

## ABSTRACT

Title of Thesis: INTEGRATED MANAGEMENT OF  
THE BOXWOOD LEAFMINER

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Degree and year: Master of Science, 1999

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Landscape managers need durable, effective, and safe methods for controlling key pests of valued plants in both landscape and nursery settings. The boxwood leafminer (*Monarthropalpus flavus*, Diptera: Cecidomyiidae) is a serious pest of boxwoods. Boxwoods (*Buxus sp.*) are a key plant in suburban Maryland landscapes. They are the second most common woody ornamental plant in these settings. In a recent study almost 43% of boxwoods surveyed required treatment for leafminer infestation. Boxwood leafminers also pose a serious problem in historical gardens, such as Longwood Gardens, PA, Dumbarton Oaks and the US National Arboretum in Washington, DC. At the present time, there is a lack of a

comprehensive, environmentally sound, management program for the boxwood leafminer.

The first step toward an effective management strategy is a better understanding of the boxwood leafminer's life cycle. Over the summers of 1994-1995, leafminer populations were surveyed and life cycles documented and correlated with growing degree days. The first growing degree day developmental chart for boxwood leafminer was developed.

Various pesticides were tested in 1995. Different chemicals and application times were evaluated for control of both adults and larvae. At present it appears that application of a translaminar pesticide such as Avid or Merit at adult emergence (growing degree day 352) provides the best control.

Resistant cultivars appear to be the most durable, simplest method to control the leafminer. Some cultivars are highly resistant to boxwood leafminer attack while others are highly susceptible. The third goal of my project was to identify resistant cultivars. This was accomplished by first observing natural variation in leafminer populations in the field. Next I caged ovipositing adults on terminal branches of various cultivars of boxwood, and measured survival of larvae. All cultivars received heavy

oviposition with equal frequency, although survival rates were very different.

Finally, I tested the hypothesis that leafminers could discriminate among resistant and susceptible cultivars. To test this emerging adults were caged with different cultivars of boxwood and allowed to select plants for oviposition. Plants were then analyzed to determine acceptance of various host plants. I found that although survival on different cultivars can vary dramatically, leafminers were unable to distinguish between suitable and unsuitable host plants.

INTEGRATED MANAGEMENT OF THE BOXWOOD LEAFMINER

by

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Thesis submitted to the Faculty of the Graduate School of the  
University of Maryland, College Park in partial fulfillment  
of the requirements for the degree of

Master of Science

1999

CI

MD

Dept. of Entomology

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## ACKNOWLEDGMENTS

I would like to thank my family and friends who have stood by me and encouraged (nagged) me through this long and at times, arduous process. Thanks to my adviser Mike Raupp for his patient guidance. I owe a great debt to Jennie Wicker and Janet Kintz for their help in designing and implementing my field experiments. Also thanks to Bruce Steward and Lynn Batdorf for their advice and assistance over the years. Thanks also to Galen Dively and Lee Hellman for their support as committee members.

This project would not have been possible without the generosity of numerous organizations. Among them Longwood Gardens, The Boxwood Society, The US National Arboretum, Pesticide Environmental Stewardship Program - EPA, Saunders Brothers Nurseries, and The University of Maryland Department of Entomology.

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# CHAPTER 1

## INTRODUCTION AND OBJECTIVES

### INTRODUCTION

Nursery production and landscape maintenance industries need reliable approaches for managing key pests of valued plants. Boxwoods, *Buxus sp.*, are man's oldest cultivated ornamental plant and are also among the most prized, useful, and valued of all woody shrubs produced by the nursery industry (Batdorf 1994). They are important components of estate, historic, and residential landscapes. Traditionally, boxwoods have also been used in numerous folk medicines (Greive 1971). In western and southern Europe, boxwood has been used as a vermifuge and a purgative (Chiej 1984). The wood of boxwood is one of the densest, hardest known woods and is prized by model builders, cabinet makers, and woodcarvers. In classical times the unique wood of boxwood was known as dudgeon. Its non-expansive, uniform nature makes it a common material for measuring devices, musical instruments, and mathematical instruments (Greive 1971).

The boxwood leafminer, *Monarthropalpus flavus* (Schrank) is a key insect pest of boxwoods in both nurseries and landscapes (Schrank, in Gagne 1989). A common misnomer for this pest is *Monarthropalpus buxi*

(Laboulbene 1873), *M. flavus* is the correct name. A survey of Maryland landscapes in 1982 found that while boxwoods in residential settings accounted for only 8.8% of total plants, boxwood leafminers accounted for almost 25% of total pest problems (Raupp 1985). In various settings, almost 43% of landscape boxwoods show leafminer problems and required treatment (Raupp 1984). Cultivars of American boxwood, *Buxus sempervirens* *Arborescens*, are severely damaged by this insect which causes damage in its larval stage by mining and galling parenchyma tissue of boxwood leaves. Mined leaves are discolored and blistered which reduces the aesthetic quality of the plant. In heavy infestations, leaves senesce and drop prematurely rendering the canopy thin and unsightly. Heavily infested plants are more susceptible to cold injury and winter kill. Heavy infestation also attracts predatory birds that rip open the galls to eat the larvae. They can remove many of the miners, but collateral damage from their feeding is usually worse than that of the leafminers.

At the present time, control of the boxwood leafminer is unreliable due to a lack of knowledge regarding the choice and optimal timing of pesticide applications. Historically, timely applications of molasses plus nicotine sulfate, fumigation with hydrogen cyanide gas, or even dipping smaller plants in boiling water have provided adequate control of the leafminer (Hamilton 1925). Sulfur dusts have been used against adults

with moderate success, and applications of arsenic have been attempted with minimal success (Hamilton 1925). The main problem with dusts and molasses-based sprays is their removal by rain and wind. Since adults emerge over a two week period, it is difficult to keep materials on the plant long enough to kill all of the adults. DDT was recommended for control of adults as they emerged and walked through the material (Barnes 1948). DDT and molasses/nicotine sulfate were applied at the first sign of adult emergence. Cyanide fumigation was done in the fall when plant growth had slowed to reduce damage to plant tissue.

Modern control is usually attempted with a contact insecticide for adults and systemic insecticide to control larvae (Brewer 1980, Batdorf 1994). Brewer (1980) tested Soldep, pirimiphos-methyl (Actellic), and omethoate (Folimat). Pirimiphos-methyl seemed to provide reasonably good control. Schread (1967) obtained effective control with late (July 22) applications of diazinon (Diazinon) and even later (August 4) applications of dimethoate. Carbaryl (Sevin) (also applied July 22) was found to give less control. Late applications of dimethoate were not very effective when tested in the summer of 1994 at Dumbarton Oaks (P. Page, personal communication). Newer pesticides such as avermectin (Avid) and imidacloprid (Merit) are currently under examination for potential usefulness. Preliminary studies indicated that avermectin and imidacloprid both provide exceptional control (d'Eustachio, unpublished). A common

feature of all these methods is a limited time period for effective control (Hamilton 1925, Brewer 1980, Batdorf 1994, Relf 1994). Application of pesticides coinciding with emergence of adult leafminers is important, although not essential for effective control.

The best tactic for achieving long-term control of boxwood leafminer is to plant resistant cultivars of boxwood. Resistance has been documented as far back as the turn of the century (Chaine 1913). However, there has never been an in depth experiment to evaluate cultivar resistance. Most previous studies lack quantitative data regarding the levels of susceptibility. Brewer (1980) attempted to test differences in resistance in 1980, but an enormous amount of winter kill disrupted his studies. Moreover, he selected cultivars not very common to American growers (Brewer *et al.* 1980).

Boxwoods have very distinctive allelochemistry. There have been numerous papers written on the subject of boxwood alkaloid chemistry (Atta-ur-Rahman *et al.*, 1992, Atta-ur-Rahman 1991, Dzhakeli 1990). Some of the chemicals isolated from boxwood include numerous steroid alkaloids, flavinoid glycosides, and flavinoids all of which are potentially biologically active against many herbivores (Rosenthal and Berenbaum 1991). Varying levels of allelochemicals, leaf toughness, or other factors could give some

cultivars resistance to boxwood leafminer while making others more susceptible.

## OBJECTIVES

The objectives of this project were threefold. First, I developed a growing degree day model to correlate various life history events of boxwood leafminer with heat accumulation. The goal was to develop a model useful for timing control tactics such as insecticide applications. Second, I evaluated the efficacy of several insecticides to reduce boxwood leafminer populations. Third, I attempted to evaluate various cultivars of boxwood for their resistance to leafminer attack. This was done by first surveying plants in the field and noting the levels at which various cultivars were attacked. Next, I compared the ability of boxwood leafminer to survive in various cultivars of boxwood. I also evaluated the mechanism by which certain cultivars resist attack. I was interested in determining if leafminers avoided certain cultivars for oviposition or if leafminers failed to develop in certain cultivars.

## CHAPTER 2

### LIFE CYCLE AND NATURAL ENEMIES

#### LIFE CYCLE OF THE BOXWOOD LEAFMINER

The boxwood leafminer is a small, fragile cecidomyiid that lives most of its life inside the leaves of boxwood. It was first identified by Schrank (in Gagne, 1989). Laboulbene gave a detailed description of its morphology and life cycle in 1873 (Laboulbene 1873). His description included an excellent set of drawings of adults, larvae, and gall damage. Orange adults lay eggs through the underside of newly expanded leaves. They have not been observed to feed from oviposition scars as do some leafminers (Barnes 1948). The period of adult emergence has long been thought of as the best time for the application of control tactics (Hamilton 1925, Barnes 1948, Brewer 1984).

Emergence of adults is strongly correlated with the spring leaf flush, much like that of other leafminers such as the holly leafminer (*Phytomyza ilicicola*) (Potter 1989). Boxwood leafminer adults emerge in late April and early May. Adults are bright orange, delicate, nematoceros flies that look rather like small orange mosquitoes. Boxwood leafminer adults are quite noticeable against the dark green background provided by mature

boxwoods, and their emergence is difficult to miss. Even in lightly infested plants adults form a dense cloud around their host. This is an ephemeral stage and adults only live for about a day. They quickly mate and oviposit. Female boxwood leafminers have a sharp, pointed ovipositor to insert their eggs into the underside of boxwood leaves. They spend several minutes twisting their ovipositor into the leaf tissue to accomplish this task. Females were observed to take from 3-8 minutes to oviposit (Hamilton 1925). Oviposition only occurs on new growth which appears to be critical for larval survival.

Larva will only survive on new growth, and no oviposition scars are observed on old growth. This is most likely due to the generally higher food quality of younger growth (Raupp and Denno 1983). Potter (1986) observed that holly leafminer development was closely linked to the presence of various structural and chemical defense mechanisms in holly. Quantitative allelochemicals such as tannins and lignins tend to be at lower levels in younger leaves, and younger leaves are noticeably more tender (Raupp and Denno 1983, Potter 1986, d'Eustachio personal observation). Additionally, it has been hypothesized that gall-formers can only form galls on plant material that is still growing (Washburn and Cornell 1981, Potter and Redmond 1989). Although with boxwood leafminer, gall development accelerates when leaves are relatively mature in late fall and early winter. Brewer (1980) found that larval survivorship decreased significantly if



adults were forced to wait 1-2 weeks after leaves had expanded before being allowed to oviposit. This is possibly due to the leaves being too old and tough for leafminers to effectively utilize.

