moths were placed in outside conditions (ambient temperature, daylength) but were protected against rain. After two to four weeks all fruits were examined for the total number of eggs laid and emerged larvae. Due to the gradual



(x) (y) (z) - fruit assignment inside the box

[ ] - placement of containers with water and honey and water

Figure 1. Position of fruit inside the boxes: a) one host design, b) two hosts design, c) three hosts design. Arrows indicated changed positions of fruit within replications.

availability of lesser appleworm male and female adults, the duration of different combinations and replications varies. Exact dates are shown in Table 1.

Results were collected by counting number of emerged larvae and number of non hatched eggs. Data were compared by the use of an index of preference as described by Tabashnik et al. (1981) (modified by author for experiment):

$$\text{Index of preference} \frac{\text{number of larvae and eggs collected from } x \,/\, \text{or } y \,/\, \text{or } z}{\text{number of larva and eggs colected from } x \,/\, y \,/\, z \,/\, \text{ and } x \,/\, y \,/\, z_{a}} \times 100\ \%$$

a) depending on combinations

In the above index values close to 50% show no preference, below 50% show a tendency to prefer alternate host(s), and above 50 % suggest the tested fruit is preferred as an ovipositional site for lesser appleworm.

Table 1. Starting and ending dates for first and second series replications of lesser appleworm oviposition preference experiment.

Combination		Dates						
		First series				Second series		
		Rep. 1	Rep. 2	Rep. 3	Rep.4	Rep. 1	Rep. 2	Rep.3
1. Apples	Start	30-Jul	30-Jul	30-Jul	30-Jul	20-Aug	20-Aug	20-Aug
	End	25-Aug	25-Aug	25-Aug	25-Aug	10-Sep	10-Sep	10-Sep
2. Plums	Start	6-Aug	8-Aug	6-Aug	8-Aug	13-Aug	23-Aug	23-Aug
	End	25-Aug	25-Aug	25-Aug	25-Aug	10-Sep	10-Sep	10-Sep
3. Hawthorn	Start	8-Aug	8-Aug	8-Aug	8-Aug	20-Aug	20-Aug	20-Aug
	End	25-Aug	25-Aug	25-Aug	25-Aug	10-Sep	10-Sep	10-Sep
4. Apples + Plums	Start	28-Jul	28-Jul	28-Jul	28-Jul	26-Aug	26-Aug	26-Aug
	End	25-Aug	25-Aug	25-Aug	25-Aug	10-Sep	10-Sep	10-Sep
5. Apples + Hawt.	Start	8-Aug	2-Aug	2-Aug	8-Aug	18-Aug	18-Aug	18-Aug
	End	25-Aug	25-Aug	25-Aug	25-Aug	10-Sep	10-Sep	10-Sep
6. Plums + Hawt.	Start	2-Aug	9-Aug	10-Aug	8-Aug	18-Aug	18-Aug	18-Aug
	End	25-Aug	25-Aug	25-Aug	25-Aug	10-Sep	10-Sep	10-Sep
7. App.+Pl.+ Hawt.	Start	30-Jul	2-Aug	2-Aug	2-Aug	24-Aug	24-Aug	24-Aug
	End	25-Aug	25-Aug	25-Aug	25-Aug	10-Sep	10-Sep	10-Sep

The indices of difference in number of eggs laid between series and among different combinations were subjected to analysis of variance (ANOVA) and Tukey's HSD (T-Method) tests (P = 0.05). For comparison of numbers of lesser appleworm progeny in the presence of specific food categories within series, analysis of variance (ANOVA) and SNK test (P=0.05) were used.

The observations of total number of eggs per one female were also analyzed and the Kolmogorow - Smirnow test was used for testing the normality of the observed frequency distribution (Sokal & Rohlf 1981).

Results and discussion.

This study was based on the assumption that insect females when exposed to many potential hosts will show a hierarchy in their preference (Thompson & Pallmyr 1991, Beach & Todd 1988, Sharma & Norris 1991). Use of simultaneous choice trials permitted for lowering the bias from variation in oviposition preference that may occur among females (Tabashnik et al. 1981).

The mean number and range of progeny of lesser appleworm in the different treatment combinations of fruit are shown in Table 2. During the first series of experiments the highest number of lesser appleworm larvae and eggs were found in the combination including apples and plums. Trials of plums, hawthorns, apples plus hawthorns, apples plus plums, and apples plus plums plus hawthorns did not show a significant difference in observed female fecundity. Females in treatment combinations of apples alone, and plums plus hawthorns laid significantly ($P < 0.05$) lower numbers of eggs than in the apples plus plums treatment combination. During the second series of

Table 2. Oviposition preference by lesser appleworm in simultaneus choice experiment.

Combination	First series[1]			Second series[2]			Total
	Mean ± SEM	Significance[a]	Range	Mean ± SEM	Significance[a]	Range	Mean ± SEM
1. Apples	10.75 ±12.47	b	0 - 23	9.00 ± 5.13	a	6 -14	10.00 ± 9.22
2. Plums	16.25 ± 10.28	ab	4 - 27	1.00 ± 1.00	a	0 - 2	9.72 ± 10.93
3. Hawthorn	21.75 ± 5.97	ab	13 - 26	9.67 ± 2.88	a	8 - 13	16.57 ± 7.89
4. Apple	19.25 ± 15.50	a	3 - 39	11.67 ± 17.67	a	0 - 32	16.00 ± 14.36
Plum	19.25 ± 15.37		5 - 41	1.67 ± 2.89		0 - 5	11.71 ± 14.47
5. Apples	12.50 ± 9.47	ab	3 - 25	6.67 ± 9.87	a	0 - 18	10.00 ± 9.33
Hawthorn	11.75 ± 8.92		1 - 22	5.00 ± 7.00		0 - 13	8.86 ± 8.32
6. Plums	2.00 ± 0.82	b	1 - 3	0.67 ± 0.57	a	0 - 1	1.43 ± 0.98
Hawthorn	6.75 ± 4.35		3 - 13	3.00 ± 3.00		0 - 6	5.14 ± 4.06
7. Apple	12.00 ± 6.63	ab	3 - 19	2.33 ± 2.08	a	0 - 4	7.85 ± 7.08
Plums	4.50 ± 3.11		2 - 9	3.00 ± 2.00		1 - 5	4.00 ± 2.58
Hawthorn	7.75 ± 3.59		3 - 11	4.33 ± 0.58		4 - 5	6.86 ± 3.63

1- data from 4 replications
2- data from 3 replication
a -caltulated for cumulative data for all fruit in combination

experiments significant differences ($P < 0.05$) among treatment combinations were not observed. At the same time there were significant ($P < 0.05$) differences in female fecundity between the first and second series experiments (Figure 2). The lack of significant differences among treatment combination during the second series experiment may be a result of many factors. This second series was conducted later during the summer, when atmospheric conditions were different. Maturity difference of fresh fruit that was used in the two experiments may also be important. Jaenike (1990) showed that the chemical structure of plants may play an important role in determining its usability and exploration by insects.

Among the four treatment combinations that included apples as one of the offered ovipositional sites (Table 3), only apples in combination with hawthorns during the first and second series experiments, and with plums during second series experiments were preferred as an ovipositional site. Given a choice among two other possible sites, apples alone were significantly ($P < 0.05$) not preferred by lesser appleworm females. Plums were chosen predominantly over other fruit only in combination with apples, but the level of preference was low. Plums in both series were a non - preferred ovipositional site when hawthorns, or apples plus hawthorn alternatives were available (Table 3). Significantly more ($P < 0.05$) larvae and eggs were observed during both series on hawthorn fruit compared to plums when those were the only choice. In the same time hawthorns was not preferred as an ovipositional site when apples were present. In a hawthorns plus apples plus plums combination indexes of preference for hawthorns were 33.38 % and 51.9 %. A similar lack of strong ovipositional preference was demonstrated by the butterfly *Colias eurytheme* (Tabashnik et al. 1981), soybean looper, *Pseudoplusia*

