THE BIONOMICS OF LITHOCOLLETIS BLANCARDELLA (Lepidoptera: Gracillariidae) IN MICHIGAN

Thesis for the Degree of M. S. MICHIGAN STATE UNIVERSITY JAMES DWIGHT DUTCHER 1975



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ABSTRACT

The spotted tentiform leafminer of apple, <u>Litho-colletis blancardella</u> Fabricius, has three discrete generations per season in southwestern Michigan apple orchards. Five hymenopterous parasitoids were identified as natural enemies of the larval stages of this leafminer. A chrysopid larva was observed as a natural predator of the adult leafminer. In an untreated plot of Jonathan apple trees, <u>Malus pumila</u> Mill., the total effective leaf area was reduced by 32% by the leafmining larvae during the 1974 season. Many broad spectrum and systemic insecticides were effective in controlling this leafminer. Certain carbamate and organophosphate insecticides caused a significant reduction in parasitism of the larvae.

THE BIONOMICS OF <u>LITHOCOLLETIS</u> <u>BLANCARDELLA</u> (Lepidoptera: Gracillariidae) IN MICHIGAN

Ву

James Dwight Dutcher

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INTRODUCTION

In recent years the spotted tentiform leafminer,

<u>Lithocolletis blancardella</u> Fabricius, has infested many
commercial apple orchards in Michigan despite extensive
insecticide spray schedules. This study was conducted
to determine the pest status of the leafminer and its
bionomics in Michigan.

Personnel of the Cooperative Michigan Apple Pest

Management Project, Michigan State University, found low

levels of L. blancardella in commercial and abandoned

orchards in Benzie, Berrien, Ionia, Kent, Manistee, Mason,

Newaygo and Oceana counties. In addition infestations

were found in Allegan, Barry, Charlevoix, Emmet, Ingham,

losco, Kalamazoo and Mecosta counties. All collecting sites

were in apple, Malus pumila Mill., orchards. This leafminer

appears to have a statewide distribution [Figure 1].

The description of <u>L</u>. <u>blancardella</u> given by Pottinger and LeRoux (1971) was used by Newman* to verify the identity of one adult female and one adult male reared from pupae collected at Fennville, Michigan. To increase the certainty of the species determination, measurements of the genitalia of 6 male and 2 female adults were compared with the measurements noted by Pottinger and LeRoux. It was determined that the insect causing spotted tentiform leafmines in Michigan

^{*} Mr. John Newman is an insect identification specialist for the Michigan State University Cooperative Extension Service.

apple orchards is L. blancardella.

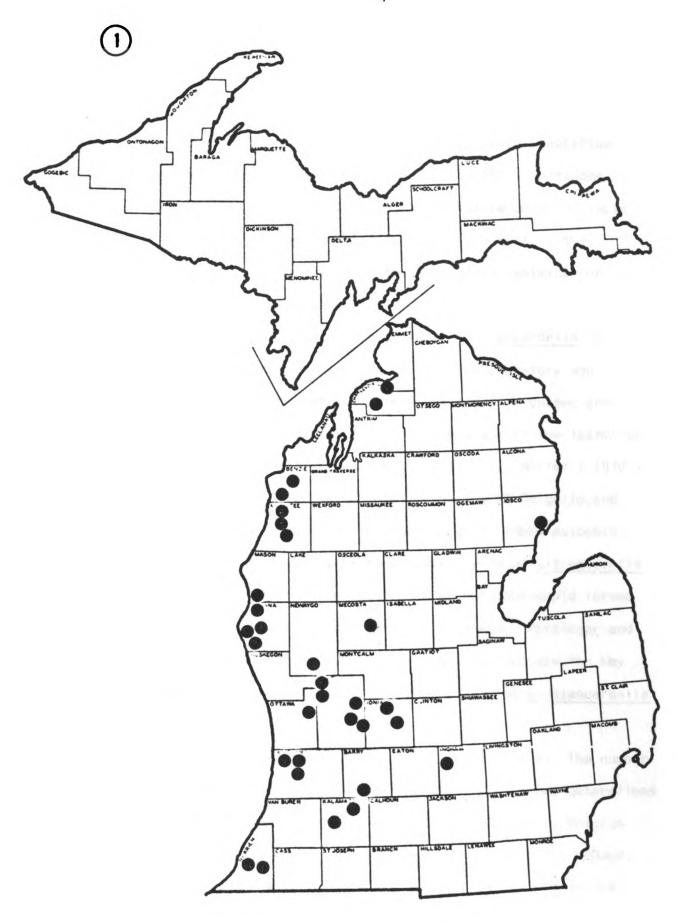
Several hymenopterous parasitoids and a chrysopid predator were observed attacking the various life stages of this leafminer in an untreated orchard at Hickory Corners, Barry Co., Michigan. The degree of parasitism and predation in the leafminer population was measured at the end of each of the leafminer's 3 discrete generations.

The seasonal development of this leafminer was monitored during 1973 and 1974 at 2 untreated apple orchards at
Richland, Kalamazoo Co., and Hickory Corners, Michigan.
Larval and adult behavior were also observed at these sites.

Certain broad spectrum insecticides were applied on a biweekly schedule at 2 apple orchards in Allegan Co., Michigan, to determine the effect of these compounds on the leafminer population density. Three systemic insecticides were applied as single applications to measure the efficacy of these compounds against the larval stages of the leafminer.

The observation that male moths are preferentially attracted to caged virgin female moths indicates that mating may be initiated by a chemical stimulus or pheromone which is released by the female moth.

FIGURE 1. - Collecting sites of \underline{L} . $\underline{blancardella}$ in Michigan, 1973 - 1974.