Eggs are small, gelatinous, translucent and about the size of a leaf parenchyma cell. They are placed deeply into the leaf tissue through the abaxial surface of the leaves. It has been estimated that females lay an average of 20 eggs (Hamilton 1925). Oviposition causes a distinct scar on the underside of the leaves which is visible to the naked eye. Shining a light source through the leaf makes oviposition scars even more apparent. Boxwood leafminer eggs hatch in mid June.

First instar larvae are about the same size as eggs. They are horse-shoe shaped and relatively featureless. Second instar larvae appear in July and are distinguishable by the larvae "straightening out" and growing a bit larger. Second instar larvae grow remarkably larger, and this stage lasts until late August and early September. This is when gall formation begins. Hypertrophied cells are visible in the parenchyma layer of leaves. By mid August, the gall is visible as a pair of tiny bumps proximal and distal to the central vein of the leaf, on either side of the oviposition scar. This placement of gall tissue is possibly due to distribution of some unknown gall-forming factor moving through the secondary veins of the leaf.

By late August and early September, larvae have molted into their third instar. This is marked by the appearance of the sternal "breastbone" or spatulum. Third instar is the stadium that boxwood leafminers pass the winter. Boxwood leafminers continue to grow and develop through the winter months, albeit rather slowly. There is little winter mortality.

In March, larvae molt into the fourth instar. The breastbone becomes notably longer and forms a distinctive "T" shape at its posterior end. This is the stage at which most economic damage to boxwood occurs. Galls grow to their full size and the leaves become yellow or brown where galls have formed. Opening a gall will reveal specialized gall tissue clearly visible to the naked eye. Larvae are quite large (~2mm) and will writhe and roll about if disturbed. There are still very few features visible at low magnification besides the breastbone. Examination at higher magnification reveals an eversible head and mouthparts. Clearly visible under low magnification are a pair of single segmented antennal tubercles. Higher magnification reveals the mouthparts, including mandibles, maxilla, a labrum, and labium. Mouthparts are all very small, slightly sclerotized and probably not very powerful. It has been suggested that larva feed by piercing gall cells and consuming the liquid contents. As no fecal matter is readily visible in the galls, it is unlikely larvae feed on anything solid. Near the end of the fourth instar, larvae carve a small, one cell thick "window" in the underside of the leaf. Usually each larva will make its own

window, but sometimes larvae will share a window. After window formation is complete, the larva pupates.

Fourth instar larvae pupate in mid-April. Pupae first appear as a light orange color and darken as they mature. Pupae have exserted and free legs, distinct wingpads, eyes and can move about if disturbed. Eyes, antennae and wingpads turn from light red to near black during development.

At the conclusion of the pupal stage, pupae push their way out of the gall through the window and hang by their posterior end as the adult ecdyses through a suture in the thorax. Several researchers, including Chaine (1913), and Brewer (1981) have shown that adult emergence usually occurs in the first few hours of daylight. Adults emerge rather quickly and are completely free of the pupal cast in about ten minutes. Within five minutes of emergence, wings have expanded and the adult is able to fly. It is believed that they quickly mate and females start ovipositing soon after. Haste is important as adults do not feed and can survive only for a day or two.

## NATURAL ENEMIES OF THE BOXWOOD LEAFMINER

In their review of boxwood leafminers, Brewer et al. (1984) reported few natural enemies for the boxwood leafminer. Their research was conducted in Czechoslovakia. They found only a few hymenopterous parasites (poss. Hymenoptera: Eulphidae *Tetrastichus flora* (Girault) (Krombein et. Al, 1979)). The only predators that seem to have a significant effect on boxwood leafminer populations are predatory birds, primarily titmice (family Paridae). As noted earlier, they do serious damage to the plants to locate the mature larvae, and can destroy most of the infested leaves on a given plant. Damage done by birds appears more significant than that done by insects. I detected small wasp pupae in a few galls, but they were so fragile that any attempt to remove them from the gall resulted in their destruction. These were very rare, and less than 15 were found out of several thousand galls analyzed. No other larval predators were observed, however, adults were often trapped in a spiders web.

## CHAPTER 3

### GROWING DEGREE DAY MODEL

#### METHODS

Growing degree day models are an important tool for predicting periods of pest activity and administering control tactics for many pests of woody plants in landscapes (Shettler 1995, Davidson and Raupp 1994). At each of two locations studied, Longwood Gardens, PA and the US National Arboretum in Washington DC, weather stations (specifically a max-min recording thermometer) were used to record daily minimum and maximum temperatures. The averaging method, where a simple mathematical equation ( $GDD = ((\text{max} + \text{min})/2) - (\text{threshold temp})$ ) (Shettler (1995)) was used to determine the growing degree day units for a given day. Using the averaging method I calculated the number of degree days accumulated daily at each site starting March 1 for the years 1994 and 1995. A 50°F threshold temperature was used for calculations. This temperature was selected as a threshold due to the widespread adoption of this base temperature for pest prediction in the northeastern United States.

Observations were made for the key life history events of adult emergence and flight, oviposition, egg hatch and larval development. To

measure the development of boxwood leafminers from egg to adult, 20 leaves were collected each week from 2 stands of 5 plants each in Longwood Gardens and also from a group of three plants in the US National Arboretum. Each week leaf samples were dissected to determine the stadia of the larvae. Adult flight was noted by simply recording the number of plants sampled with swarming adults. This data was plotted against the growing degree day measurements of heat accumulation. These events were correlated with heat accumulation to construct a predictive model. Techniques for all of these processes were tested and refined in 1994 and a second data set was collected in 1995 to construct degree day models. (table 1, figures 1-7).

## RESULTS AND DISCUSSION

Development of boxwood leafminer is shown below in Table 1. This is the first time boxwood leafminer development has been documented using a growing degree day method. The chart shows the first date of appearance for each stadia, average date of first appearance and the approximate julian dates of developmental events. Adult flight was measured by simply observing the dramatic emergence of adults. The remaining graphs show the development of the boxwood leafminer. As is common with many insects, the early stadia are well synchronized, with later stadia more spread out. The first instar to appear in the 3<sup>rd</sup> instar, in which the

boxwood leafminer overwinters. In early March-mid April (GDD 80) the larva molt into the 4<sup>th</sup> instar. They molt into the pupal stage in April (GDD 310). Adults begin to appear around GDD 352, with peak emergence

It should also be noted that a few larval stragglers still in early instars were found throughout the year. Although they were still alive (responding if prodded), they usually did not survive the winter. If they did survive, they failed to molt in time to reach adulthood.

Table 1. Table of boxwood leafminer stadia correlated with Growing Degree Days (average measurements for the years 1994-1995). The peak emergence is shown with the standard error.

GDD TABLE

Stadia	1st Appearance (GDD)	Peak emergence GDD (SE)	Number of GDD in stadia	Approximate Dates
4 <sup>th</sup>	0	80 (27)	80	early March- mid April
Pupa	46	310 (66)	230	mid April - May
Adult	352	440 (127)	N/A	May
Egg	352	748 (104)	308	mid-late May
1 <sup>st</sup>	679	1106 (258)	358	early June
2 <sup>nd</sup>	1236	2459 (270)	1353	late June - July
3 <sup>rd</sup>	2443	3287 (161)	828*	August - March

\* The boxwood leafminer overwinters in the third instar. This number reflects the number of GDDs spent in the late summer.



Figure 1: Growing Degree Days for various stadia of boxwood leafminer estimated at two sites. Plotted points represent mean peak emergence dates and vertical lines represent standard errors.

# Boxwood Leafminer

## Development of Stadia

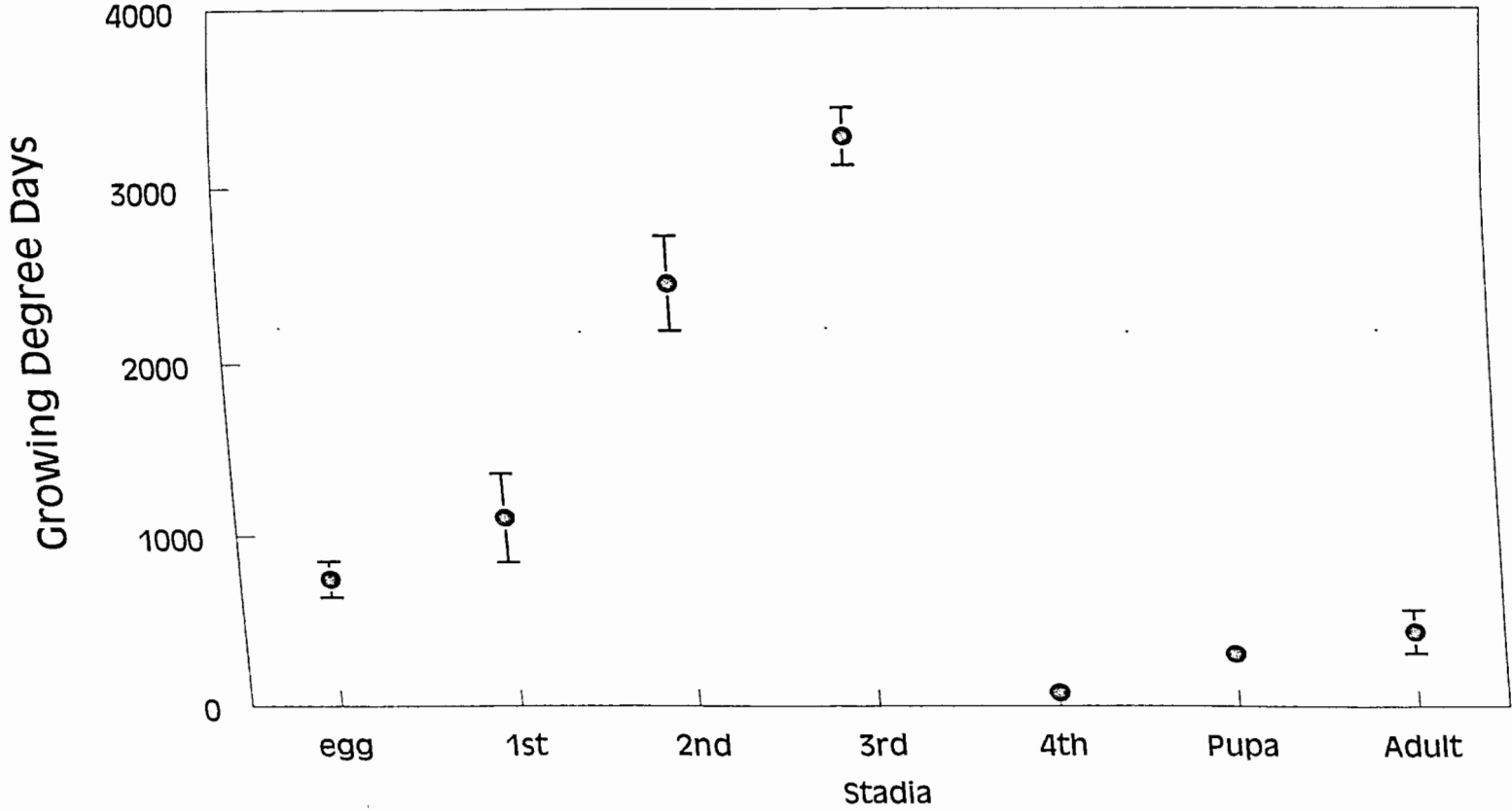


Figure 2: Boxwood leafminer development. Period of adult boxwood leafminer emergence observed at two locations. The growing degree day is listed on the X axis. The percentage of plants with adults is plotted on the Y axis.