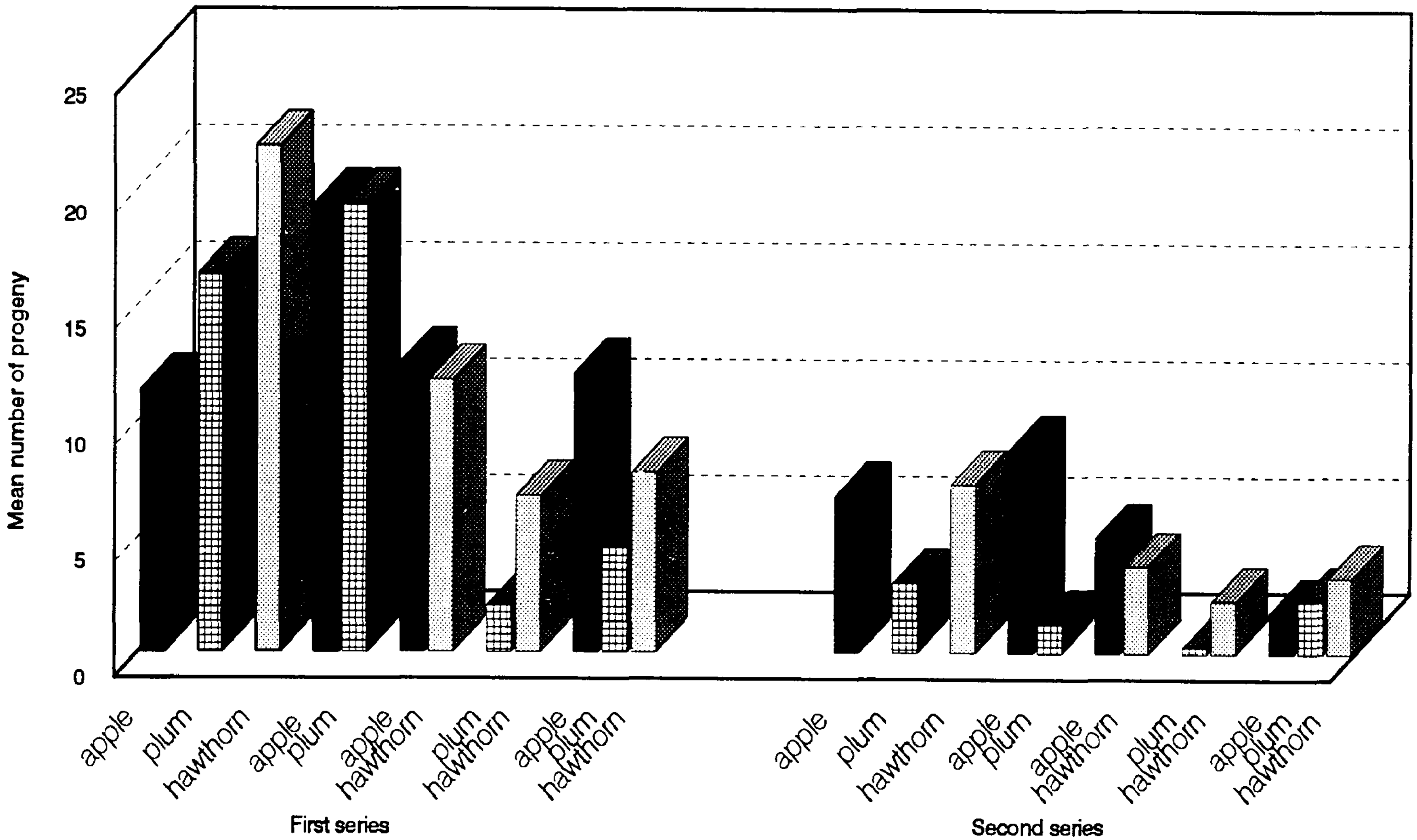


Figure 2. Comparison of mean number of lesser appleworm progeny per combination during first and second series experiments

Table 3. Indexes of preferences for lesser appleworm oviposition on three different hosts.

Combination	First series [b, c]	Second series [b, c]
1 a) *Apples* [a]	100 a	100a
1 b) *Apples* + Plums	47.28 b	78.83 ab
1 c) *Apples* + Hawthorn	54.75 b	52.70 ab
1 d) *Apples* + Plums + Hawthorn	47.60 b	19.53 b
2 a) *Plums*	100 a	100 a
2 b) *Plums* + Apples	52.72 b	21.17 a
2 c) *Plums* + Hawthorn	25.20 c	38.07 a
2 d) *Plums* + Apples + Hawthorn	19.03 c	28.57 a
3 a) *Hawthorn*	100 a	100 a
3 b) *Hawthorn* + Apples	45.50 bc	47.30 a
3 c) *Hawthorn* + Plums	74.78 ab	61.90 a
3 d) *Hawthorn* + Apples + Plums	33.38 c	51.90 a

a - in an italics - host for comparison
b- data analysed used anlysis of variance (ANOVA) and Tukey's (HSD) test at $P < 0.05$
c - when no eggs were laid no preference was assumed (index of preference =50%)

includens (Walker) (Beach & Todd 1988) and by beet armyworm, *Spodoptera exigua* (Hübner) on cultivars of chrysanthemum (Yoshida & Parrella 1991). Insects use wide arrays of stimuli for choosing possible oviposition site. For example, females of codling moth, *Cydia pomonella* (L.), commonly known fruit pest, during their search for an ovipositional site will more likely oviposit on fruit that have some level of alpha farnesene isomer than on fruit without it (Wearing & Hutchins 1973). There are no data available in the literature indicating which factor(s) may lead lesser appleworm females in their choice of ovipositional site.

The results show that lesser appleworm will accept cultivated hosts as well as wild ones. The data did not answer the question of which factors played the most important role in host discrimination for lesser appleworm. Small arena size may have had a negative impact on the importance of prealighting host discrimination by females (Renwick 1989). Recognition and acceptance of the host plant by gravid females after landing depends on numerous physical and chemical stimuli such as surface structure or presence of specific chemical compounds (Renwick & Chew 1994).

Hawthorn, which has served as a native host for lesser appleworm (Chapman & Lienk 1971, Wellhouse 1920) for many years appears to be preferred over plums. The use of apples as larval food during colony rearing may have had some effect on female choice between different hosts and apples. An insect's previous experience from larval stage may be important for acceptance of oviposition site. One of North America's the most important native apple pests, the apple maggot *Rhagoletis pomonella* (Walsh), will more likely oviposite on a fruit type with which it has previous experience (Papaj & Prokopy 1988).

Single females used for observations laid different numbers of eggs. In two replications during first series of experiments and in two replications during the second series, no single egg or larva was observed on the fruit. Of the remaining females (n = 45) that oviposited during the experiment 64.4 percent (n = 29) laid less than 16 eggs. The highest observed number of eggs per single female was 64. There is no information in the literature about possible lesser appleworm female fecundity. In the old literature Quaintance (1908), Foster & Jones (1909), and Taylor (1909) mentioned fruit surface as a preferred ovipositional site; however, no data on the number of eggs per single female was mentioned. Figure 3 shows the frequency of eggs per single female and the results of a test for normality of distribution.

More research is needed on the evaluation of food preference and larval performance of lesser appleworm on different hosts. No-choice trials with many different hosts may contribute to a better understanding of adult ovipositional preference. Sequential choice trials which allow insects to make contact with more than one host, which may more closely reflect what naturally happens during the host search behavior (Singer 1986), may explain the complexities of lesser appleworm oviposition behavior more thoroughly.

In the real orchard situations, when only cultivated hosts are available, lesser appleworm will likely use them for completing development. The presence of hawthorn trees in the surrounding areas may have an effect on lesser appleworm performance. Rely on conditions, lesser appleworm may choose its host. Monocultural abundance of orchard fruits may turn a wild hawthorn insect population into a fruit pest. Presently, this situation is probably avoided by the use of chemical control of orchard pests. However,

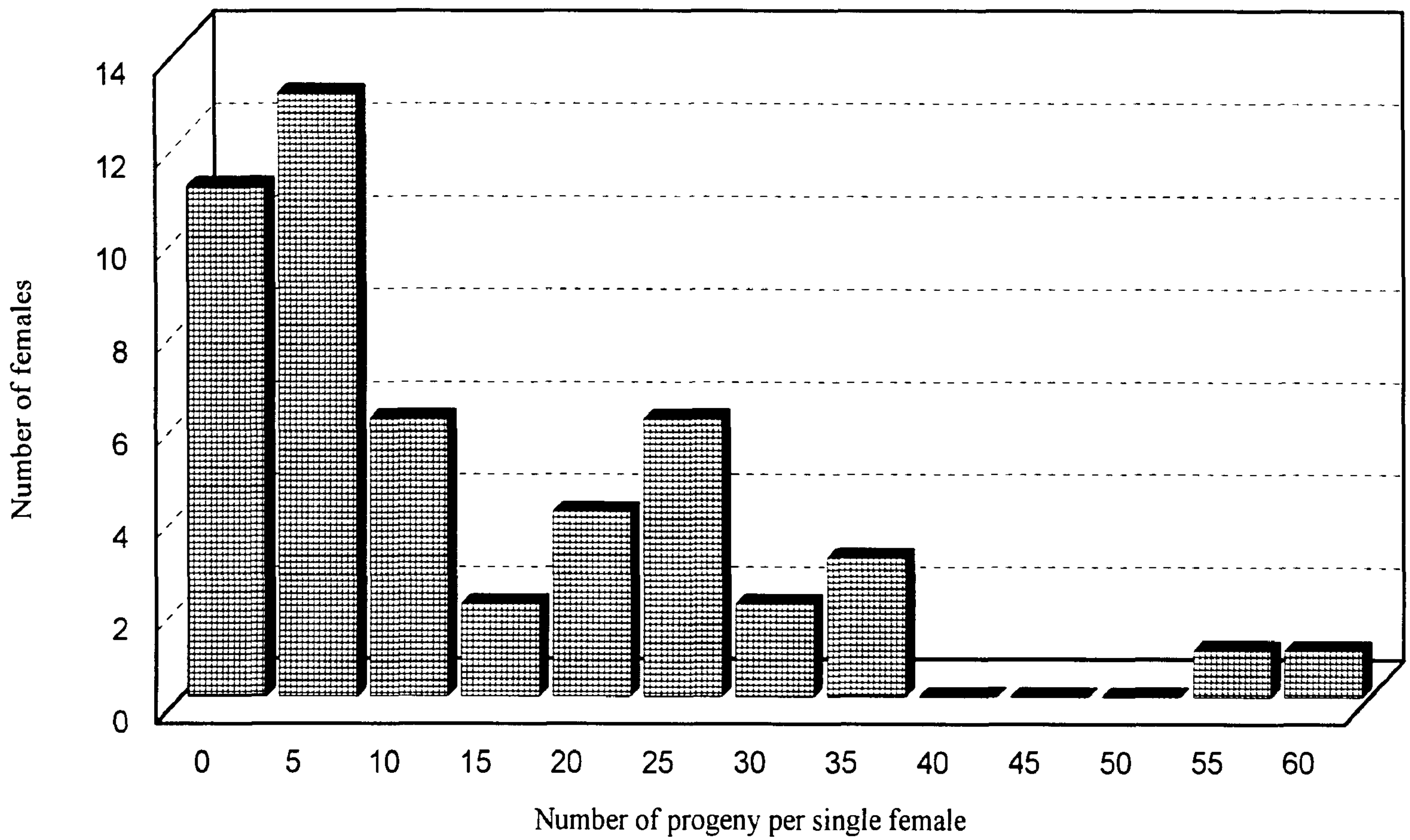


Figure 3. Frequency histogram of lesser appleworm *Grapholita prunivora* (Walsh) females fecundity when reared in simultaneous choice situation

use of non-chemical methods for fruit pest control may invite insects such as the lesser appleworm to benefit from this new available space.