LITERATURE REVIEW

L. blancardella was originally described by Fabricius in 1781 and was redescribed by Bankes in 1899 (Pottinger and LeRoux, 1971). This insect was first recorded on apple in the United States by Slingerland and Crosby (1914). Hutson (1938) was the first to record this leafminer on apples in Michigan.

The parasitoid natural enemies of <u>L</u>. <u>blancardella</u> in Europe are listed by Fulmek (1962). The predatory and parasitoid natural enemies of this leafminer in Quebec are listed and catagorized as to which life stage of the leafminer is attacked by Pottinger and LeRoux (1971). Miller (1970) has written a key to the neararctic species of Pnigalio and Sympiesis. His studies on the host range of these eulophid ectoparasitoids reveal many to be parasitoids of <u>Lithocolletis</u> spp. Hering (1951) has observed predatory chrysopid larvae attacking leafmining insects within the mines. Pottinger and LeRoux (1971) state that predators and weather are the key mortality factors regulating the populations of <u>L</u>. <u>blancardella</u> in Quebec.

L. <u>blancardella</u> has a multivoltine life cycle. The number of generations per year varies with location. Three generations were completed in Switzerland (Baggliolini, 1960), Belgium (Soenen and Aerts, 1953) and Quebec (Pottinger and LeRoux, 1971). Four and occasionally 5 generations were completed

in northern Italy (Kremer, 1963). In Quebec the 3 generations were discrete. In warmer climates the last generations, i.e. second and third in Virginia (Beckham, et al, 1950) and second, third and fourth in northern Italy (Kremer, 1963), were not separate. The terminology proposed by Schurr and Rings (1964) is used in this study to distinguish the following stages of the life history of L. blancardella:

Overwintering pupae

Spring generation adults

First generation eggs, larvae and pupae

Second generation eggs, larvae and pupae

Third generation eggs and larvae.

The life history and habits of this leafminer have been observed in detail by Pottinger and LeRoux (1971) and Hering (1951). Their observations are noted here briefly. The spring generation adults emerge from their overwintering sites on the orchard floor during the first warm days of spring. The eggs are distributed randomly on the lower surface of the apple leaves. They [Figure 3] are dome shaped with the flat surface affixed to the leaf, average 0.4 mm in diameter and have a reticulate surface.

The larval stage consists of 5 instars and 2 distinct morphological forms. Instars I, II, and III are dorsoventrally compressed, apodous and plasmophagous and are sapfeeders [Figure 4]. Instars IV and V are eruciform and are

tissue-feeders [Figure 5]. The mining habits of the larvae vary with each instar. The first instar larva breaks through the egg chorion, enters the leaf directly and begins mining the area around the point of entry. The second instar larva mines away from this point and forms a linear mine or ophionome (Hering, 1951) between the lower epidermis and the spongy mesophyll of the leaf. The third instar larva extends the lateral margins of the mine to form an oval blotch mine or stigmatonome (Hering, 1951). The fourth instar larva connects the lateral margins of the mine together with moist silken threads. As these threads dehydrate they shorten, bringing the lateral margins of the mine together and creating longitudinal folds in the lower epidermis of the leaf in the mined area [Figure 7]. At this point the mine is given greater depth to compensate for the larva's cylindrical shape and is called a tentiform mine or ptychonome (Hering, 1951). The fifth instar larva increases the depth of the mine by spinning more silk between the lateral margins of the mine.

The first three instars feed entirely on the protoplasm of the spongy mesophyll cells (plasmophagous) which are sheared open with the larva's specialized mouthparts. The mouthparts of the last two instars are modified to facilitate their tissue-feeding (histophagous) habit. Grandi (1959)

explains this duality of form in detail. Fourth instar larvae feed primarily on the spongy mesophyll tissue and rarely feed through to the upper epidermis of the leaf. The fifth instar larvae feed on both spongy and columnar mesophyll tissue and selectively ignore the vascular tissue of the leaf. This instar often feeds up to but never through the upper epidermis of the leaf. The mature mine when viewed from the top has a spotted appearance due to the irregular feeding sites of the larva.

The larva prepares itself for pupation by voiding itself of solid waste and spinning a slight cocoon at one end of the mine. Pupation [Figure 6] occurs within the mine. At the time of emergence the pupa pushes through the lower epidermis of the leaf [Figure 7] and the adult [Figure 8] emerges. Adults are weak flyers and flight activity is confined to a short period at dusk when mating occurs. During the day they rest on the leaves and trunk of apple trees only flying when disturbed.

A heavy infestation of <u>L</u>. <u>blancardella</u> can cause stunting of fruit growth, reduced terminal growth, early leaf fall and reduced fruit set the following season (Kremer, 1963). The leafmining larvae decrease the yield of apple trees by lowering the photosynthetic area of the leaves, leading to premature ripening and drop of the fruit (Pottinger and LeRoux, 1971). As leafminers affect yield indirectly,

it is extremely difficult to prove cause and effect in field studies. The preceding authors do not give any data to support their statements.

Kremer (1963) tested many insecticides against

L. blancardella and found the tissue-feeding larvae to
be more resistant to the toxicants than the sap-feeding
larvae. Beckham, et al (1950) found that regular
application of DDT for codling moth, Carpocapsa pomonella
Linn., control also controlled L. blancardella.

Attention has been drawn to the precisely timed flight period of <u>L</u>. <u>blancardella</u> which occurs during a 2-hr. period at dusk. The moths are fairly inactive during the day and after the flight period (Pottinger and LeRoux, 1971). Pottinger and LeRoux believe this behavior is due to a response to the reduction in sunlight at dusk.