**Arb95** = US National Arboretum 1995  
**LW95** = Longwood Gardens PA 1995

### Period of Adult Emergence

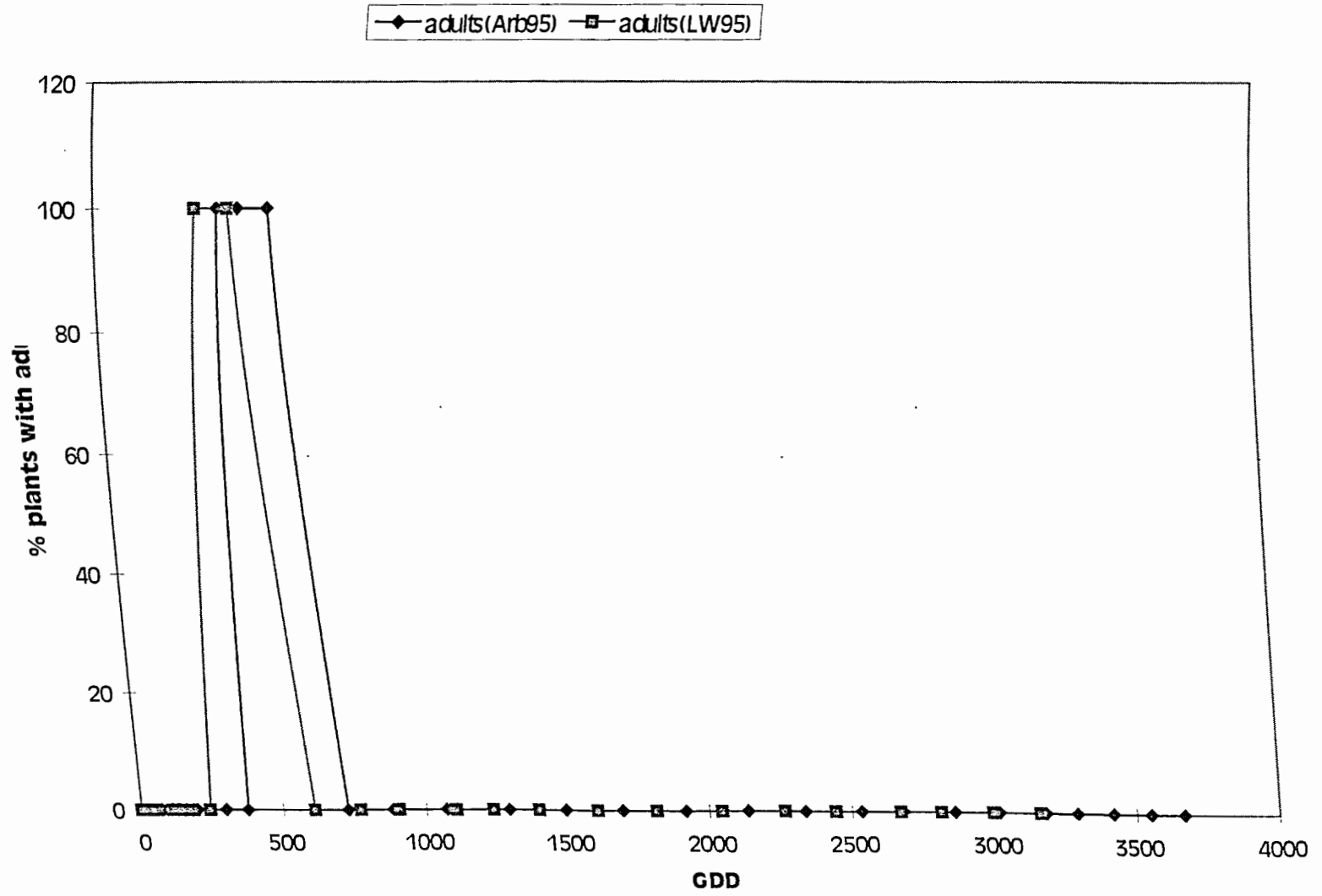


Figure 3: Average number of boxwood leafminer eggs in each sample of 20 leaves per site observed at two locations. The growing degree day is listed on the X axis. The number of eggs observed is plotted on the Y axis.

**Arb95** = US National Arboretum 1995  
**LW94** = Longwood Gardens PA 1994  
**LW95** = Longwood Gardens PA 1995

# Eggs

◆ egg (Arb95)    □ egg (LW94)    ▲ egg (LW95)

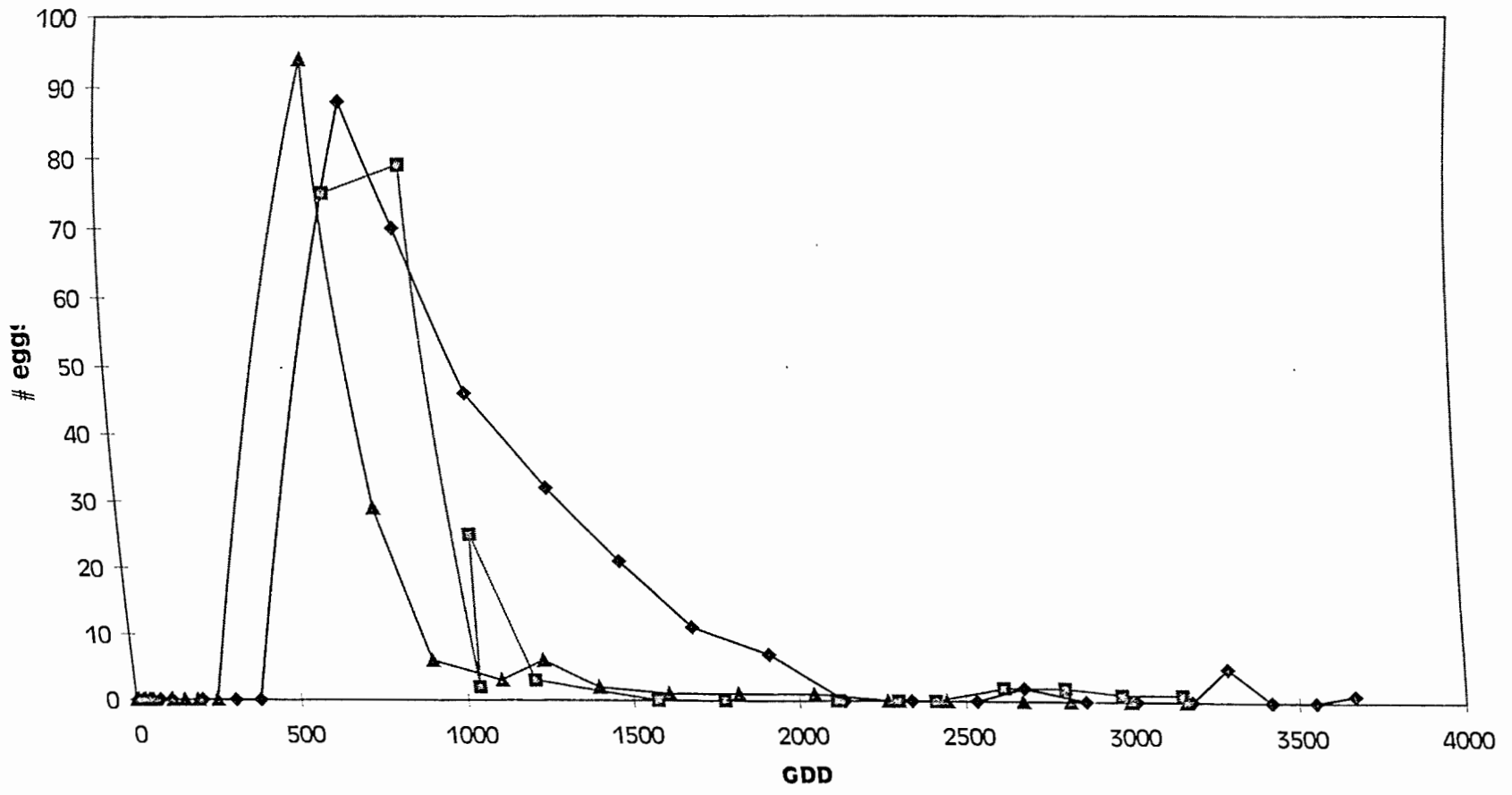


Figure 4: Boxwood leafminer development. Number of first instar boxwood leafminer larvae per sample of 20 leaves per site observed at two locations. The growing degree day is listed on the X axis. The number of larvae observed is plotted on the Y axis.