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CHAPTER 6

Larval and pupal characters for identification of lesser appleworm *Grapholita prunivora* (Walsh), oriental fruit moth *G. molesta* (Busck), and cherry fruitworm *G. packardi* Zeller (Lepidoptera : Tortricidae)

Introduction

Current trends in fruit protection against pests require that pesticides will be applied only when they are necessary. Integrated pest management practices or its single components are primary components of fruit protection methods in the majority of North American orchards. The primary activities employed for achieving the goal of lowering the use of pesticides are: monitoring, utilization of multiple management tactics, action thresholds, and IPM education or use of IPM communication systems (Whalon & Croft 1984). Monitoring and sampling of pest populations are two of the most important elements in the process of decision making in IPM. The correct estimation of pest density, its economical threshold and phenological forecasts are elements on which the IPM decision making processes rely the most (Binns & Nyrop 1992). Use of effective sampling procedures and the ability to correctly identify insects in the field are also important elements for effective pest control.

Most commercially grown plants are under continuous attack from various insect pests. Most of the potential plant enemies have already been described and identified. Some however, especially ones that occur sporadically, may cause a problem when for some reason they will appear in a plantation and start to cause important damage to the crop. Insects that can use a plant as a secondary, accidental or incidental host (Chapman 1973) are a good examples of how such a situation can occur. Identification and separation of insect life stages in many situations are the most crucial first steps in protection from damage.

Identification and proper arrangement of an organism into the right classification is a crucial first step during any research activity. Only when the object of the research is properly identified can one try to find reasonable and effective ways of dealing with a problem (Danks 1988). Examples of the importance of taxonomy and systematics in integrated pest management programs in Southeast Asia are broadly discussed by Hardy (1982). The correct identification of a pest by itself may be a solution to a problem, because the necessary information may be often found in the literature if one knows which species to search for.

Although the identifications of adult pest insects, is relatively accomplishable, especially for insects that are common in a given area, identification of larval or pupal stages is often much more difficult. Detailed examination, often using powerful microscopes may be necessary for separating closely related species.

With our current knowledge, the larvae of three internal apple feeders: the lesser appleworm, *Grapholita prunivora* (Walsh), the cherry fruitworm, *Grapholita packardi* Zeller and the oriental fruit moth, *Grapholita molesta* (Busck) are very difficult to distinguish and can only be separated using characters such as size and coloration retained after boiling them in hot water and preserving in 70 % ethanol (Chapman & Lienk 1971, Brown 1987). The number of crochets on ventral and anal prolegs was also used to separated the two first species from the later one by MacKay (1959), but this is an ambiguous characteristic.

The current study focused on identifying potential characters for identification of larval and pupal stages of these three species, and diagnoses of fruit injuries caused by lesser appleworm, oriental fruit moth and cherry fruitworm larvae. From the fruit growers point of view, larval injury recognition may be of the same importance, especially since fruit injuries are usually the first sign of a pest presence in the system.

Observations in the field , laboratory, and under light and scanning electron microscope were used in search of dependable characters for correct pest diagnosis.

Materials and Methods

Insect colony. During the summers of 1992 - 1994, apples were collected from abandoned orchards located in Douglas, Shelby, Kalamazoo, and Casnovia, MI. Larvae collected from apples were reared in growth chamber at 16 : 8 h photoperiod, and temperature 25: 16 °C respectively for development of insects. Emerged adults were collected and placed into 40.3 cm L x 27.7 cm W x 15.1 cm H plastic boxes (Sterilite Co. USA) with nine apples arranged in three groups of three fruits. Approximately 10 males and 10 females were released in each box. Moths in each box were supplied with small plastic containers with water and a water and honey solution replaced every second day. During July, rearing boxes were kept outside under ambient conditions, while starting from August growth chambers were used for insect rearing. Twilight conditions were provided by a one 75 W light bulb placed in the middle of the growth chamber turned on for two hours daily. Larvae collected from fruits were reared in Petri dishes, using pieces of apple as food source. Each Petri dish had two filter paper disks placed inside for a pupation site. Every 3 to 4 days drops of water were added to the filter paper for providing moisture. After eclosion adults were carefully transferred to a new box with fresh fruits.

During fall and winter I used apples collected during late summer (August, September) from pesticide - free orchards and they were placed in cold storage. Four apples varieties were used for maintaining moth colonies: "Red Delicious", "Empire", "Golden Delicious", and "Granny Smith". The last variety was used mainly during winter, when no other apple variety could provide a food source.

Pupae. Pupae of lesser appleworm, oriental fruit moth and cherry fruitworm were collected from the same apple blocks as for starting colony. All pupae removed from fruits were placed in plastic vials with a loose stopper for air flow. Each pupa was examined under a light microscope (Carl Zeiss, Germany), using a 10 x graduated ocular eyepiece. Two hundreds units under microscope equaled 10 mm.

Three diagnostic characters were measured on each individual: a) length of pupa from anterior of frontal ridges to posterior tip of anal segment, b) width of dorsal part of mesowing behind prothorax, and c) width of dorsal part of anal segment. These parts were chosen as the characteristic elements, being important in the optical image of the pupae. Each pupa was categorized as to species and sex. Assignment of each pupa into male or female group was based on the number of independently movable abdominal segments. Male pupae have four, whereas female pupae have three movable abdominal segments (Adler 1991). Pupae were placed in vials until adult emergence when species and sex assignment were verified. After emergence, adults were used to augment the insect colony.

Larvae. Larval measurements were made only on larvae collected out of established moth colonies. After leaving the fruit and moving between filter papers inside Petri dishes, last instar larvae were collected and preserved using KAAD or hot water and stored in 70 % ethanol. The number and structure of prongs on the anal comb, the number of crochets on last pair of ventral prolegs, and the number of crochets on anal prolegs were determined under 50 x magnification using light microscope (Carl Zeiss, Germany). Voucher specimens have been deposited in the Center for Insect Diversity Study in Department of Entomology at Michigan State University.

Scanning electron microscopy. Pupae of the three examined species were also observed on a JEOL scanning electron microscope (JSM - 6400V) at the Michigan State University Center for Electron Optics. Pupae collected from known moth colonies were fixed in glutaraldehyde with buffer, washed in phosphate buffer, and gradually dehydrated through a series of 25%, 50 %, 75%, 95% and 100% ethanol using 30 minutes per step. Samples were then critical-point dried (Flegler et al. 1993). Samples were mounted on stubs, using double-sided tape, and graphite was painted on the edges of stubs. All samples were sputter coated with gold. Samples were then examined at 10kV and 39 mm working distance at various magnifications.

Fruit injury characteristics. In addition to pupal and larval characters an attempt to diagnose fruit injuries among the three species was conducted. Using similar methods as for the lesser appleworm colony establishment, cherry fruitworm and oriental fruit moth colonies were established on apple as a food source. Observations of external and internal fruit injury pattern were made on fruits infested by larvae.

For comparison of larval and pupal characters, multivariate statistics were applied. Correlation coefficients among different characters within the species were applied for the analysis of diagnosed forms (Daly 1985).

Results and Discussion.

Pupal characters. Lesser appleworm pupal characters were measured on n = 110 female and n = 104 male pupae. Length of male pupae averaged 5.07 ± 0.31 mm and ranged from 4.1 mm to 6.0 mm; in females it ranged from 4.1 mm to 5.55 mm with average of 4.93 ± 0.3 mm. The width of mesowing on dorsal side in both sexes ranged from 0.8 mm to 1.65 mm, with the width of the anal segments ranging from 0.25 mm to 0.5 mm (Table 1).

Oriental fruit moth characters were measured on n = 37 male and n = 18 female. Length ranged from 5.15 mm to 7.6 mm averaging 6.09 ± 0.32 mm for males and 6.6 ± 0.63 mm for females. Oriental fruit moth pupal mesowing width ranged from 1.2 mm to 2.0 mm averaging 1.64 ± 0.13 mm for males and 1.76 ± 0.19 mm for females, with the anal segment width ranged from 0.4 mm to 0.65 mm.

Measurement of n = 12 males and n = 10 females of cherry fruitworm pupae showed their length ranged from 4.3 mm to 5.35 mm, mesowing width ranged from 0.9 mm to 1.4 mm, and anal segments width ranged from 0.3 mm to 0.5 mm (Table 1).

The size of measured pupae is in general agreement with data presented by Adler (1991), who examined four unsexed individuals, and found that lesser appleworm pupae

Table 1. Comparison of pupal length, mesowing width, and anal segment width of lesser appleworm (LAW), oriental fruit moth (OFM), and cherry fruitworm (CFW).