EXPERIMENTAL METHODS AND MATERIALS

A. Natural Enemies

Pupae of various hymenopterous parasitoids were extracted from mines of \underline{L} . $\underline{blancardella}$ and reared in plastic petri dishes (4" dia.). Moistened tissue paper was placed in the petri dishes to prevent dessication of the pupae. Emerged adults were preserved in 90% ethanol solution and sent to Martinat* for identification [Figures 9 and 10].

Predatory chrysopid larvae were collected in the field and reared on larvae and pupae of \underline{L} . <u>blancardella</u> which were extracted from the mines. The feeding rate of the chrysopid larvae was measured as the number of leafminer larvae consumed per hour [Figure 12].

A "muscadine" (Steinhaus, 1967) fungus [Figure II] which was prevalent in field plots was brought into the laboratory. Leafminer tissue-feeding larvae which were infected with this fungus were introduced into the mines of healthy tissue-feeding larvae on a one-to-one basis. Five such mines were placed in a plastic petri dish (4" dia.) and then placed in a controlled environment chamber. The experiment was replicated 3 times with 3 control petri dishes containing 5 unadulterated mines each.

In a block of 20 McIntosh apples at Richland, the degree

^{*} Mr. Peter Martinat is a graduate student in the Department of Entomology at Michigan State University who specializes in Taxonomy.

of mortality caused by each of the distinguishable mortality factors was measured at the end of each generation of the leafminer. A 100 mine sample was dissected by peeling away the lower leaf epidermis and the contents of each mine were recorded on each sample date [Table 1].

B. Seasonal Development

The development of each generation of <u>L</u>. <u>blancardella</u> was monitored in 2 untreated apple orchards at Richland and Hickory Corners, Michigan. Samples were taken at Richland from July 18, 1973 until July 16, 1974. The degree of parasitism increased to a high level during the 1974 season so that accurate monitoring of the development of the leafminer was not possible. At this time the sampling site was changed to the Hickory Corners orchard which is about 7 miles northeast of Richland. Jonathan variety apples were sampled at each site.

A 100 mine sample was taken at Richland from a 20 tree plot on each sample date. The sample was examined the same day and the number of leafminers in each of the following stages of development was counted:

- I. Sap-feeders
- 2. Tissue-feeders

- 3. Pupae includes all non-feeding stages between instar V and the adult.
- 4. Emerged adults includes the number of empty pupal cases found protruding from the mines of the current generation [Figure 7].

The number of leafminers which were parasitized by any of the distinguishable natural enemies also was recorded on each sample date. Sampling continued until November 4, 1973.

Spring generation adult emergence was monitored in 1974 using 4 cardboard traps which were given a light coating of Tanglefoot* adhesive. The 12" square boards were placed on top of the leaf litter, adhesive side down, around the base of a single tree which was heavily infested the previous year. The boards were put out on April 16, 1974 and were checked for adults on each sampling date until June 12 [Table 2a].

Leaf sampling started again on May 13 and continued until all third generation larvae either pupated or stopped feeding on November 5, 1974. A 100 mine sample from 20 trees was taken on each sample date from Hickory Corners [Table 2b].

C. Effect of Mining on the Host

A 500 leaf sample was picked at random from a plot of 12 Jonathan trees at the Hickory Corners orchard at the end

^{*} Manufactured by the Tanglefoot Co., Grand Rapids, Michigan.
Active ingredients: Polybutenes and hydrogenated castor oil.

of each generation during 1974. The number of mines per sample was recorded and the average number of mines per leaf was calculated. A sample of 30+ mines was selected at random from the former sample and the lower epidermis of the mined area was dissected away. Care was taken to dissect away only mines of the current generation. The lower surfaces of the mined leaves were photocopied and the leaf copies were cut out and weighed. The mined areas of the leaf copies were then cut out and weighed. The ratio of the mine weights (of the paper photocopies) and the whole leaf weights was multiplied by the average number of mines per leaf and considered as an estimate of the leaf area damaged by each generation of the leafminer [Table 3].

The reaction of the leaf to the intrusion of the leafminer was observed under a compound microscope. A 1-cm latitudinal section was cut from a fresh leafmine and placed in a 10% solution of saffranin dye. The section was placed in a microtome made from an 8-mm bolt and nut. Paraffin was poured over the specimen and a thin section was cut across the mine. The section was then placed in xylol to dissolve the paraffin and was then mounted on a glass microscope slide. The section was scanned under the microscope [Figures 14 and 15].

D. Chemical Control

Certain carbamate and organophosphate insecticides were tested during the 1974 season to determine the effect of these compounds on the population density of $\underline{\mathsf{L}}$. $\underline{\mathsf{blan-cardella}}$. The insecticides were applied in 4 different plots which differed in apple variety. Specifics of each plot are as follows:

- Plot I Apple variety: Red Delicious Location: Fennville, Michigan Spray schedule: A*
- Plot 2 Apple variety: McIntosh Location: Fennville Spray schedule: A*
- Plot 3 Apple variety: Jonathan Location: Fennville Spray schedule: A*
- Plot 4 Apple variety: Jonathan Location: Douglas, Michigan Spray schedule: B*

There were 3 replications for each treatment in each plot.

A 50 leaf cluster sample was taken from each replicate of each treatment. The number of mines per sample was considered as an estimate of the population density of <u>L. blancardella</u> in each tree. Each replicate consisted of a single treated tree [Table 4].

The following experimental and registered broad spectrum insecticides were applied with a John Bean air blast sprayer:

^{*} For spray schedule 'A' application dates were: 5/24, 6/7, 6/20, 7/3, 7/17, 7/31, 8/28, 9/11. For spray schedule 'B' application dates were: 6/3, 6/17, 7/1, 7/15, 7/29, 8/12, 8/26, 9/9.