**Arb95** = US National Arboretum 1995

**LW94** = Longwood Gardens PA 1994

**LW95** = Longwood Gardens PA 1995

# 1st Instar

◆ 1st(Arb95)    □ 1st(LW94)    ▲ 1st(LW95)

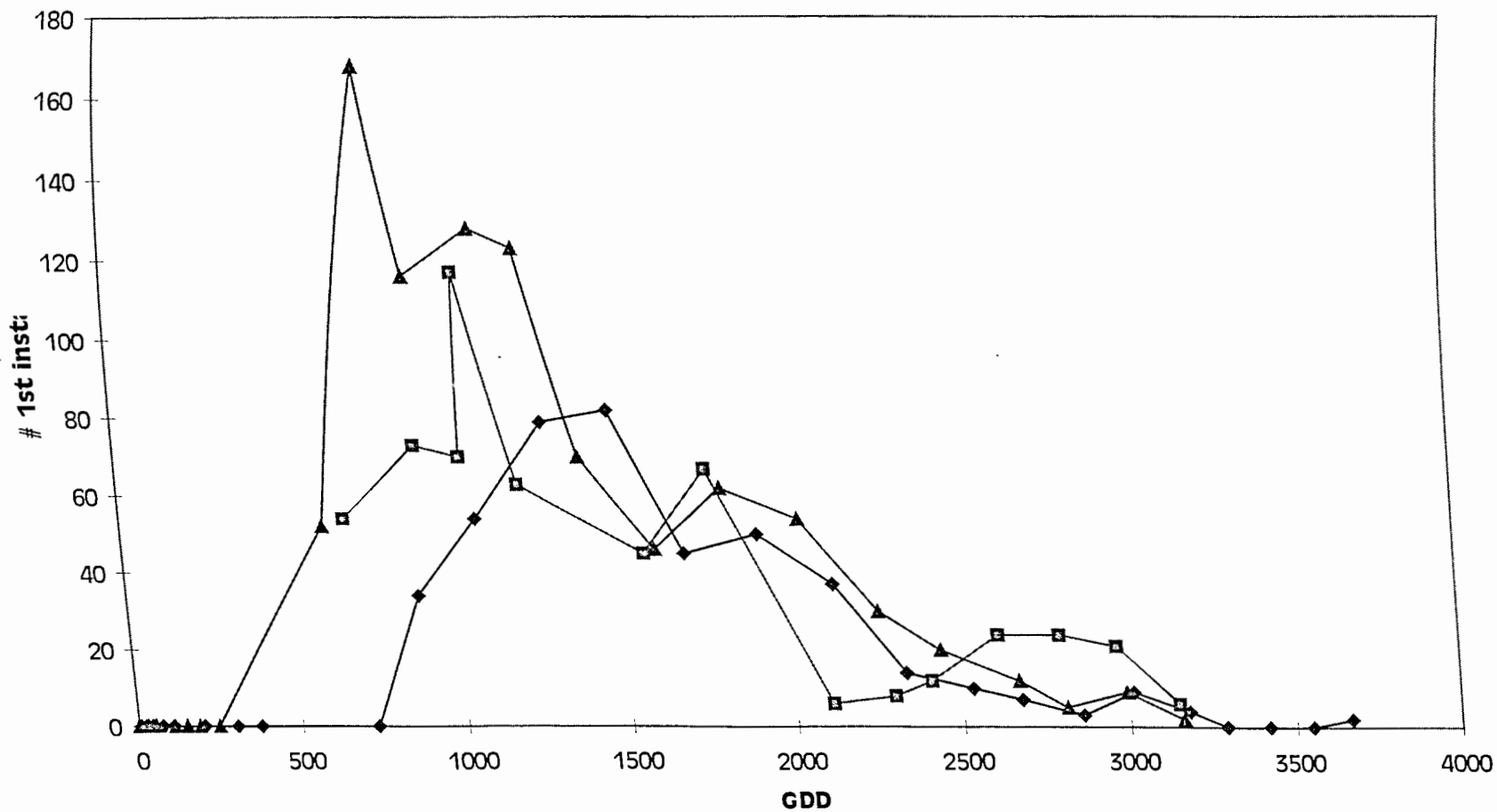




Figure 5: Number of second instar boxwood leafminer larvae per sample of 20 leaves per site observed at two locations. The growing degree day is listed on the X axis. The number of larvae observed is plotted on the Y axis.

**Arb95** = US National Arboretum 1995  
**LW94** = Longwood Gardens PA 1994  
**LW95** = Longwood Gardens PA 1995

### 2nd Instar

◆ 2nd (Arb95)    □ 2nd (LW94)    ▲ 2nd (LW95)

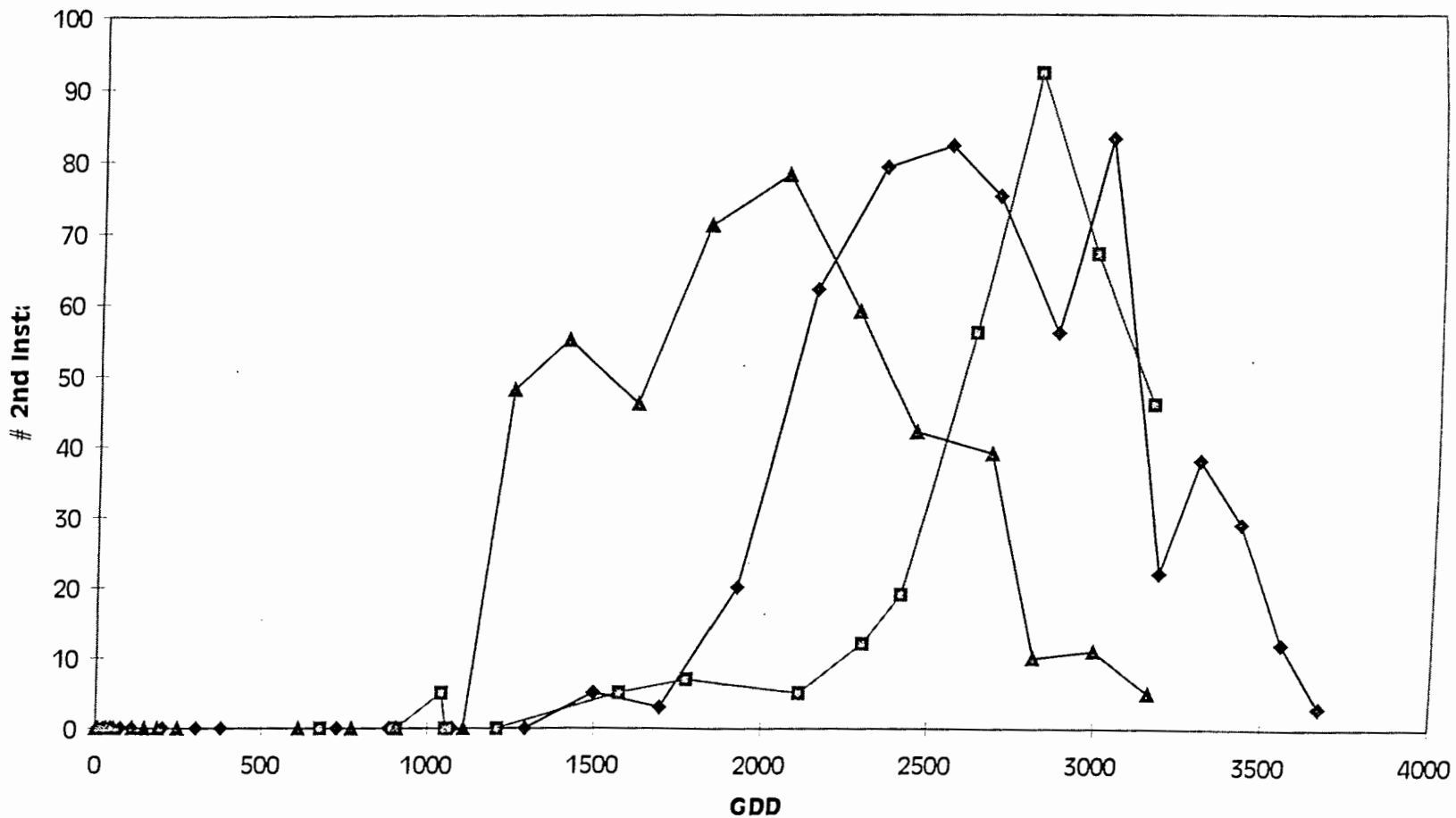


Figure 6: Number of third instar boxwood leafminer larvae per sample of 20 leaves per site. Observed at two locations. The growing degree day is listed on the X axis. The number of larvae observed is plotted on the Y axis.

Arb95 = US National Arboretum 1995  
LW94 = Longwood Gardens PA 1994  
LW95 = Longwood Gardens PA 1995

**3rd Instar**

—◆— 3rd (A1b95) —□— 3rd (LW94) —▲— 3rd (LW95)

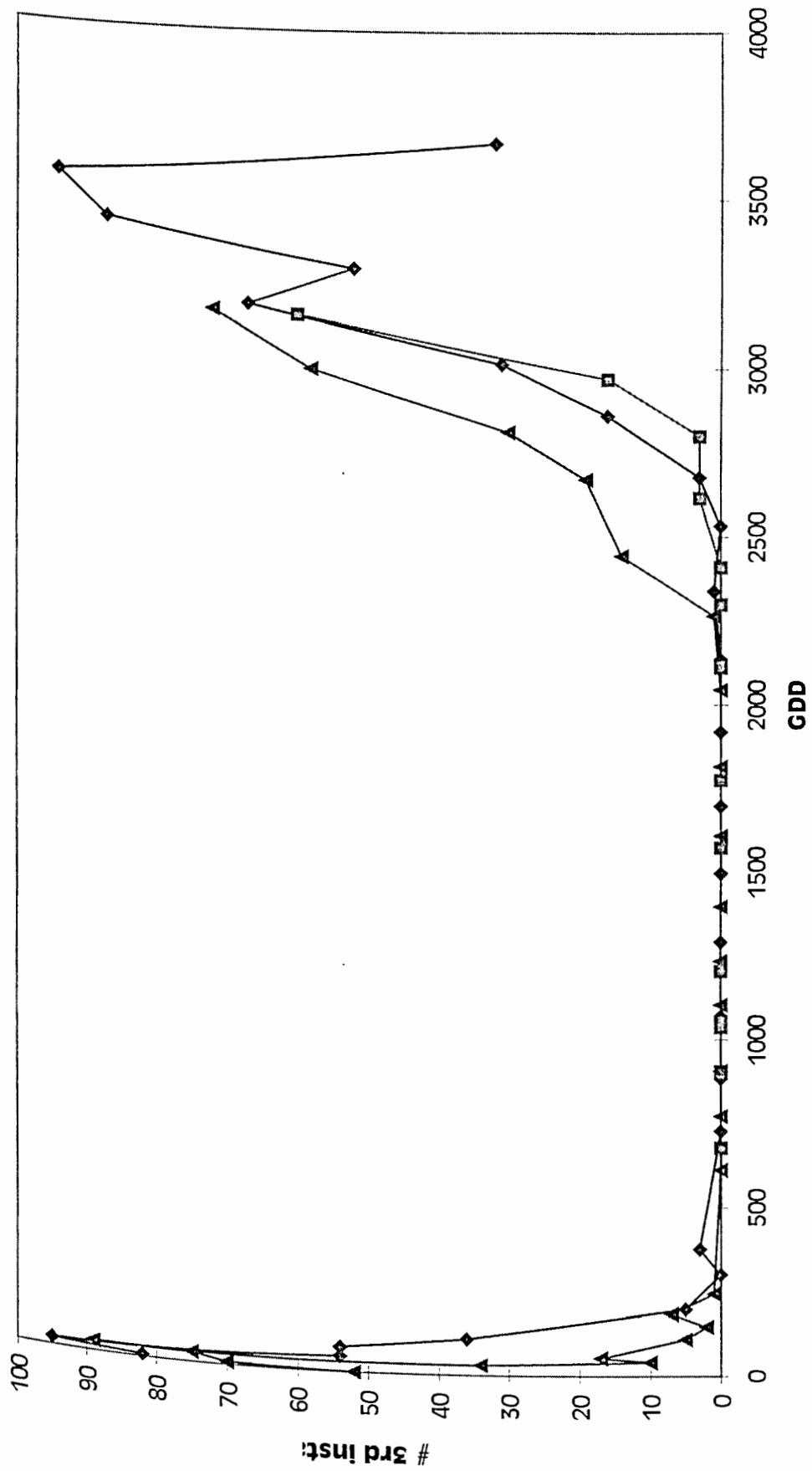


Figure 7: Number of fourth instar boxwood leafminer larvae per sample of 20 leaves per site observed at two locations. The growing degree day is listed on the X axis. The number of larvae observed is plotted on the Y axis.