Species	Sex	Character	*n*	Mean[a]	SD	Range[a]	Mode[a]
LAW	male	length	104	5.07	0.31	4.1 -6.0	4.95
		mesowing width	104	1.39	0.11	0.95 -1.6	1.45
		anal segm. width	104	0.37	0.04	0.25 - 0.5	0.35
	female	length	110	4.93	0.3	4.1 - 5.55	4.95
		mesowing width	110	1.35	0.12	0.8 - 1.65	1.3
		anal segm. width	110	0.35	0.04	0.3 - 0.5	0.35
OFM	male	length	37	6.09	0.32	5.4 - 6.75	6
		mesowing width	37	1.64	0.13	1.2 - 1.9	1.7
		anal segm. width	37	0.51	0.06	0.4 - 0.65	0.4
	female	length	18	6.6	0.63	5.15 - 7.6	6.9
		mesowing width	18	1.76	0.19	1.35 - 2	1.6
		anal segm. width	18	0.57	0.06	0.4 - 0.65	0.6
CFW	male	length	12	4.87	0.32	4.3 - 5.35	4.85
		mesowing width	12	1.2	0.12	1.05 - 1.4	1.3
		anal segm. width	12	0.38	0.05	0.3 -0.5	0.35
	female	length	10	4.82	0.22	4.3 - 5.05	5.05
		mesowing width	10	1.14	0.12	0.9 - 1.3	1.2
		anal segm. width	10	0.34	0.04	0.3 -0.4	0.3

[a] - all data are in mm

are in length range of 4.5 mm to 6.0 mm. Similarly small differences between my data and Adler (1991) exist for measurements of cherry fruitworm and oriental fruit moth. Adler (1991) used a color as an additional character for pupae diagnosis but I did not find color useful as a character for separating these three species. Color of pupae can change based on the development of internal moth structures, and it is extremely difficult to use color descriptions (various shades of brown) as a character for identification.

Correlation coefficient "r" for characters within species showed how difficult it is to separate pupae of lesser appleworm and cherry fruitworm from pupae of oriental fruit moth (Table 2). The highest r values were observed for oriental fruit moth pupal length and width of mesowing ($r = 0.84$). Other characters within species showed various values of correlation coefficients. They ranged from $r = 0.39$ for cherry fruitworm length and anal segment width, to $r = 0.69$ also for cherry fruitworm pupae, but for correlation cofficient of anal segment width and mesowing width.

On the other hand using only visual observations of the general shape of pupae, and individual relations of three characters: pupal length, mesowing width, anal segment width, and number of movable abdominal segments, I classified correctly 91.5 percent of pupae into species and sex (Table 3). This test showed that one can relatively well separate the pupae of oriental fruit moth from those of lesser appleworm and cherry fruitworm. More problems arise during separation of the last two species. Some extreme values of pupal characters are responsible for the misidentification of a few lesser appleworm and oriental fruit moth. Two dimensional plots of pupal length and width of mesowing and anal segment show the differences that will allow one after getting some

Table 2. Correlations coefficients for three pupal characters: length, width of mesowing, and width of anal segment for lesser appleworm (LAW), oriental fruit moth (OFM), and cherry fruitworm (CFW).

	LAWlen.	LAWmes.	LAWanal
LAWlen[a]	1		
LAWmes[b]	0.438835	1	
LAWanal[c]	0.410816	0.364996	1
	OFMlen.	OFMmes.	OFManal
OFMlen.	1		
OFMmes.	0.839728	1	
OFManal	0.543713	0.477736	1
	CFWlen.	CFWmes.	CFWanal
CFWlen.	1		
CFWmes.	0.626319	1	
CFWanal	0.390674	0.696313	1

[a] - length of pupae from anterior of frontal ridges to posterior tip of anal segment

[b] - width of dorsal part of mesowing behind prothorax

[c] - width of dorsal part of anal segment

Table 3. Accuracy of species identification using visual characters to categorize pupae of lesser appleworm (LAW), oriental fruit moth (OFM), and cherry fruitworm (CFW)

Species	Predicted [a]	Observed			Percent
		LAW	OFM	CFW	correct
LAW	213	201	3	9	94.37
OFM	60	8	52	0	86.7
CFW	18	5	0	13	72.2
Total		214	55	22	91.4

[a] - number of expected pupae that belongs to a given species. Observation based only on general appearance of the pupa

experience to correctly identify pupae (Figure 1 and Figure 2). The oriental fruit moth pupae are generally bigger and wider in appearance than lesser appleworm or cherry fruitworm. Among examined oriental fruit moth specimens, 92.6 percent of the pupae had a ratio of pupal length to width of mesowing between 3.28 and 3.98 (Figure 3).Cherry fruitworm pupae are the thinnest, most delicate in comparison with the other two species. Although there were low numbers of cherry fruitworm pupae examined, the ratio of pupal length and width of mesowing for 95.2 percent of pupae varied from 3.9 to 4.54 (Figure 3). Understanding of those subtle differences may be especially important for field identifications without the possibility of using magnifying equipment.

Additional hints for identification include differences in larval pupation behavior. Full grown larvae of lesser appleworm leave the fruit to prepare a pupation site. They may use numerous available sites such as leaves, corrugated paper or scars on fruit tissue. Often, when they use dry leaves for making a cocoon, they form a characteristic "folded envelope" with flat bottom and convex top part (Figure 4). In the laboratory, when larvae were provided with corrugated paper, they formed a small cocoon on the side of the tunnel using a top layer of paper. The oriental fruit moth larva closes the whole tunnel and use it entirely for making a much larger cocoon, similar to codling moth. There were no detailed observations of cherry fruitworm pupation behavior during the experiment, due to a low numbers of specimens.

Even using scanning electron microscopy as a tool, I was not able to find definitive pupal difference among the three studied species. My goal was to find differences under high magnification, that can be observable even without using electron microscopy. Observing cherry fruitworm pupa's dorsal side, two rows of spines are

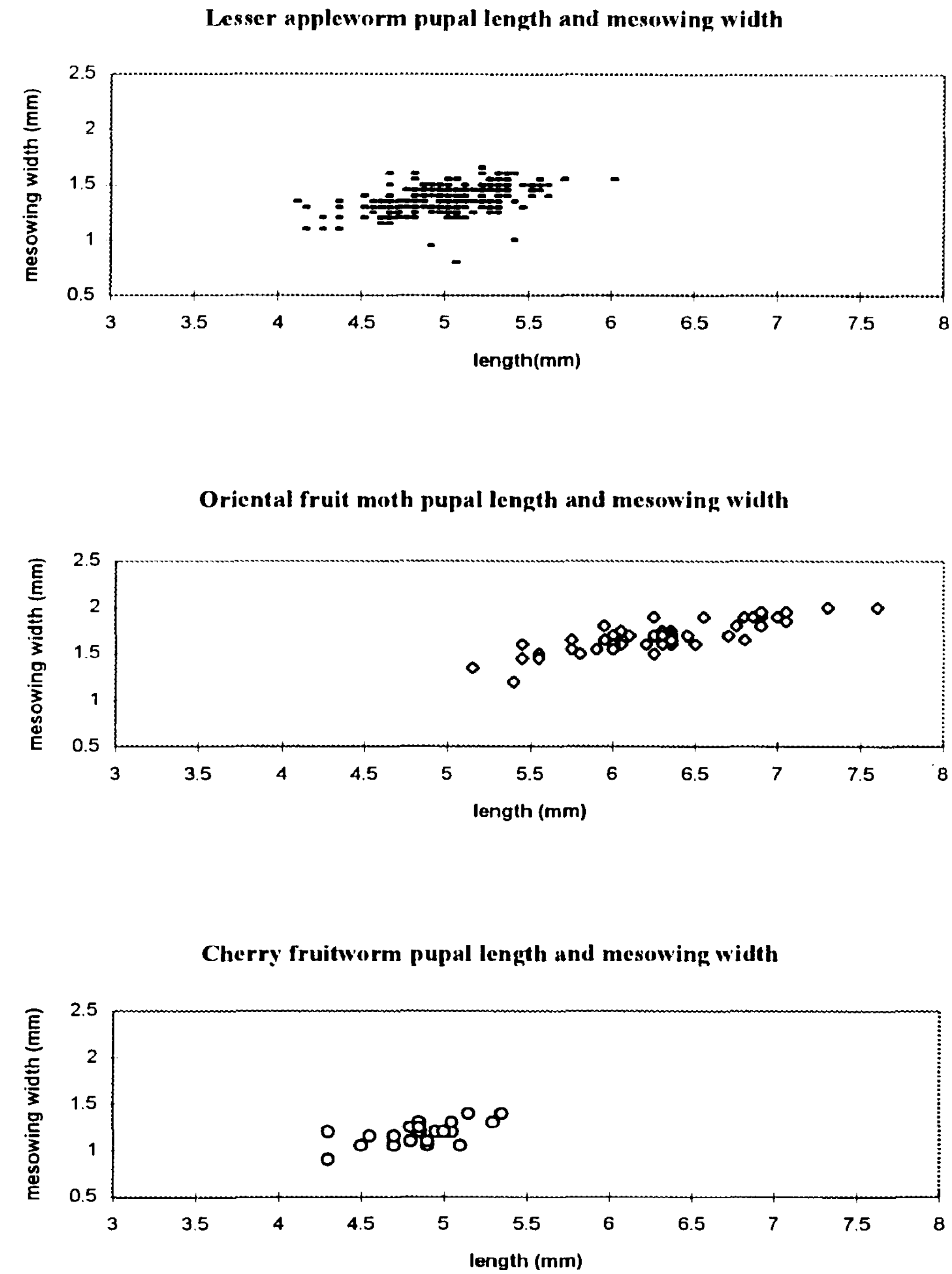


Figure 1. Comparison of pupal length and mesowing width of three internal fruit feeders : lesser appleworm, oriental fruit moth, and cherry fruitworm.