San 197 - organophosphate, Sandez Wander, Inc.

San 201 - organophosphate, Sandez Wander, Inc.

DS 15647 - carbamate, Diamond Shamrock Chemical Co.

CGA 18809 - organophosphate, Ciba - Geigy Corp.

CA 6900 - E. M. Laboratories, Inc.

CME 74020 - E. M. Laboratories, Inc.

Mobil 9087 - Mobil Oil Co.

Lorsban - chlorpyrifos, organophosphate, Dow Chemical Co.

Lannate - methomyl, carbamate, DuPont Chemical Co.

Parathion - thiophos, organophosphate, Monsanto Chemical Co.

Guthion - azinphos methyl, organophosphate, Chemagro Corp.

To determine the effect of systemic insecticides on the larval stages of $\underline{\mathsf{L}}$. $\underline{\mathsf{blancardella}}$ the following compounds were applied in a randomized complete block design with a hand held spray gun:

Systox - demeton, organophosphate, Chemagro Corp.

Meta-systox R - oxydemeton methyl, organophosphate, Chemagro Corp.

Cygon - dimethoate, organophosphate, American Cyanamid Co.

Each replicate consists of one side of a single tree. There were 3 replicates per treatment and azinphos methyl was used as a standard for comparison.

Treatment blocks were designed to reduce variance due to population density measured in the pre-spray counts [Figure 16]. Leafminer density was determined in the pre-spray

counts by recording the number of leafmines in a sample of 10 leaf clusters [Figure 16]. The effect of these systemic insecticides was measured in two separate trials on the sapfeeders and the tissue-feeders. Jonathan apple variety was used in each trial. At Hickory Corners, the sprays were applied while the larvae were in the sap-feeder stage on August 20, 1974. Six days after treatment a 15 mine sample was collected from each replicate and the percent mortality was determined by dissecting the mines in the laboratory. At Richland, the sprays were applied while the larvae were in the tissue-feeder stage on June 27, 1974. Sixteen days after treatment a 25 mine sample was taken from each replicate and the percent mortality was determined by dissecting the mines in the laboratory. To reduce the variance due to natural mortality of the leafminer and to gain a better estimate of the effect of the insecticides, the efficacy of each material was determined using the formula proposed by Abbott (1925) as presented by Unterstenhofer (1963).

Efficacy =
$$\frac{\text{no. living leafminers}}{\text{living leafminers in the control}} = \frac{\text{no. living leafminers}}{\text{living leafminers in the control}} \times 100$$

The percent mortality and efficacy data for these trials is given in Tables 5a and 5b.

The effect of certain broad spectrum sprays on the degree of parasitism in the leafminer population was determined at the Douglas orchard (plot 4). The degree of parasitism by

Apanteles ornigis and eulophid parasitoids was measured in treatment replicates which had a sufficient number of mines for sampling. For this determination a sample of leafmines was collected from each replicate and examined in the laboratory. The sample size varied from 20 to 50 mines depending on the availability of material [Table 6].

E. Miscellaneous Studies on the Adult Leafminer

- a. Fecundity A sample of 35 leafmines was collected and placed into a single 18" X 6" nylon mesh sleeve. The sleeve was then placed around a 12" branch of apple. The leafminers in the sleeve were in the pupal stage. Four weeks after the sleeve was put into the field the sleeve was removed along with the enclosed branch. The number of mines on the leaves of the branch and the number of empty female and male pupal cases in the 35 mine sample were counted.
- b. Parthenogenesis A sample of 25 female pupae was placed into a petri dish (4" dia.) and were then reared to adults. In 3 weeks 20 females emerged which were placed in a nylon sleeve similar to the one used in the fecundity experiment. The sleeve was placed around an apple twig which was cut from the tree and placed in a jar of water. After all the females had died (2 weeks) the leaves of the twig were examined for eggs and leafmines.

c. Pheromone studies - On August 2, 1973 virgin female moths of <u>L</u>. <u>blancardella</u> were placed into 2 cylindrical plastic cages (6 females per cage) measuring I-cm deep by 2-cm in diameter with one end covered by a fine mesh nylon screen. Each cage was placed inside of a 3M 'Sectar' trap which is commonly used to trap other orchard pests via pheromones. The 2 traps were set out in the field along with 2 empty control traps at the Richland orchard.

RESULTS AND DISCUSSION

A. Natural Enemies

The following hymenopterous parasitoids were found to attack the larval stages of L. blancardella in Michigan:

Braconidae Apanteles ornigis Weed

Eulophidae Sympiesis conica (Provancher)

Sympiesis marylandensis Girault

Sympiesis sp.

Pnigalio flavipes (Ashmead)

Predacious chrysopid larvae were observed to be natural predators of adult <u>L. blancardella</u>, attacking them during the daytime soon after they emerged. These chrysopid larvae were able to consume an average of 7 leafminer larvae per hour in the laboratory. These predators were only identified to the family level as rearing them to the adult stage was not accomplished.

Of the 15 mines infected with the "muscadine" fungus,
5 healthy larvae became overgrown by the fungus. One healthy
larvae in the 15 control mines became infected. When these
infected larvae where transferred to 6 healthy sap-feeder mines
no further infections occured.

The counts of the mortality factors of \underline{L} . $\underline{blancardella}$ at the end of each generation [Table I] reveal a decrease

Table 1. - Counts of the mortality factors of <u>L. blancardella</u> from a 100 mine sample taken at the end of each generation of the leafminer, Richland, Michigan, 1974.