**Arb95** = US National Arboretum 1995

**LW95** = Longwood Gardens PA 1995

### 4th Instar

◆ 4th (Arb95)    □ 4th (LW95)

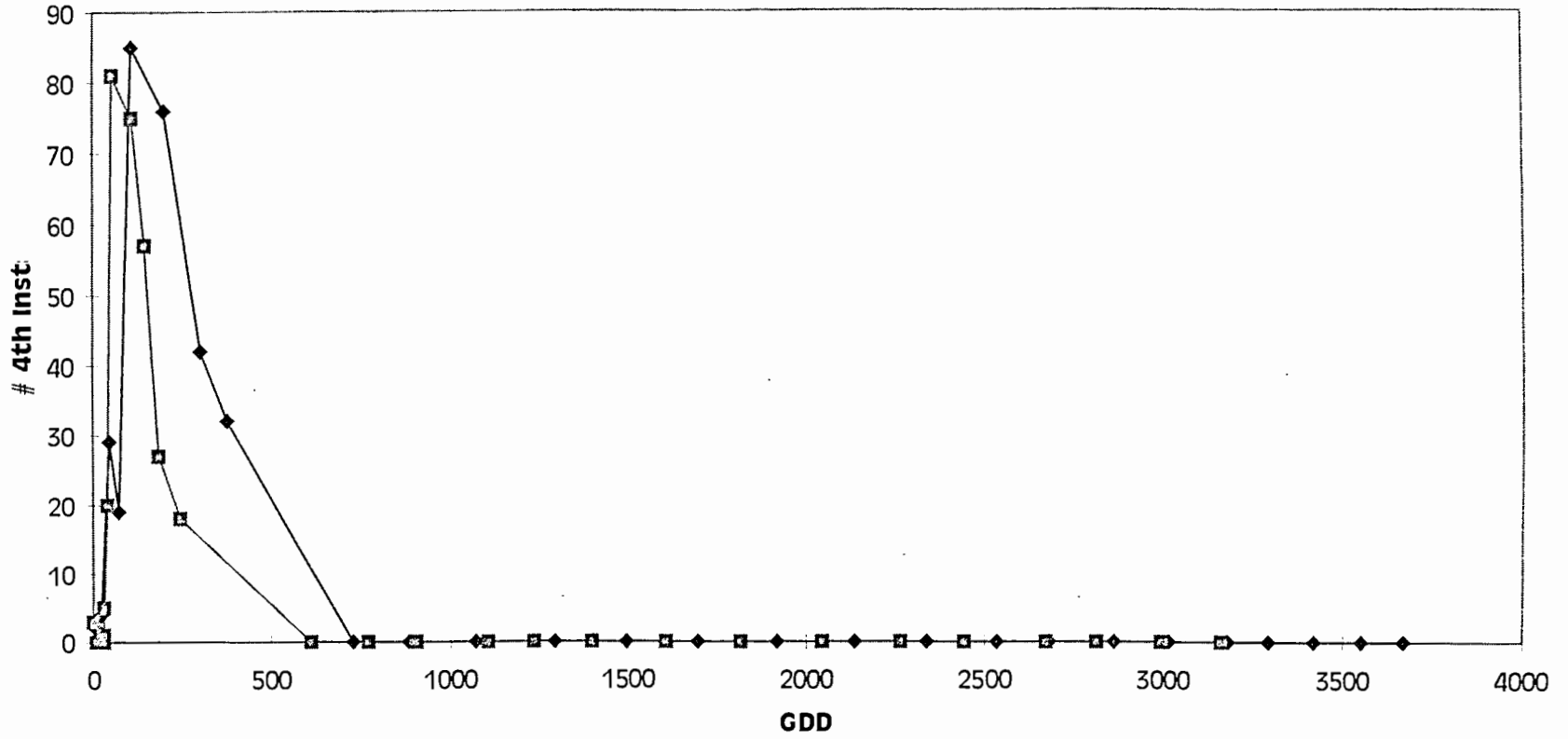
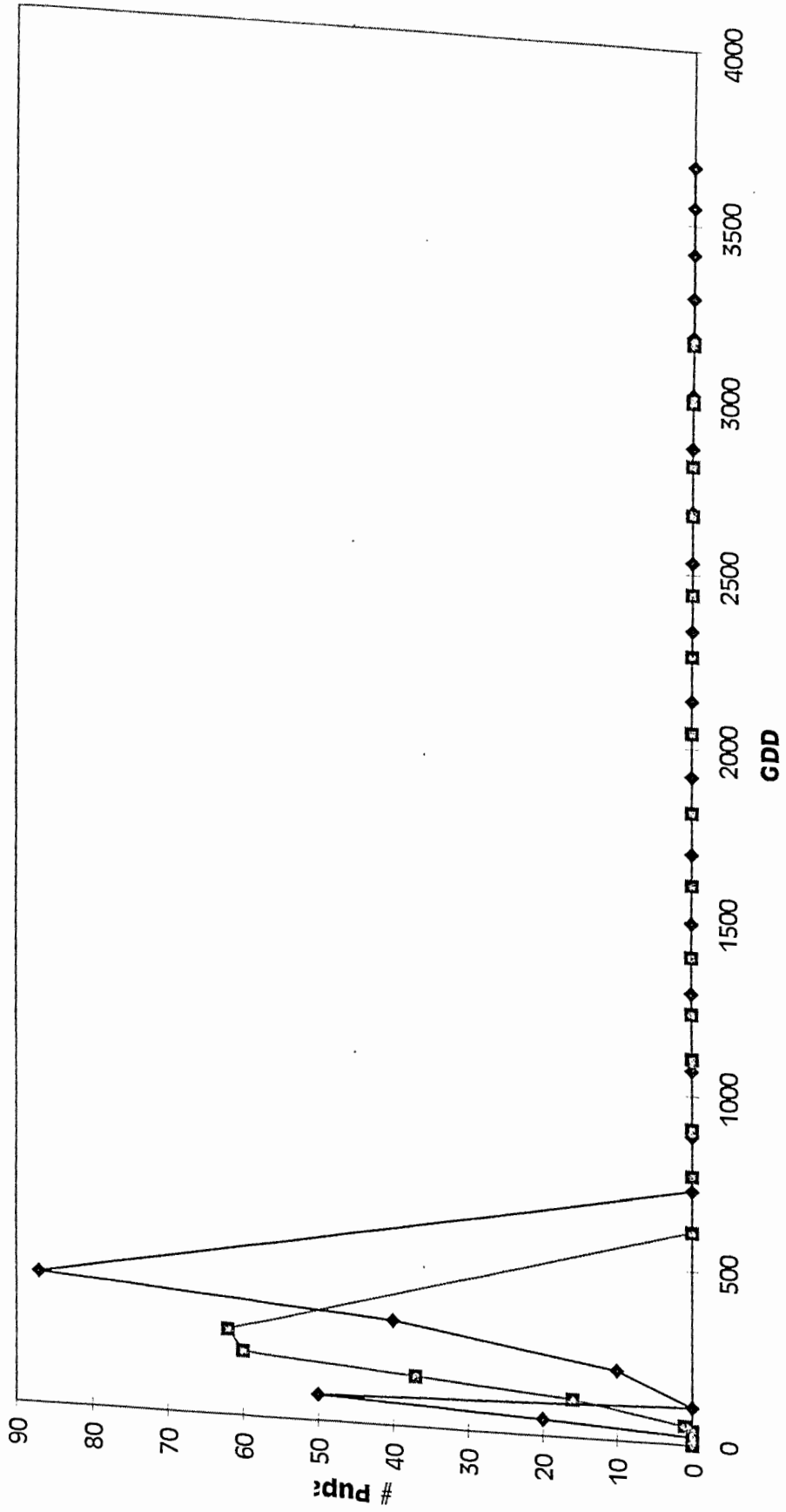


Figure 8: Number of boxwood leafminer pupae per sample of 20 leaves per site observed at two locations. The growing degree day is listed on the X axis. The number of larvae observed is plotted on the Y axis.

**Arb95** = US National Arboretum 1995  
**LW95** = Longwood Gardens PA 1995

# Pupa

◆ pupa (Afb95)    □ pupa (LW95)





## CHAPTER 4

### CHEMICAL CONTROLS

#### METHODS

Two trials were conducted to test the efficacy of different pesticides applied at different stages of boxwood leafminer development. The first trial tested early application of Avid (avermectin) (emulsion) and Merit (imidacloprid)(wetable powder), the second trial examined the effect of late application of Avid, Merit, and Orthene (acephate)(wetable powder).

For the early trial, 10 plants (*Buxus sempervirens* 'Arborescenes') were sprayed at the first sign of adult emergence in late April. Each plant recieved a single application of one of the two insecticides tested. A control group of 5 plants was sprayed with water and spreader/sticker only. Both Avid and Merit were used at concentrations recommended for leafminer control (0.1oz/gallon for Avid, 3 tablespoons (1.5oz)/gallon for Merit, both with an eyedropper full of spreader-sticker adjunct). Each plant was sprayed to a point slightly beyond leaf drip using a two gallon hand sprayer. Ten leaves were harvested from each plant in September. The number of surviving larvae was compared among different pesticide treatments. To determine if pesticide treatments affected oviposition behavior, the number

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For the early trial, 10 plants (*Buxus sempervirens* 'Arborescenes') were sprayed at the first sign of adult emergence in late April. Each plant recieved a single application of one of the two insecticides tested. A control group of 5 plants was sprayed with water and spreader/sticker only. Both Avid and Merit were used at concentrations recommended for leafminer control (0.1oz/gallon for Avid, 3 tablespoons (1.5oz)/gallon for Merit, both with an eyedropper full of spreader-sticker adjunct). Each plant was sprayed to a point slightly beyond leaf drip using a two gallon hand sprayer. Ten leaves were harvested from each plant in September. The number of surviving larvae was compared among different pesticide treatments. To determine if pesticide treatments affected oviposition behavior, the number

of ovipositions was measured by counting the number of oviposition scars on the underside of the leaves. Oviposition scars remain visible for the entire lifetime of a leaf and are only observed on the current years growth.

A second trial was initiated in mid July to test late application of pesticide. Fifteen Plants were selected and treated with Avid, Merit, or Orthene. Five plants were treated with water and spreader sticker as a control. Plants were sprayed slightly beyond leaf drip. Concentrations were recommended for leafminer control(0.1oz/gallon for Avid, 3 tablespoons (1.5oz)/gallon for Merit, 3 tablespoons (1.5oz)/gallon for Orthene; all with an eyedropper full of spreader-sticker adjunct). Leaves were harvested the day of treatment prior to treatment to assess initial pest densities, then sampled in September to determine pesticide efficacy. Ten leaves per plant were dissected using the same technique described previously.