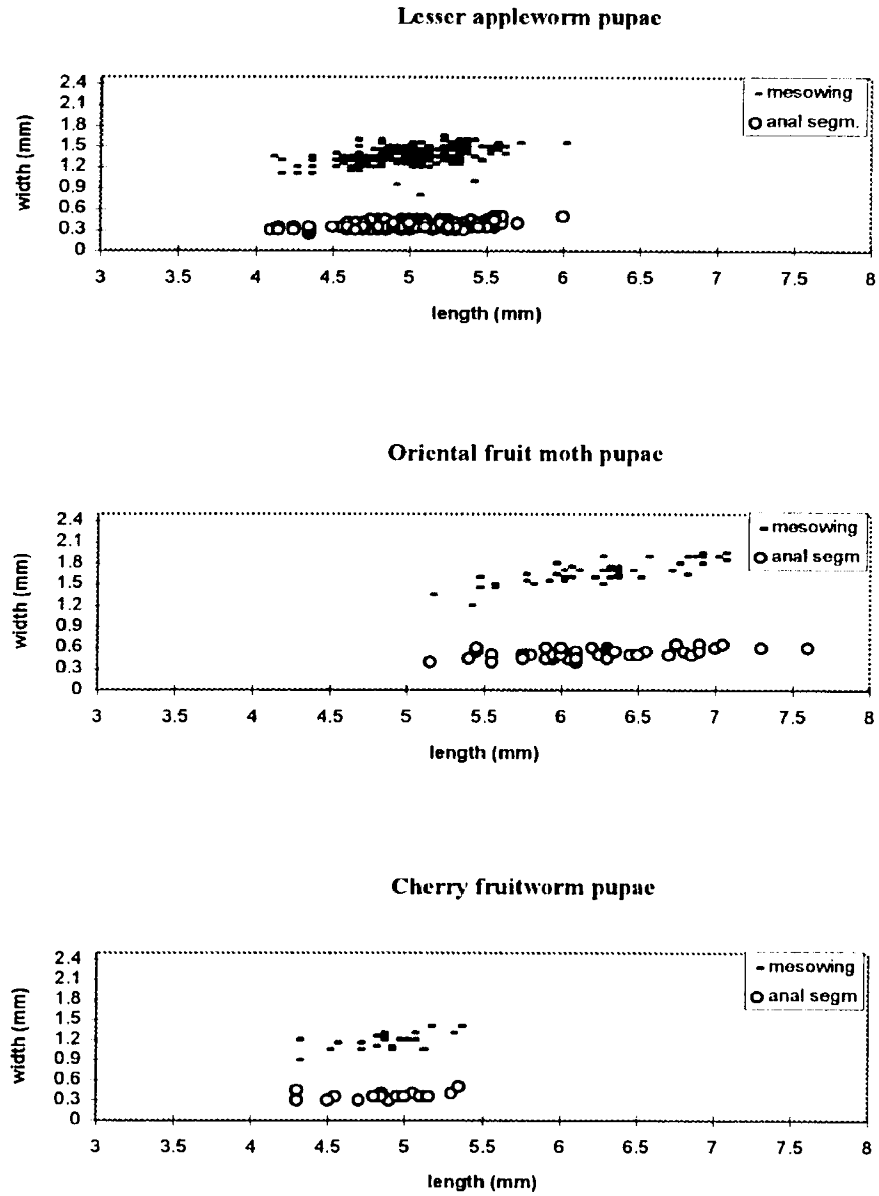


Figure 2. Comparison of three pupal dimensions: length, mesowing width, and anal segment width of lesser appleworm, oriental fruit moth, and cherry fruitworm.

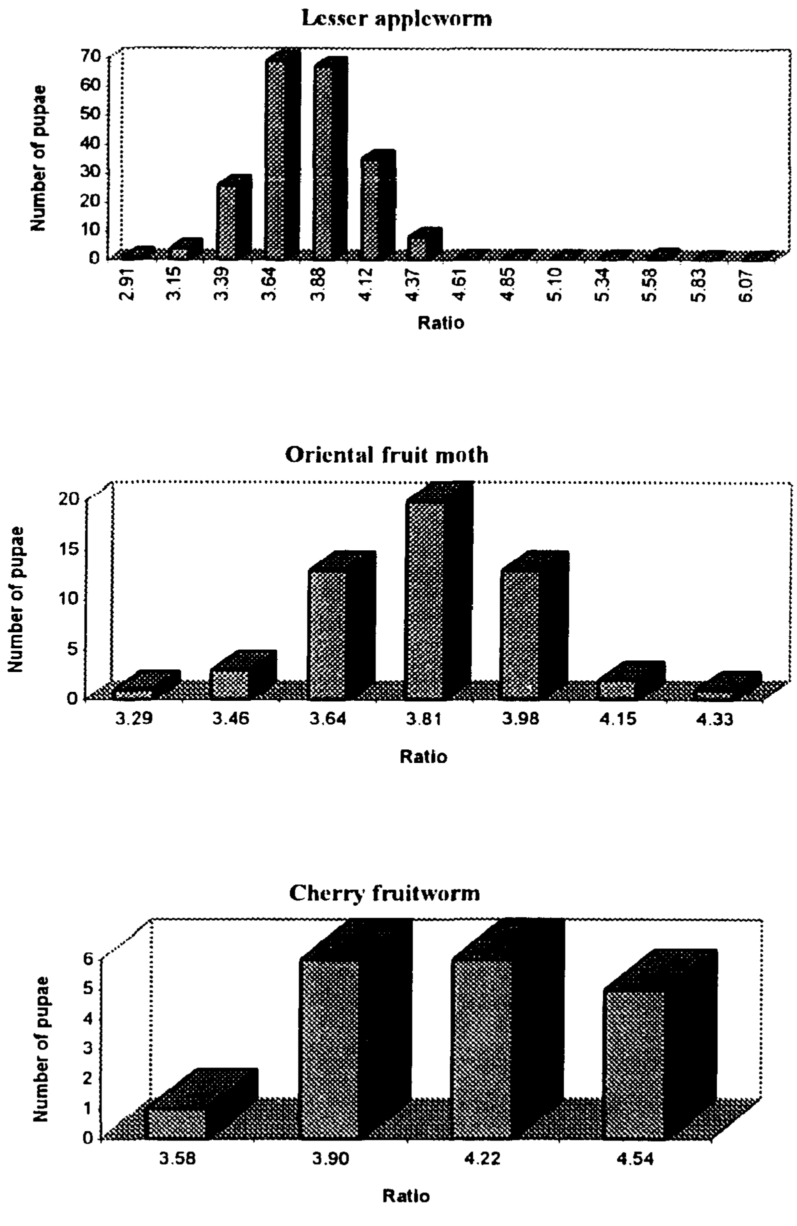


Figure 3. Frequency of ratios between pupal length and width of mesowing for lesser appleworm, oriental fruit moth, and cherry fruitworm pupae