Mortality factor	Sampling date and generation July 16, 1st Sept. 3, 2nd Nov. 5, 3rd (no. per sample)				
Apanteles ornigis	32	I 4	18		
All eulophids	16	49	17		
Hyperparasitoid*	12	5	0		
" Muscadine" fungus	14	16	8		
Unknown causes**	16	11	19		
Emerged adults and living pupae	10	5	38		

^{*} Attempts to rear the pupae of this insect failed so identification was not possible. However, its biology is very similar to the hyperparasitoid described by Beckham (1950) and Pottinger and LeRoux (1971) of the genus Horimenus.

** Mortality in this catagory may have been caused by predation or dehydration of the larvae.

in the incidence of parasitism by \underline{A} . originis and an increase in the incidence of eulophid parasitism after the first generation. Mortality was lowest in the third generation.

B. Seasonal Development

Tables 2a and 2b show the field data for the 1973 and 1974 seasons. Table 2b is annotated with significant field observations. Figures 2a, 2b and 2c are plots of the development of the life stages in 1974. The data show that the generations of the leafminer are discrete in southwestern Michigan. The degree of parasitism seems to be least during the sap-feeder stage of the leafminer. Spring generation adult emergence occurs during the first warm (70+°) days of spring.

C. Effect of Mining on the Host

A total leaf area reduction of 31.7% was realized by the end of the third generation at Hickory Corners in 1974. The average area per mine increases with each generation [Table 3]. First generation mines appeared on the central leaves and water sprouts more frequently than on the outer canopy. Second and third generation mines were fairly evenly distributed throughout the canopy.

Even though the larvae specifically ignore the vascular

Table 2a. - Counts of spring generation adults from 4 adhesive traps distributed on top of the leaf litter around a single apple tree, Richland, 1974.

ample date	No. of adults trapped			
oril 23	0			
26	264			
ay 2	50			
13	61			
20	57			
ine I	5			
6	3			
10	0			

Table 2b. - The development of each generation of <u>L. blancardella</u> through time, recorded as the number of individuals in each life stage per 100 mine sample, Richland and Hickory Corners, Michigan, 1973 and 1974.

<u>Life</u> stages							
Sample date	Sap- feeders	Tissue- feeders	Pupae	Emerged adults	Parasitized leafminers		
1973							
July 18 25 31	5 7 8	20 15 24	2 0 0	3 25 14	70 53 54		
Aug. 2 6 9 14 16	3 0 1 0 5	44 25 20 11 12	20 10 3 8 0	6 15 12 32 15	27 50 64 49 68		
Sept. 5 17 29	90 60	4 37	0 0 1	0 0	48 6 3 I		
Oct. 6 20 27 Nov. 4	0 0 0	77 58 46	3 0 20 63	0 0 0	20 42 34 11		
July 18 25 31 Aug. 2 6 9 14 16 24 Sept. 5 17 29 Oct. 6 20	8 3 0 1 0 5 22 90 60 23 0 0	15 24 44 25 20 11 12 12 4 37 75 77 58	0 0 20 10 3 8 0 11 0 0	25 14 6 15 12 32 15 17 0 0 0	53 54 27 50 64 49 68 48 6 3 1 20 42		

Table 2b. (continued)

			<u>Life</u>	stages		
Sampl date	е	Sap- feeders	Tissue- feeders	Pupae	Emerged adults	Parasitized leafminers
1974						
May	2 ^b	0 68	0	0	0	0 32a
June	20 1 6 10 12 ^e 14 18 20 22 28	46 38 23 28 16 11 8 0 4	13 22 25 32 46 36 62 42 52	0 0 3 1 4 4 6 8	0 0 0 0 0 0 0	41° 40 52 ^d 39 33 39 26 52 36
July	1 4f 10 13b 16h 22 27e	0 0 0 10 28 20 21	38 18 0 0 35 38 46	8 16 18 10 0 0	0 18 14 14 12 19	54 58 64 66 25 23
Aug.	1 7f 13 ^b 20 26	6 0 33 28 34	62 44 13 3 0	14 31 27 10 1	4 5 9 33 36	18 20 25 26 29
Sept.	3 ^e 10 18 23	49 61 49 50	10 5 16 20	0 0 0	12 10 14 15	28 24 21 15
Oct.	2 8 15 22 29	19 8 0 0	73 79 85 82 41	0 1 2 5 51	4 3 1 1 0	4 9 12 13
Nov.	5	0	2	51	0	4 79

a Dead larvae were either dehydrated or overgrown by fungus.

b Eggs in advanced stage of development first sighted.

c First eulophid larva sighted within mine [Figure 9].

d First A. ornigis cocoon sighted within mine [Figure 10].

e No adults sighted in the field.

f Adults first sighted flying in the field.

g Parasitism mainly due to A. originis.

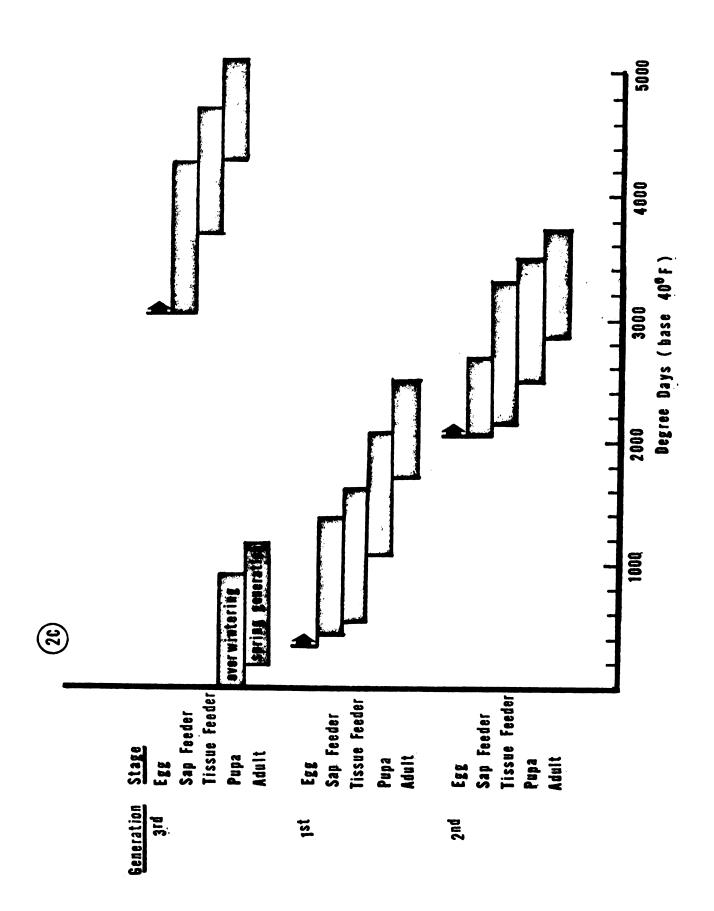
h Sampling site switched from Richland to Hickory Corners.