Larval survival and oviposition data were analyzed using the proc ANOVA function (SAS institute) for a randomized complete block design. Following the analysis of variance, a Student-Newman-Keuls test was used to separate treatment means. The level of six larvae per leaf was proposed by Hamilton (1925) as the economic threshold, and this level was used to assess the relative success of control efforts.

## RESULTS AND DISCUSSION

Recalling Hamilton's (1925) threshold of six larvae per leaf, both Avid and Merit proved quite effective when applied at the first sign of adult emergence, and showed significant levels of control. (Tables 2, 3, Figures 9, 10) Merit significantly reduced ovipositions by the leaf miner. Merit and Avid significantly reduced leafminer survival relative to controls. In practical terms, this means that plants can be protected from serious damage. There may still be a significant number of leafminers in the plants, enough to warrant additional treatments the following spring. However, early applications kept damage well below the aesthetic threshold. After a year or two of effective treatment, levels of boxwood leafminer activity have been reduced to a point that further treatment was unnecessary (d'Eustachio, personal observation).

Plants treated later in the season varied in their levels of leafminer infestation as indicated by oviposition scars (Table 4, Fig. 11). However, this variation was slight and could not be resolved with a Student-Newman-Keuls test (Fig. 11). Pesticide applications later in the season provided control only when Merit was used (Table 5, Fig 12). This could be due to a number of factors. Later in the season leaves may have hardened to the point that pesticides can no longer penetrate the leaf tissue. The waxy cuticle, which is thick on boxwoods, may have developed to the point that

water-based materials cannot enter. Also, it is possible that larvae have grown and developed enough to resist the small amount of pesticide that penetrates their galleries.

I noted during both experimental and routine pesticide applications that use of a power sprayer greatly increased the level of control. This may be due to the power sprayer applying a higher rate of material more forcefully than a hand sprayer. This may give more complete coverage to all leaf surfaces and improve the ability of the pesticide to "stick" to the leaf surface. The thick waxy cuticle of boxwood leaves, especially that of new growth, posed problems to pesticide application. It took a great deal of spreader-sticker adjuvant to "wet" new growth. Rates of up to 1pt/100gal were necessary for proper adhesion at extremely low nozzle pressures. At higher pressures, less adjuvant was necessary. A high rate of spreader-sticker application can burn leaves of some plant species but this was not observed on boxwoods, even at extremely high rates.

Table 2: Analysis of Variance Table for number of oviposition scars on plants treated with early (April) pesticide application.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	930.13	465.06	5.86	0.0167
Error	12	951.60	79.30		
Corrected Total	14	1881.73			

**Figure 9: Number of oviposition scars/leaf on plants treated with Avid and Merit at early (April) pesticide application. Bars represent means and vertical lines represent standard errors. Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p = 0.05$ .**

# Early Pesticide Application

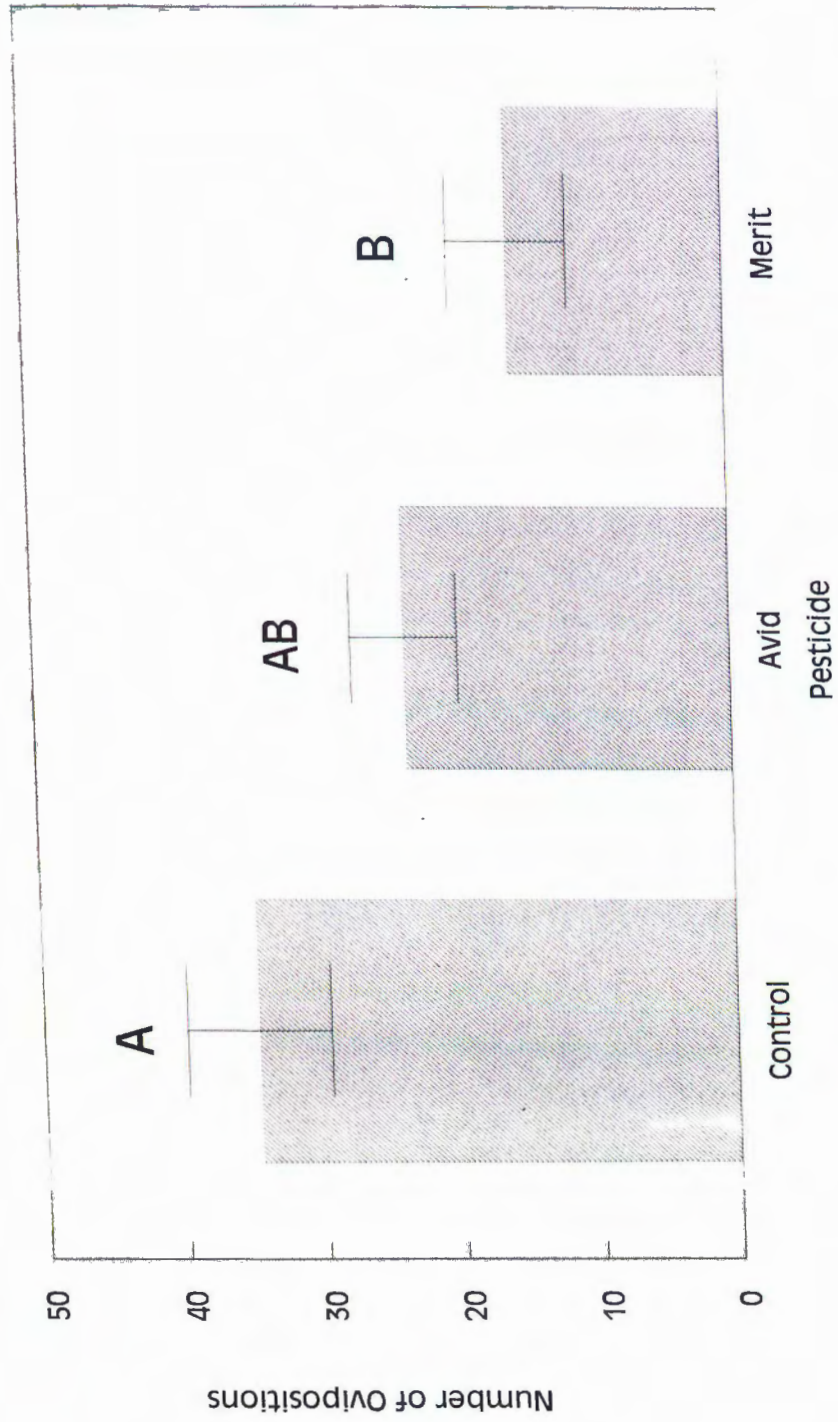




Table 3: Analysis of Variance table for number of surviving larvae on plants treated at early (April) pesticide application.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	354.69	118.23	5.01	0.017
Error	12	283.25	23.60		
Corrected Total	15	637.93			

Figure 10: Number of surviving larvae resulting early (April) pesticide application. Bars represent means and vertical lines represent standard errors. Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p = 0.05$ .

# Early Pesticide Application

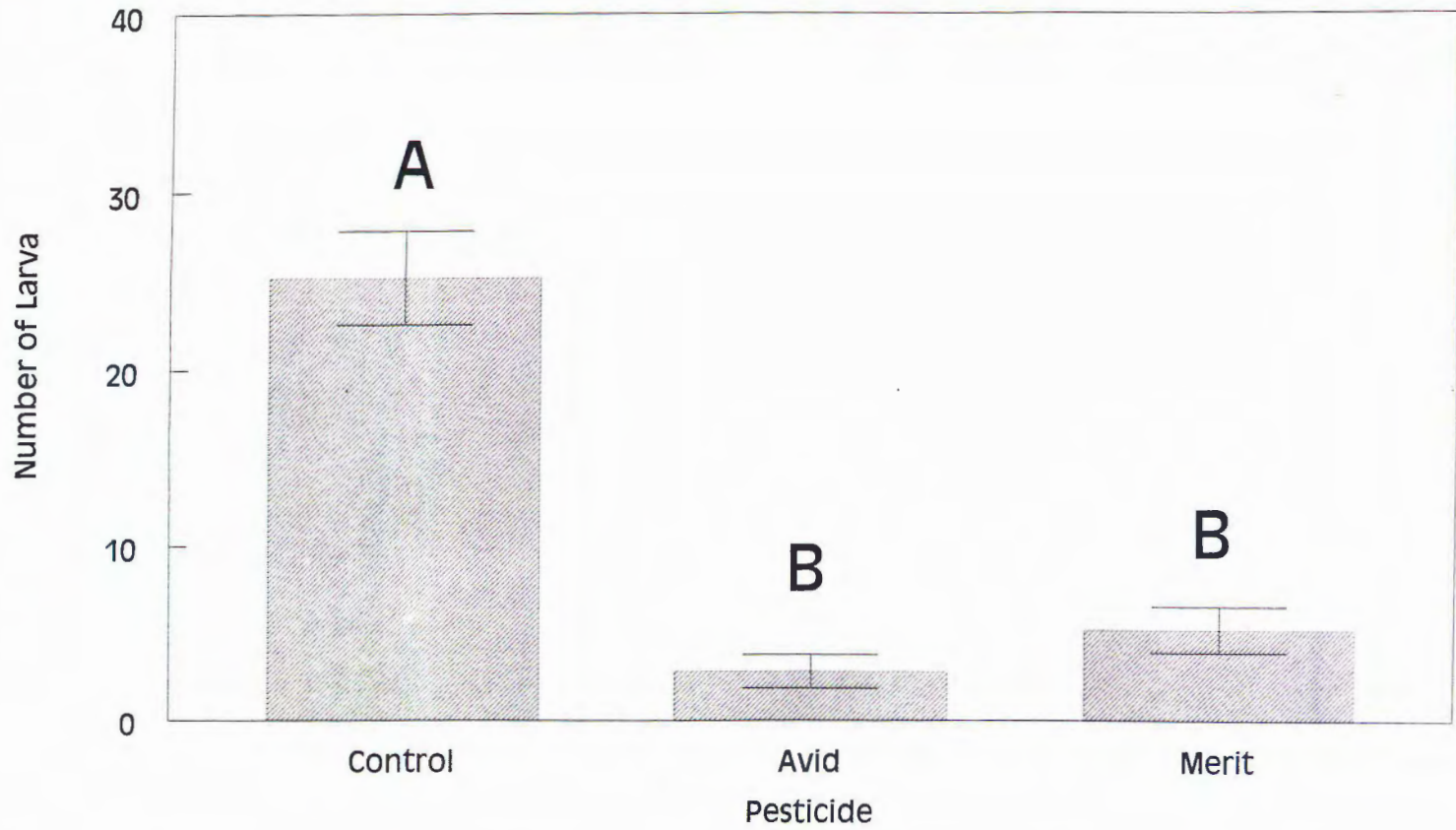


Table 4: Analysis of Variance Table for number of oviposition scars on plants treated at late (July) pesticide application.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	377.25	125.75	3.59	0.0465
Error	12	420.50	35.04		
Corrected Total	15	797.75			

Figure 11: Number of oviposition scars on plants treated with late (July) pesticide application. Bars represent means and vertical lines represent standard errors. Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p = 0.05$ .