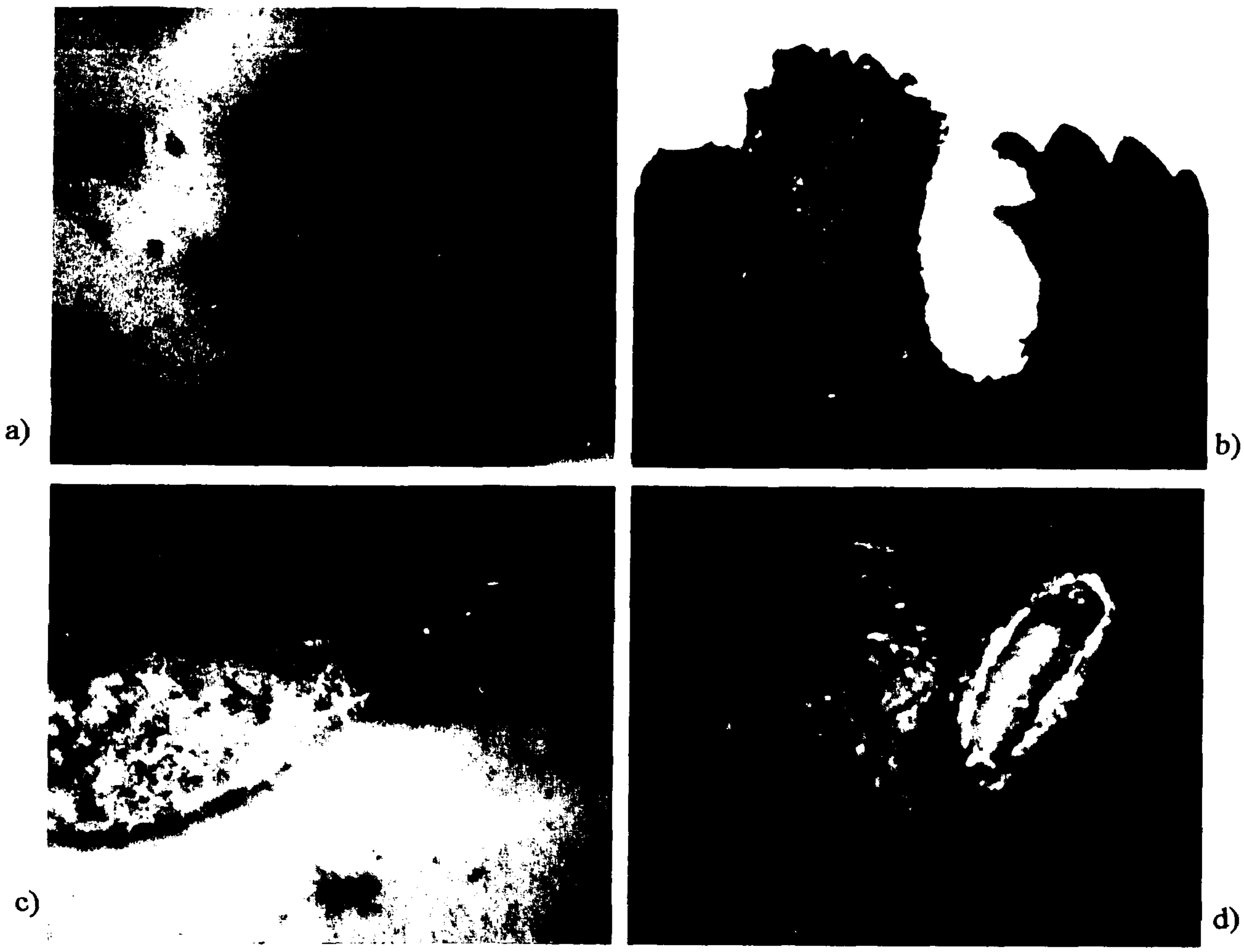
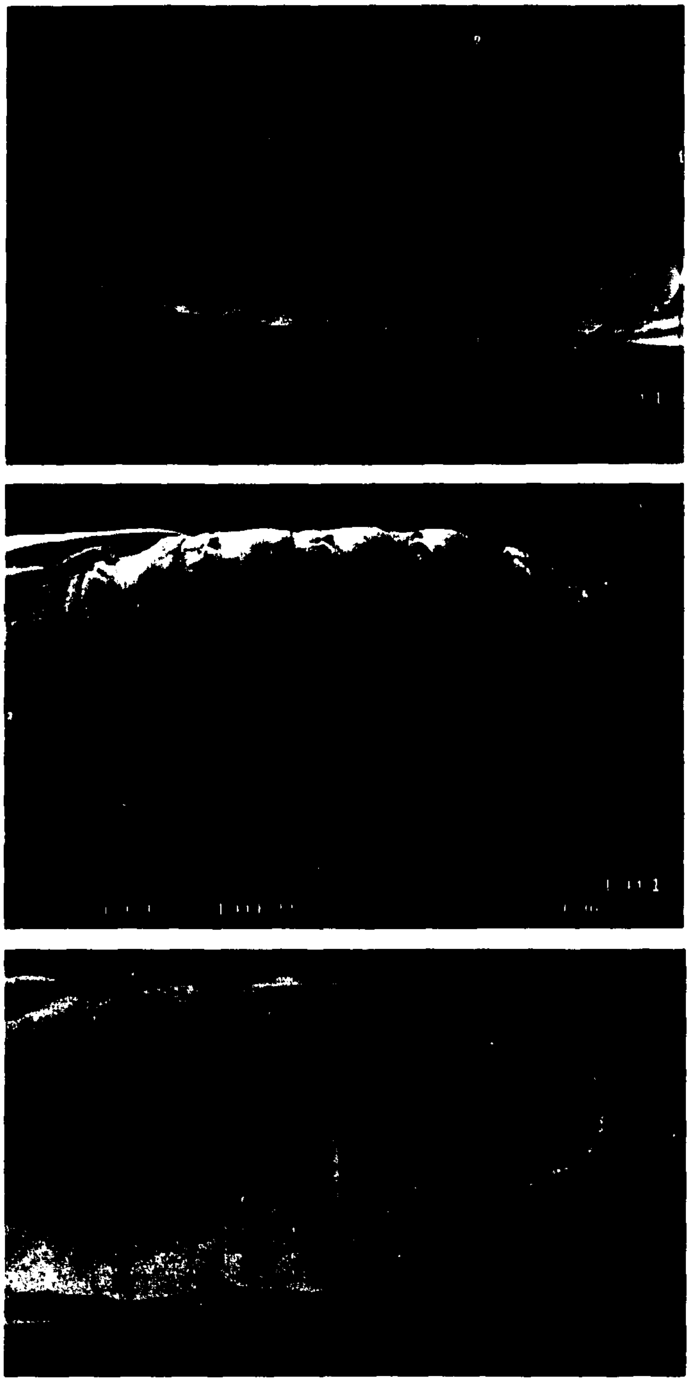


Figure 4. Lesser appleworm *Grapholita prunivora* (Walsh) pupal cocoons : a) on apple fruit, b) on leaf, c) on filter paper, and d) on hawthorn fruit.

visible on abdominal segments 2-7, and a single row on segments 8 - 9. On segments 4 -6 the anterior rows contain double rows of overlapping small spines (Figure 5). The anterior row of dorsal spines on the lesser appleworm and oriental fruit moth are composed of single row of spines. Lesser appleworm and oriental fruit moth pupae have the posterior row of dorsal spines about 50 percent smaller than anterior one and located at a distance two/third of the width of the segment toward the posterior end. Cherry fruitworm has its posterior rows of spines relatively bigger when compared to previous species, but almost two/third of size of first row and located very close to posterior edge. Adler (1991) used the number of anterior rows of spines on abdominal segments 4 - 6 as a major diagnostic character. She did not use the size of spines and their position on the segment as a character for species identification.

Larval characters. Larval identification of lesser appleworm, oriental fruit moth, and cherry fruitworm has always been difficult. In identification keys among lesser appleworm and oriental fruit moth larvae the main characters are larval size, or color difference between those two species. Chapman & Lienk (1971) in their key for apple feeders in New York found that lesser appleworm larvae retain pinkish color when stored in 70 % alcohol, and that does not happen for oriental fruit moth or cherry fruitworm larvae.

There is also an overlap in larval length and head width. According to Chapman & Lienk (1971) lesser appleworm larval head width is 0.77 - 0.85 mm and length is 7.5 - 9.5 mm, when for cherry fruitworm the head width is 0.85 - 0.94 mm, and length 7.5 - 9.0 mm. Brown (1987) in his key for tortricid larvae present on fruits use the color of



a)
b)
c)

Figure 5. Scanning electron microscope photographs of dorsal abdominal part of three tortricid pupae: a) *Grapholita prunivora* (Walsh), b) *Grapholita molesta* (Busck), and c) *Grapholita packardi* Zeller

pinacula on posterior segment and body color in alcohol as main characters to distinguish between cherry fruitworm and lesser appleworm.

To find other characters for identifying the lesser appleworm, cherry fruitworm and oriental fruit moth larvae, I measured the anal comb configuration and counted the number of crochets on the prolegs. Crochets are small hooks arranged in row or circles around the edge of the planta (Brown 1987). All three species showed differences in the number of crochets on the last pair of ventral prolegs and on the anal prolegs (terminology as in Brown 1987). Crochets on the ventral prolegs are arranged in an uniordinal circle or oval, and crochets on anal prolegs are arranged in uniordinal half oval row. Lesser appleworm larvae (n = 40) had an average of 14.91 crochets on the anal prolegs (Table 4); cherry fruitworm (n = 7) had 17.8, and oriental fruit moth (n = 29) had 23.77 crochets. On the last pair of ventral prolegs they had respectively: lesser appleworm - 24.33, (n = 39); cherry fruitworm - 26.21, (n = 7); and oriental fruit moth - 35.46 (n = 27) (Table 4). Except for cherry fruitworm right and left anal prolegs, the number of crochets on each proleg and pair of prolegs shows positive correlation (Table 5). The number of crochets on ventral prolegs in lesser appleworm larvae (range 21 - 30) is different from that in oriental fruit moth (range 26 - 41), with one exception, when on oriental fruit moth larva had only 26 crochets on its last ventral right proleg (Figure 6). This character can be used as a good character for larval identification. In the case of the cherry fruitworm the number of crochets on prolegs were included in the range of those of the lesser appleworm which makes it impossible to use this character for identification of those two species. A scatter plot of number of crochets among three species shows

Table 4. Comparison table of number of crochets on last pair of ventral prolegs and on anal prolegs on lesser appleworm (LAW), oriental fruit moth (OFM), and cherry fruitworm (CFW)

Species	n	Left	Right	x (L + R)	SD	Range a	Mode
		Number of crochets on anal pair of prolegs					L - R
LAW	40	14.98	14.85	14.91	2.05	10 - 19	13 - 15
OFM	29	23.14	24.39	23.77	2.8	17 - 29	23 - 23
CFW	7	17.43	18.17	17.8	1.54	15 - 20	17 - 18
		Number of crochets on last pair of ventral prolegs					
LAW	39	24.31	24.36	24.33	2.17	21 - 30	22 -24
OFM	27	35.26	35.67	35.46	3.17	26 - 41	38 - 35
CFW	7	26.14	26.28	26.21	2.55	23 - 30	28 - 26

[a] - range from left and right prolegs

Table 5. Correlation coefficients for number of crochets on last pair of ventral prolegs and on anal prolegs for larvae of lesser appleworm (LAW), oriental fruit moth (OFM),and cherry fruitworm (CFW).