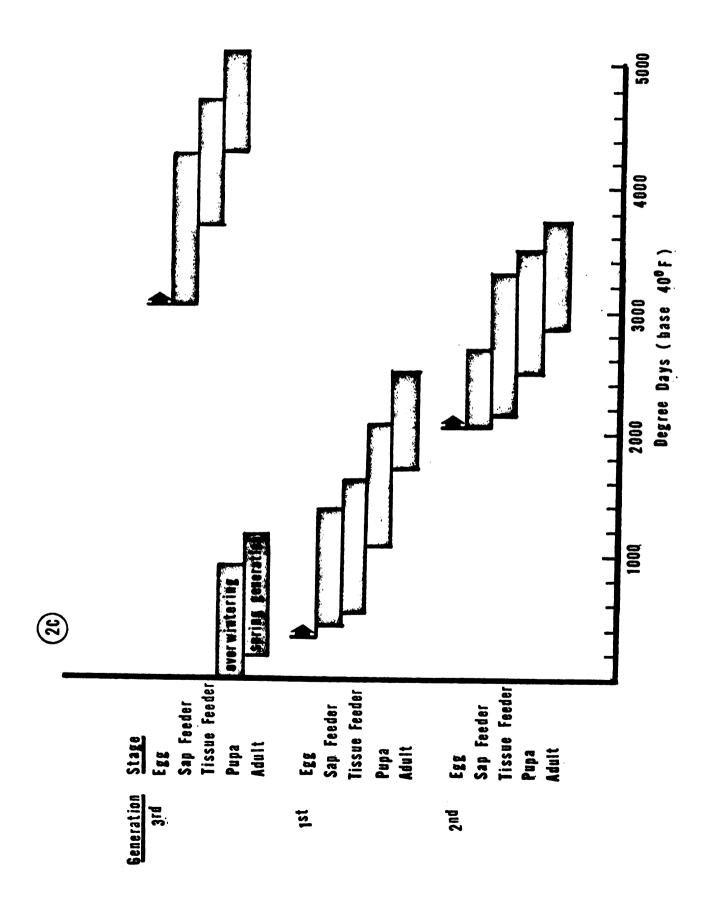
FIGURES 2a, 2b and 2c - Seasonal development of \underline{L} . <u>blancardella</u>, Richland and Hickory Corners, Michigan, 1974.

Figure 2a is a plot of the percent of the unparasitized sample [Table 2b] in the adult and sap-feeder stages versus sampling date. Solid line represents the adult stage and the dotted line represents the sap-feeder stage.

Figure 2b is a similar plot of the tissue-feeder and pupa stages. Solid line represents the tissue-feeder stage and the dotted line represents the pupa stage.

Figure 2c is a plot of the development of each stage of each generation of the leafminer as it relates to temperature. Temperature data were provided by the National Weather Service which monitors daily temperatures at Hickory Corners, Michigan. Degree days were calculated from daily minimum and maximum temperatures using a base temperature of 40° F. Degree days were accumulated from April I, 1974 until November 5, 1974.





epidermis from the spongy mesophyll causes dessication of the mined area after the adult emerges. In severe infestations, i.e. 5 or more mines per leaf, the leaf becomes folded to the extent that effective leaf area is reduced.

While scanning the parenchyma cells inside the mines of new leaves under a microscope, clusters of ca. 20 to 30 hypertrophied parenchyma cells are often observed protruding outward toward the lower epidermis. These cells are ca. 4X the size of normal parenchyma cells. Close examination of the parenchyma cells along the outer margin of the mine reveals many areas of cell regeneration [Figures 14 and 15]. This indicates that the leaf is able to repair itself if the leafmining larva dies.

Reduction in leaf area is minimal after the first two generations [Table 3]. The greatest damage occurs after the third generation. It is doubtful that a significant reduction in yield will occur during the season of a heavy infestation since most of the damage occurs so late in the season. Such an infestation, however, will cause reduced vigor and possibly reduced yield the following season.

D. Chemical Control

All broad spectrum insecticides at the Fennville site

(plots I, 2 and 3) gave excellent control of L. blancardella

Table 3. - Estimates of the leaf area damaged by each generation of L. blancardella larvae, Hickory Corners, Michigan, 1974.

Sample date	Genera- tion	Mines/ 500 leaves (no.)	Mines sampled (no.)	Mean area/ mine (cm ²)	Estimated damage (% leaf area)
July I	lst	75	34	0.81	2.1
August 7	2nd	284	33	0.90	5.7
October	16 3rd	1262	35	0.92	23.9

[Table 4]. This can be attributed to the early and continual coverage of the foliage. At the time of the first application, spring generation adults were emerging and first generation larvae were in the sap-feeder stage. Infestations of the sprayed trees were minimal and not of economic importance.