## Late Pesticide Application

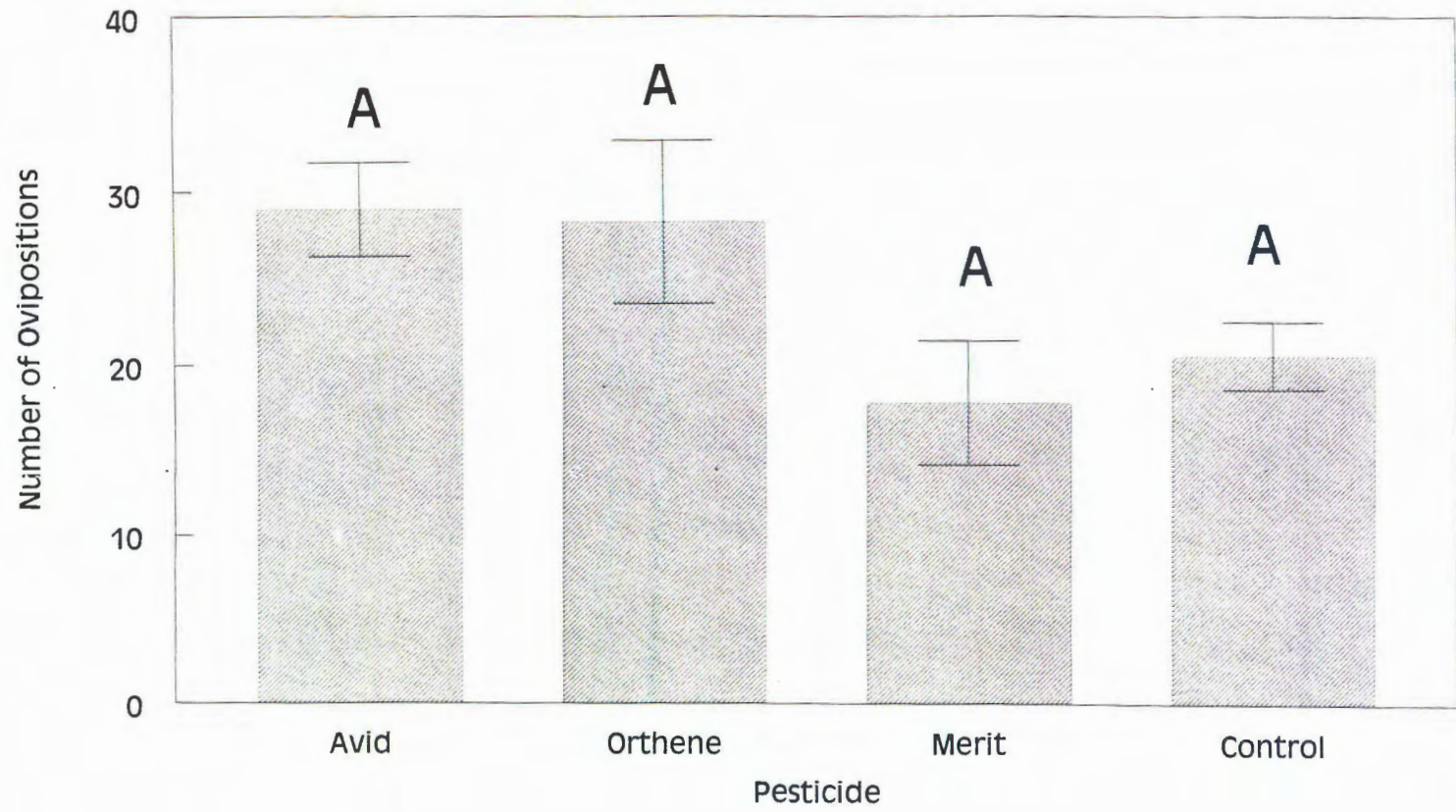


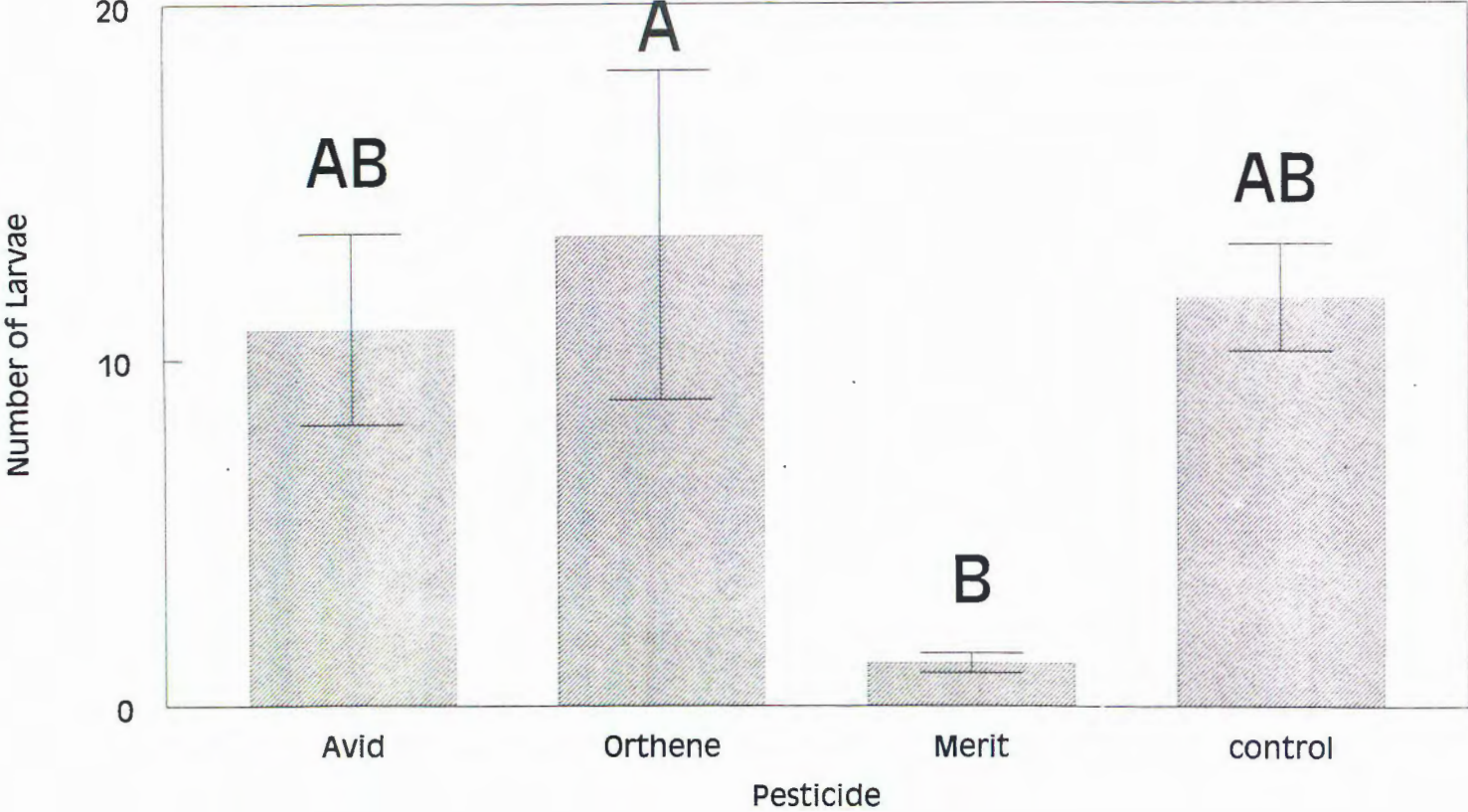
Table 5: Analysis of Variance Table for larvae surviving on plants treated at late (July) pesticide application.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	225.50	75.17	4.21	0.03
Error	12	214.50	17.88		
Corrected Total	15	440.00			

Figure 12: **Number of larvae surviving treatment with late (July) application of pesticide.** Bars represent means and vertical lines represent standard errors. Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p=0.05$ .



# Late Pesticide



## CHAPTER 5

### CULTIVAR RESISTANCE

#### METHODS

##### FIELD SURVEY

In the springs of 1994 and 1997, numerous cultivars were surveyed at the US National Arboretum to evaluate levels of resistance in plants grown in a common garden which had been exposed to ambient levels of boxwood leafminer. Results from the 1997 survey are shown. Three plants from each cultivar were selected, and five leaves were harvested from each plant. The following cultivars were sampled: *Buxus sempervirens* 'Arborescens,' 'Myrtifolia,' 'Belleville,' 'Suffruticosa,' 'Pyramidalis,' 'Handsworthiensis' 'Vardar Valley,' *Buxus microphylla* var. *japonica* 'National', and *B. microphylla* var. *japonica*. To help control for differences in leaf age, leaves of the same plastochron index (position on the branch) were selected from each infested branch. The leaves were dissected, and the number of oviposition scars, and mature larvae were recorded. Data were analyzed using the ANOVA procedure and followed by an Student-Newman-Keuls analysis to separate means (SAS institute).

## LARVAL SURVIVAL

Variation in the ability of different cultivars to support leafminer survival was evaluated further by exposing cultivars to ovipositing leafminers in cages and observing larval survival. Terminal branchlets containing emerging leafminer adults were placed in water pics and caged in aerial cages on the new growth of nine different cultivars of boxwood. Cages were made of nylon mesh with a plastic support to help them hold their shape. This allowed plenty of airflow around the branches. Plastic cages were tested and rejected due to the fact that the high humidity caused by the lack of airflow through the cages cause all of the leaves in them to die from *Macrophoma* infection. The following cultivars were evaluated: *Buxus sempervirens* 'Arborescenes,' 'Myrtifolia,' 'Belleville,' 'Suffruticosa,' 'Handsworthiensis,' 'Vardar Valley,' and *Buxus microphylla* cv. *japonica* . Three plants of each cultivar were chosen and three inclusion cages were randomly placed on each plant. Adults emerging from the excised branchlets oviposited on the newly expanded leaves enclosed in the aerial cages. Some samples were lost due to cages breaking.

Mortality of leafminers was determined by dissecting all leaves in the cage, counting the number of surviving larvae and comparing that to the number of oviposition scars. The results were analyzed as a randomized

complete block using GLM then separating the means using a Student-Newman-Keuls to determine if oviposition and survival differed among cultivars (SAS Institute 1990).

#### OVIPOSITIONAL PREFERENCE

To measure the ability of adult leafminers to discriminate between suitable and unsuitable hosts for oviposition, emerging adult flies were confined in cages and allowed to oviposit on four different cultivars of boxwood in both choice and no choice experiments. The plants chosen were the cultivars *Buxus sempervirens* 'Arborescens', *B. sempervirens* 'Suffruticosa', *B. sempervirens* 'Vardar Valley' and *Buxus* 'Green Beauty'. These were selected on the basis of previous trials, popularity, in the market and availability. Prior to the experiments, leaves containing mature pupae were collected on May 15th from Longwood gardens PA by cutting terminal branches from heavily infested plants and placing them in water pics. The branches were refrigerated until they were needed.