Lesser appleworm

Correlation coefficients

	anal L.	*anal R.*	*ventral L.*	*ventral R*
anal L.[a]	1			
anal R.[b]	0.717985	1		
ventral L.	0.455088	0.457138	1	
ventral R.	0.421467	0.477823	0.677166	1

Oriental fruit moth

	anal L.	*anal R.*	*ventral L.*	*ventral R.*
anal L.	1			
anal R.	0.82079	1		
ventral L.	0.472525	0.673335	1	
ventral R.	0.370251	0.563121	0.633814	1

Cherry fruitworm

	anal L.	*anal R.*	*ventral L.*	*vevtral R.*
anal L.	1			
anal R.	-0.10775	1		
ventral L.	0.419965	0.566352	1	
ventral R.	0.26367	0.824465	0.895044	1

[a] - crochets on left proleg

[b] - crochets on right proleg

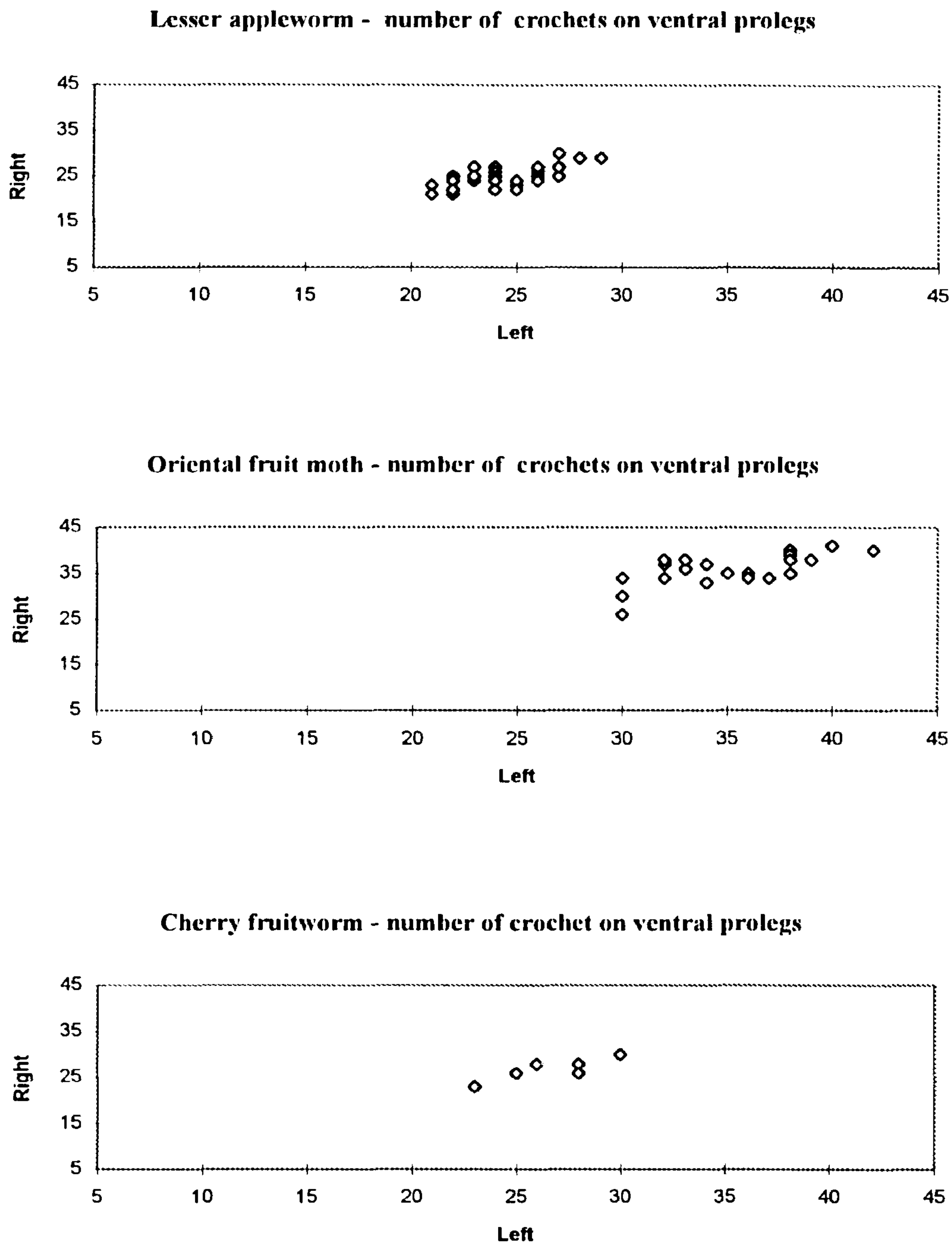


Figure 6. Comparison of number of crochets on last pair of ventral prolegs in lesser appleworm (LAW), oriental fruit moth (OFM), and cherry fruitworm (CFW)

pattern that may be useful for species separation, but overlapping numbers decrease the value of this character (Figure 7).

MacKay (1959) in her attempt to separate those species, used the number of crochets and ratio of spinneret length and width as an useful way for separation of oriental fruit moth larvae from larvae of lesser appleworm and cherry fruitworm, but she also found it impossible to separate the later two species based on crochet difference.

Anal comb. All three species have a moderately developed an anal comb. The anal comb is defined as a mesal sclerotized prong ventrad of the anal plate and adjacent to the anus (Stehr 1987). The function of the anal comb is not well defined. Frost (1919) compared the advantages and disadvantages of presence of anal comb for external and internal plant feeders, but no definite conclusions were found. Stehr (1987) define anal comb function as "used to eject frass" (pp. 711). Prongs are straight, of various lengths. Individuals of lesser appleworm larvae have the number of prongs from 4 to 9, oriental fruit moth from 4 to 7, and cherry fruitworm from 4 to 6. All analyzed specimens show a large difference in the prong arrangement (Table 6). On some studied specimens only prongs of the same size were observed, but the majority of larvae had various prongs arrangements. There were no detectable differences among species that can be use for larvae identification.

Fruit injury. All three examined species, along with the codling moth are grouped by orchardists as internal fruit feeders. All of them feed inside the fruit, but the damage they cause is different. Lesser appleworm larvae enter the fruit at any point, but usually at the calyx or stem end. Larvae usually borrow through the skin and feed exclusively under the skin. In contrast to the oriental fruit moth or codling moth it does not tunnel to

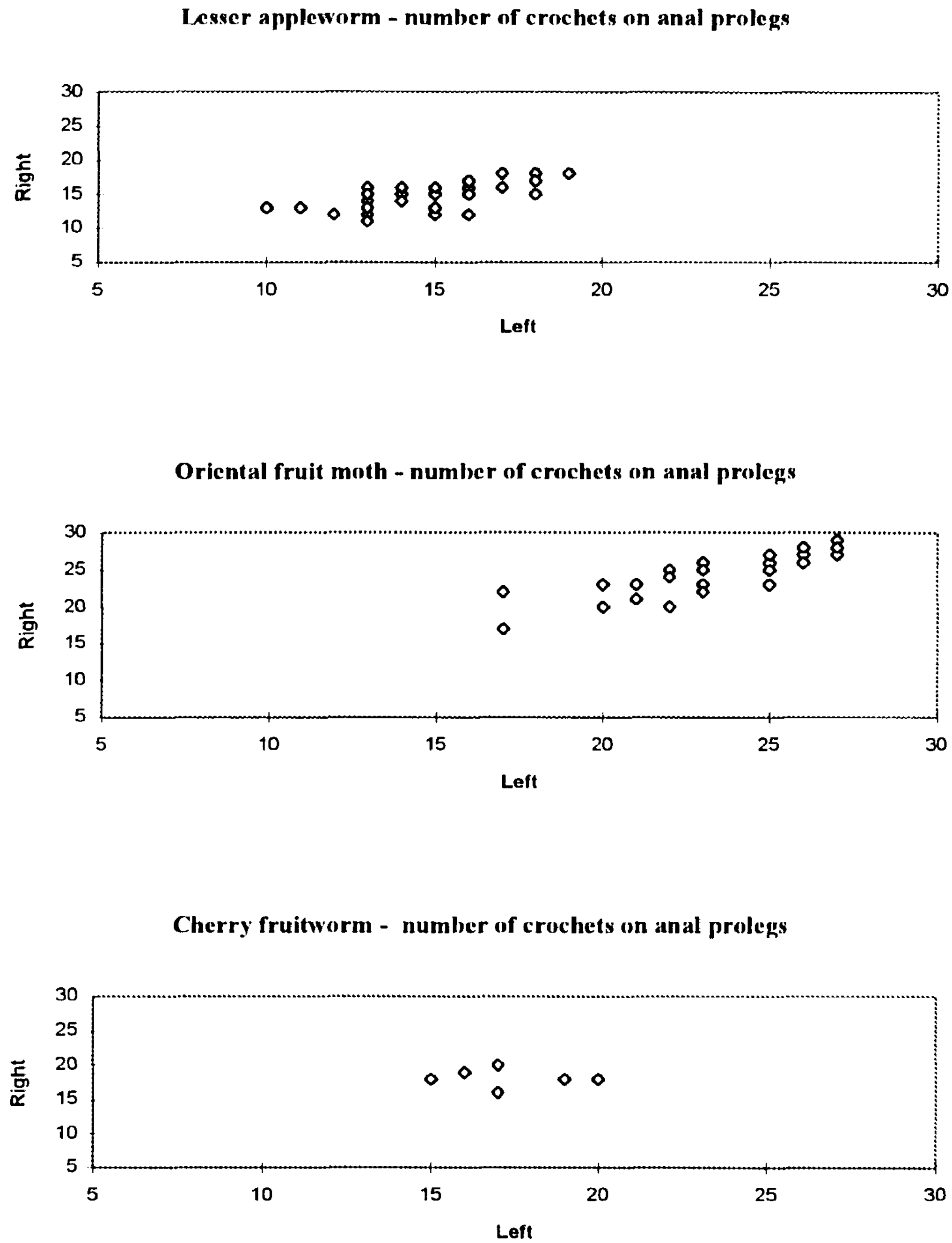


Figure 7. Comparison of number of crochets on anal pair of prolegs in lesser appleworm (LAW),oriental fruit moth (OFM), and cherry fruitworm (CFW).