At Douglas (plot 4) all treatments were significantly lower with respect to leafminer infestation than the control. No significant difference was found between the high and low rates of San 197, San 201 or DS 15647. The population density in the DS 15647, single application, treatment was greater than in the DS 15647, regular schedule, treatment. No significant difference was found between the 2 formulations of methomyl. These data indicate that continuous coverage of the foliage with DS 15647 gives better control than a single application of this compound. Satisfactory control of <u>L. blancardella</u> can be achieved using the reduced rates of San 197, San 201 and DS 15647.

The systemic insecticides were generally more effective against the sap-feeders than the tissue-feeders [Tables 5a and 5b]. Oxydemeton methyl and dimethoate caused higher mortality of the sap-feeders than demeton. No significant difference was found between the azinphos methyl and demeton treatments in the sap-feeder plot with respect to mortality.

Table 4. - Field evaluations of indicated insecticides applied as foliar treatments to control <u>L. blancardella</u> on apple, Fennville and Douglas, Michigan, 1974.

Plot	Treatment and formulation	oz.	Al/100 gal.	Mean no. leafmines 50 leaf clusters*
	0			51.7 -
	Control CGA 18809, 50 WP		4	51.7 a 0.0 b
 	CGA 18809, 50 WP		4 6	0.0 b
1	Azinphos methyl, 50	WP	4	0.0 b
'	Azimphos mernyi, 30	***	7	0:0 5
2	Control			124.3 a
2	Thiophos, 2 EC		8	2.7 b
2	Thiophos, 25 WP		8	0.7 b
2	CA 6900, 4 EC		3	0.0 b
2 2	CME 74020, 25 WP		3	0.0 b
2	CME 74020, 25 WP		4	0.0 b
2	Azinphos methyl, 50	WP	4	0.0 b
3	Control			100.3 a
3 3	Chlorpyrifos, 25 WP		3	4.0 b
3	Chlorpyrifos, 25 WP		8	4. 0 b
3	Azinphos methyl, 50	WP	4	0.0 b
4	Control			40.0 a
4	DS 15647, 4 EC**		16	22.7 b
4	Mobil 9087, 2 EC***		4	14.0 bc
4	Methomyl, 1.8 EC		4	9.0 bc
4	DS 15647, 4 EC		8	7.3 bc
4	San 197, 4.3 EC		4	6.7 c
4	Methomyl, 90 WP		4	4. 7 c
4	San 201, 4.3 EC		4	3.3 c
4	San 197, 4.3 EC		8	2.0 c
4	DS 15647, 4 EC		16	2.0 c
4	San 201, 4.3 EC		8	1.7 c
4	Azinphos methyl, 50	WP	4	0.0 c

^{*} Means in the same plot which are followed by similar letters are not significantly different at the 1% level (Duncan's multiple range test).

^{**} Single application on June 3, 1974.

^{***} Applied every fourth week on June 3, July 1, July 29, and August 26, 1974.

Table 5a. - Effect of a single application of the indicated insecticide on the sap-feeder larvae of L. blancardella, Hickory Corners, Michigan, 1974.

Treatment and formulation	oz. Al/l00 gal.	Mortality of larvae (%)*	Efficacy (%)
Control		20.0 a	
Demeton, 50% EC	2.25	62.3 b	87
Azinphos methyl, 50 WP	4.00	73.3 cb	68
Dimethoate, 40% EC	6.75	88.9 c	87
Oxydemeton methyl, 25% EC	6.00	95.6 c	95

Table 5b. - Effect of a single application of the indicated insecticide on the tissue-feeder larvae of L. blancardella, Richland, Michigan, 1974.

Treatment and formulation	oz. Al/l00 gal.	Mortality of larvae (%)*	Efficacy (%)
Control		61.5 a	
Azinphos methyl, 50 WP	4.00	85.3 b	62
Demeton, 50% EC	2.25	90.7 Ь	76
Dimethoate, 40% EC	6.75	90.7 Ь	76
Oxydemeton methyl, 25% EC	6.00	90.7 b	76

^{*} Means of 3 replicates. Means followed by similar letters are not significantly different at the 1% level (Duncan's multiple range test).

However, the efficacy of azinphos methyl was lower than the efficacy of any of the systemic insecticides in both sapfeeder and tissue-feeder trials. Mortality in all treated, sap-feeder plots was significantly higher than in the control plot. This is also true of the mortality in the treated, tissue-feeder plots except that all treatment means are equivalent. No significant differences were encountered in the degree of parasitism between any of the systemic insecticide treated plots and the control plots.

Table 6 shows that parasitism by \underline{A} . ornigis was significantly reduced in plots treated with San 201 and Mobil 9087 (plot 4, Douglas, Michigan). Parasitism by \underline{A} . ornigis in plots treated with DS 15647 and methomyl was the same as in the control. Parasitism by all eulophid parasitoids was significantly lower in all treated plots than in the control plot.

E. Miscellaneous Studies on the Adult Leafminer

- a. Fecundity Of the 35 pupae placed in the nylon mesh sleeve, 25 reached the adult stage (12 males and 13 females). When the leaves enclosed in the sleeve were checked 4 weeks later, 105 linear leafmines were present.
- b. Parthenogenesis After all female moths enclosed in the sleeve died (2 weeks after the start of the experiment)

Table 6. - Effect of indicated insecticide on the parasitic Hymenoptera which parasitize the immature stages of <u>L. blancardella</u>, Douglas, Michigan, 1974.

Treatment and formulation			parasitized with: All Eulophidae*	Mean no. mines sampled
Control		13.7 a	28.7 a	38
Methomyl, 90 WP	4	12.7 a	12.0 b	27
Methomyl, I.8 EC	4	6.3 ab	12.0 b	38
DS 15647, 4 EC	8	5.7 ab	12.0 b	30
Mobil 9087, 2 EC	4	2.3 b	15.7 b	27
San 201, 4.3 EC	4	1.3 b	9.0 b	29

^{*} Means followed by similar letters are not significantly different at the 1% level (Duncan's multiple range test).

the leaves of the enclosed apple twig were checked for leafmines and leafminer eggs. No leafmines or eggs were found.

c. Pheromone studies - Although none of the data was statistically significant some replicates gave results that could not be ignored.

In the virgin female traps set out August 2, 1973,

7 male moths were caught in one trap and 2 female moths were caught in the other. No moths were caught in the 2 empty control traps. Experiments conducted by Ayers* indicate that male moths are attracted to cis-7-hexadecenyl acetate and cis,cis-7,ll-hexadecadecenyl acetate and cis-II-tetradecenyl acetate.

The lack of parthenogenesis, the equal abundance of males and females in the field and the attractiveness of virgin females and the preceding organic acetates to the male moths indicate that mating is initiated by an air-borne chemical pheromone which is released by the female moth.

Although many broad spectrum insecticides are effective against this leafminer, resistance to these materials may occur as is the case with other multivoltine fruit pests, e.g. <u>Psylla</u> pyricola on pear and Typhlocyba pomaria on apple. The

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nonspecific action of the broad spectrum insecticides also destroys the natural control mechanisms of this leafminer, i.e. the protelean parasites and predators. An integrated control approach is suggested employing correct timing of low dosage insecticide applications and the use of parasitoid-safe systemic insecticides.

To increase the accuracy of spray application timing, more research is required to elucidate the structure of the sex attractant pheromone of $\underline{\mathsf{L}}$. $\underline{\mathsf{blancardella}}$. A correlation between the emergence of the leafminer and the developmental stage of the host apple tree would give us a better understanding of the relationship between development of the insect and the phenology of the host.

FIGURES 3 through 15 -

Figures 3 through 8 depict the developmental stages of \underline{L} . plancardella.

Figures 9 through 12 depict the natural enemies of \underline{L} . blancardella.

Figures 13 through 15 depict the effect of mining on the host plant.

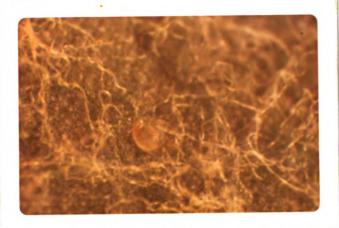


Figure 3. - Leafminer egg on lower surface of apple leaf.



Figure 4. - Sap-feeding larva on inner mine surface.



Figure 5. - Tissue-feeding larva on inner mine surface.



Figure 6. - Male pupa within slight cocoon. Note irregular feeding sites (arrow).



Figure 7. - Empty pupal case protruding from leafmine. Note folds in the leaf epidermis (arrow).



Figure 8. - Adult.

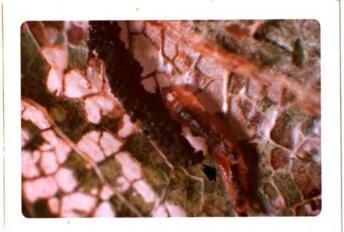


Figure 9. - Eulophid parasitoid larva (arrow) attacking tissue-feeding leafminer.

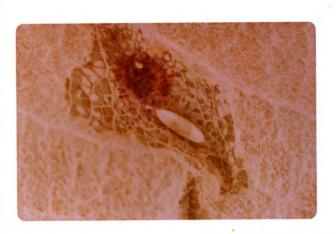


Figure 10. - Cocoon of A. ornigis as it is found in the leafmine.



Figure II. - Fungal growth on larva (arrow) and mine surface, commonly found in the mines.



Figure 12. - Chrysopid larvae attacking extracted leafminer pupa.



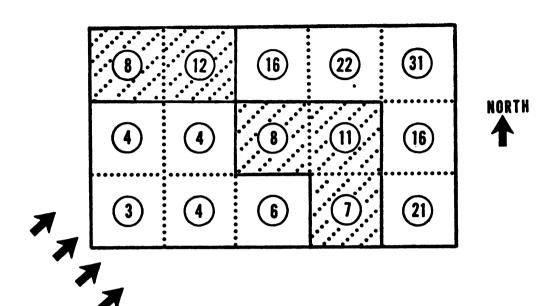
Figure 13. - Close-up view of 3 mature leafmines on a single apple leaf.





Figures 14 and 15 (left and right, resp.). - Thin cross-sections of the lateral margin of the leafmine (mag. = 100X). The upper epidermis of the apple leaf is to the right and the lower epidermis which has been separated from the rest of the leaf is to the left in each figure. Regenerating cells of the spongy mesophyll tissue of the leaf are evident between the 2 epidermal layers (arrows). Note the cellular connection (arrow) between the lower epidermis and the spongy mesophyll tissue in figure 15.

L. blancardella in a 15 tree plot of Jonathan apple trees at Richland, Michigan, used for the experiment which tested the effect of certain systemic insecticides on the mortality of the tissue-feeder larvae. Each square represents a single tree and the encircled number is the mean density (based on 3 counts per tree) of leafmines in the tree expressed as the number of mines per 10 leaf clusters. The solid lines enclose the three blocks of the randomized complete block design which was designed to remove variance due to leafmine density. Blocks I and III are plain and block II is stippled. The 4 arrows in the lower left corner represent the direction of the southwesterly prevailing winds.



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