Table 6. Configuration of prongs in anal comb in lesser appleworm (LAW), oriental fruit moth (OFM), and cherry fruitworm (CFW)

LAW	OFM	CFW
1 **5**	*1* **4** *1*	**5**
1 **4**	**2** *1* **1** *1* **1**	**3** *2*
	3 *1* **2**	**5**
1 **4** *1*	**4** *1*	**4** *1*
1 **5**	**3** *2*	
1 **4**	*1* **3** *1*	*2* **1** *3*
4 *1*	**4**	**4**
4	*1* **4**	
1 **4** *1*	*1* **4**	
3 **3** *1*	*1* **5**	
1 **1** *1* **2** *1*	*1* **4** *1*	
5 *1*	**6**	
1 **4** *1*	*1* **4**	
4	*1* **5** *1*	
2 **5**	*1* **2** *2* *1*	
1 **4** *1*	*1* **5**	
5	**6**	
4 *1*	**4** *2*	
1 **4** *2*	**5**	
4	**4**	
4	**4**	
1 **3** *1*	**4**	
1 **3** *1*	**6**	
1 **4** *1*	**4**	
1 **3** *1*	**2**	
1 **5** *1*	*1* **5**	
4 *1*	**5**	
6	**4** *2*	
4 *1* *1*	**2** *3*	
4	*1* **5** *1*	
5 *1*		
4 *1*		
2 *1* **2**		
1 **5**		
2 **4** *3*		
2 **3**		
2 **4** **2**		
4		
4		
4		

1 - numbers in italics show number of weak prongs
1 - numbers in bold show number of strong prongs

the apple seeds, although it may go deeper into the fruit. When lesser appleworm larvae tunnel into the fruit, the core line was the limit, inside of which we never saw any damage. I did not find lesser appleworm larvae feeding on seeds. Externally on fruit, a small amount of frass is visible only at the entrance point. Most of the frass remains within the tunnels under the skin. Tunnels made by lesser appleworm larvae are a snake shaped. One end of the tunnel, where the larva is present, is much wider. Lesser appleworm larvae are usually visible through the skin. Oriental fruit moth and especially fresh codling moth injury may also sometimes looks similar, however older larvae are usually tunneling inside the fruit and feeding on seeds in core area. Fruit injury characteristics of the lesser appleworm are shown on Figure 8.

Lesser appleworm larvae were never observed feeding on any other part of the tree than the fruit. It was never found feeding on leaves or young growing watersprouts. Quaintance (1908) reported this leaf feeding of larvae, but it was not found during our observations. Chapman & Lienk (1971) observed that fruit injured by the first generation of lesser appleworm drop from tree to the ground. I was unsuccessful in collecting larvae from dropped fruit, probably since the injury did not affected fruit seeds, and did not force the fruit to drop.

Conclusions. No one single character will allow for definitive identification of lesser appleworm, cherry fruitworm, and oriental fruit moth larvae and pupae. The identification of oriental fruit moth pupae due to its relatively bigger size and appearance, and to characters that overlap only on the edges with characters of the two other species is relatively accomplishable. Lesser appleworm can be identified only after careful examination of all pupal and larval characters and preferably by the kind of injury

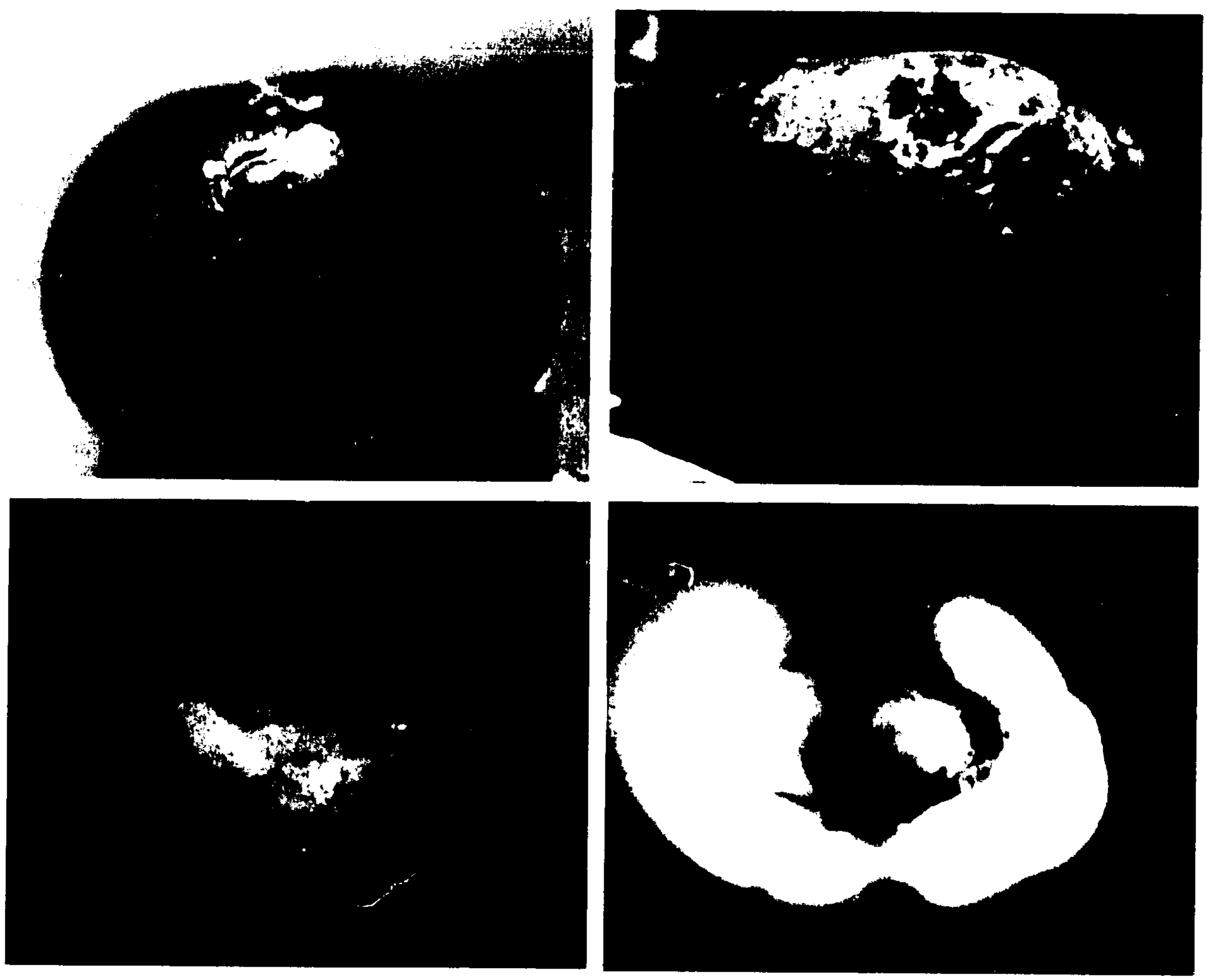


Figure 8. Apple fruits injured by leser appleworm *Grapholita prunivora* (Walsh)larvae

it caused on the fruit. For separating cherry fruitworm larvae or pupae from those of lesser appleworm, only detailed examination with special attention given to a relation among diagnostic characters and general, optical shape can be used. Although strong overlap in the examined characters exist between these two species, it is possible after getting some experience, to accomplish this task. The best results can be accomplished when we are able to deal with all three species at the same time and comparisons can be done.

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APPENDIX

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1996 - 2

Title of thesis or dissertation (or other research projects):

BIOLOGY AND PEST STATUS OF LESSER APPLEWORM GRAPHOLITA PRUNIVORA (WALSH) (LEPIDOPTERA - TORTRICIDAE) IN MICHIGAN

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)
Grzegorz Krawczyk

Date April 04.1996

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.
Copies: Included as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Grapholita prunivora (Walsh)	Michigan, Allegan Co. Douglas 130 Str. T3N,R16W, sec. 21 Col. Sept. 1994, from moth colony		8(1) 12(2) 19(3)		2				MSU
Grapholita molesta (Busck)	Michigan, Allegan Co. Douglas 130 Str. T3N,R16W, sec. 21 Col. Sept. 1994, from moth colony		25(1) 35(2) 25(3)		2 13				MSU
Grapholita packardi Zeller	Michigan, Allegan Co. Douglas 130 Str. T3N,R16W, sec. 21 Col. Sept. 1994, from moth colony		7						MSU
Cydia pomonella (Linnaeus)	Michigan, Allegan Co. Douglas 130 Str. T3N,R16W, sec. 21 Col. Sept. 1994, from moth colony		20						MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Grzegorz Krawczyk

Date April 04. 1996

Voucher No. 1996 - 2

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

[signature] April 4 1996

Curator Date

Signed for F. Stehr by J. Wilterding