To further evaluate oviposition behavior in a no choice setting, 4 potted boxwoods of the same cultivar and size were placed in 4'x4'x4' cages of fine mesh. Two terminal branches in water pics containing pupae were placed in the center of each cage to provide a source of adult flies. These

flies were allowed to emerge and oviposit for one week. Plants were then placed under a shade canopy in gravel beds at the University of Maryland greenhouse. Development was allowed to continue until September when plants were dissected and number of oviposition scars and galls were counted on the leaves. This experiment was replicated 4 times.

To compare oviposition behavior when a choice of cultivars was possible, cages were established that contained one representative of each of the four cultivars used in the study. Their placement was varied to account for possible heliotropism. All of the plants were of approximately the same size and were placed in identically sized pots. Flies were allowed to emerge and oviposit for one week. This experiment was repeated three times.

Oviposition on different cultivars was compared in the choice and no choice experiments with a randomized complete block design (SAS institute 1990). Location of plants within cages were similarly analyzed to measure if there was any effect of heliotropism.

## RESULTS AND DISCUSSION

### FIELD SURVEY

The survey showed that there was some difference in the number of ovipositions on two of the more susceptible cultivars, 'Myrtifolia' and 'Belleville'. However, there was no significant difference in the number of ovipositions on the other cultivars, regardless of susceptibility (Table 6, Fig. 13). This result is possibly due to the fact that more susceptible cultivars will have greater numbers of adults emerging nearby to oviposit on them.

There was a substantial difference in larval survival among cultivars (Table 7, Fig. 14). There were three levels of cultivar susceptibility: highly susceptible, moderately susceptible, and resistant. The cultivars *Buxus microphylla* cv. *japonica* 'National', and *Buxus sempervirens* 'Myrtifolia' were highly susceptible, supporting greater larval survival than other cultivars. 'Belleville' and 'Arborescens,' and *B. microphylla* cv. *japonica* were moderately susceptible. The varieties 'Suffruticosa,' 'Pyramidalis,' 'Handsworthiensis,' and 'Vardar Valley' were all resistant.

'Handsworthiensis' and 'Vardar Valley' had almost no surviving larvae despite a statistically similar number of ovipositions (Table 6-7, Fig. 13-14).

The most common cultivars in North America are 'Arborescenes' and 'Suffruticosa'. 'Arborescenes' is moderately susceptible, while 'Suffruticosa' is highly resistant. The less known cultivars also showed a broad range of susceptibility. 'Myrtifolia' and *Buxus microphylla* National were highly susceptible. 'Belleville' and 'Arborescenes' were moderately susceptible. 'Pyramidalis,' 'Vardar Valley' and 'Handsworthiensis'. 'Vardar Valley' and 'Handsworthiensis' were both highly resistant. It was difficult to find any surviving larvae on either plant, in fact, only three larvae were found on 'Vardar Valley' and none were found on 'Handsworthiensis', despite the large number of ovipositions on both. *B. microphylla* cv. Japonica was statistically between moderately susceptible and resistant.

#### LARVAL SURVIVAL RESULTS

The mean number of oviposition scars and number of larvae surviving to the third instar were compared among cultivars by confining ovipositing flies on boxwood branches. We found the cultivars Handsworthiensis and Vardar Valley were both highly resistant to boxwood leafminer while others were susceptible. Arborescens (American or tree box) is the most common boxwood in Maryland and often shows the highest levels of injury in the field. However, the number of oviposition scars was not significantly different among (Table 8, Fig. 15). This result supports

later ones indicating that adult leafminers fail to discriminate among cultivars of boxwood.

Leafminer survival under the aerial cage, no-choice conditions differed somewhat from those observed in the field. The cultivars 'Handsworthiensis' and 'Vardar Valley' exhibited high levels of resistance (Table 9, Fig. 16). In the field evaluations, ovipositions on 'Suffruticosa' rarely resulted in surviving larvae. However, in these no-choice tests survival on this cultivar was relatively high. 'Belleville' was a highly susceptible cultivar in the field survey but was not as highly suitable as other cultivars in this test. 'Myrtifolia' supported high levels of larval survival in this study and was heavily infested in field plots as well.

#### OVIPOSITIONAL PREFERENCE

A large number of oviposition scars were observed on all of the plants in this experiment. Within each cage, the position of the plant had no effect on the number of eggs laid (Table 10, Fig. 17). In the no-choice cage study all cultivars except the resistant *B. sempervirens* 'Vardar Valley' received similar numbers of oviposition (Table 11, Fig. 18). *Buxus sempervirens* 'Vardar Valley' received slightly fewer ovipositions in this study. This is possibly due to the fact that Vardar Valley has fewer, yet larger leaves than



the other cultivars. All of the plants were the same age, yet there was some variation in size of the plants. In contrast, the choice test revealed that there were no significant differences in the number of eggs laid on any of the four cultivars (Table 12, Fig 19). This experiment indicates that ovipositing adult boxwood leafminers are unable to choose between suitable and unsuitable cultivars. The fact that larva could only survive on certain cultivars but oviposited on all of them more or less equally implies that boxwoods display an antibiosis resistance to boxwood leafminer attack.

Many researchers have demonstrated that ovipositing female insects will choose the most suitable plant for their eggs, although this is not always the case (Karban 1992). Often there is a direct correlation between ovipositional preference and larval survival (Craig 1989). In boxwoods, there is an obvious difference in host plant suitability, and there is clearly increased survival in suitable plants. In spite of this, boxwood leafminer adults fail to select more suitable hosts over ones less suitable when given a choice. One possible reason for this may be the ephemeral nature of the boxwood leafminer adult. They only live a day or two and have a definite time constraint on how much time they can spend searching for a suitable host plant.

Inability to choose may also drive clades to adapt to new hosts. Futuyma (1983) noted that insects can change evolutionarily to adapt to

newly resistant host plants. The hessian fly, *Mayetiola destructor* (Say), which is in the same family as boxwood leafminer, has been noted to adapt to resistant wheat varieties. The advantage of adapting to a broader host range would allow females to lay more surviving eggs in their brief lifespan (Rausher 1983). However, Rausher goes on to point out several disadvantages of a broad host range, including the loss of synchronization between critical life stages in insects and plants. In boxwood leafminer, the emergence of ovipositing adults must be properly timed with spring leaf flush for the larvae to be successful (Brewer, 1984).

Another factor limiting host range expansion is that boxwood leafminer adults are not very good fliers. They may never get far from their plant of origin. If they were able to successfully colonize and survive on their "parent" plant, then the "parent" plant should be suitable for their offspring and there should be reduced pressure for expanding host range. This isolation will tend to re-enforce any selective pressure placed on the boxwood leafminers by the host plant.

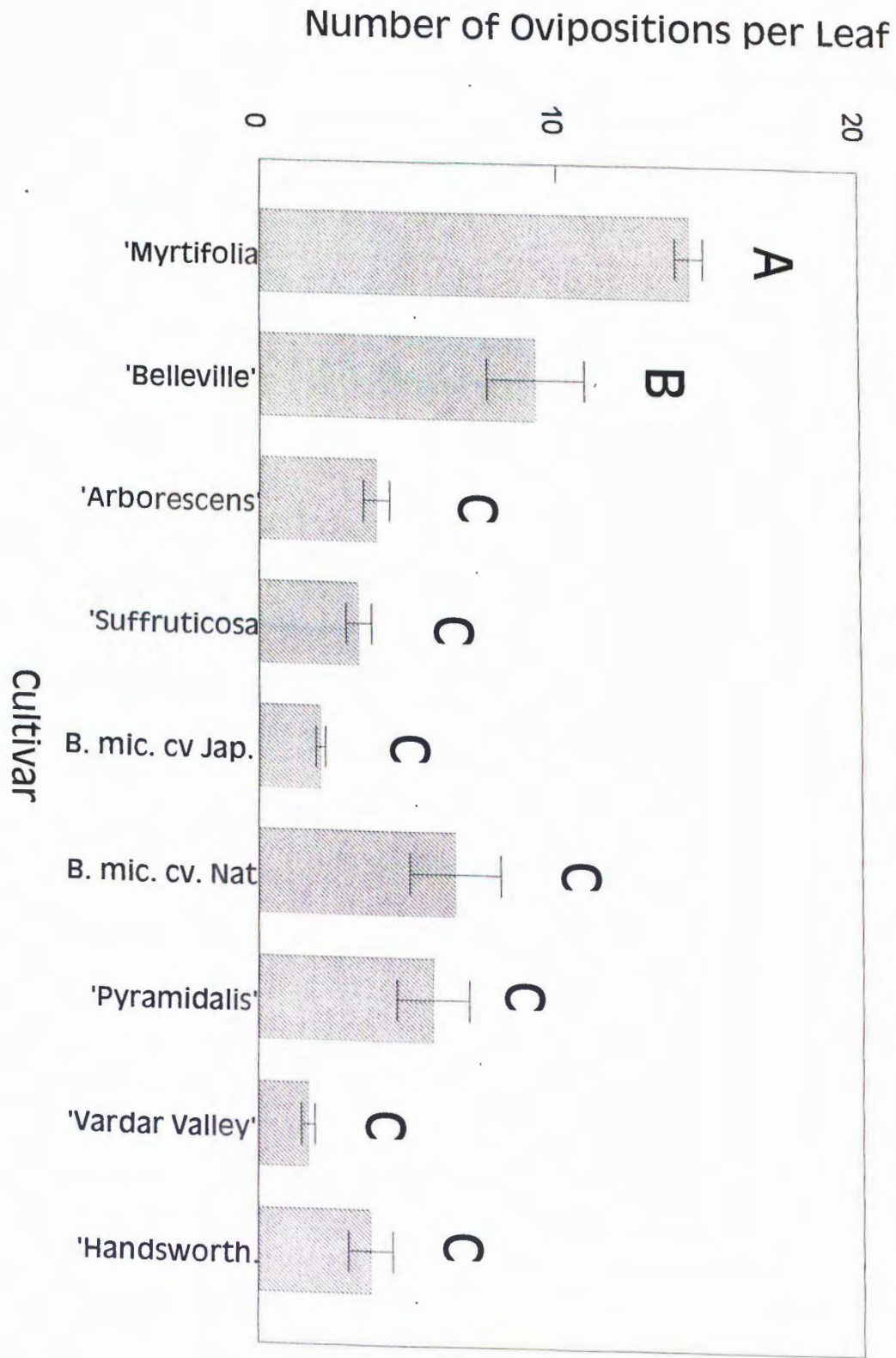
In an evolutionary light, inability to choose could actually be beneficial to boxwood leafminers. If eggs were laid predominantly on the parent plant, and it was only slightly suitable (for example having a survival rate of less than 10%) the few survivors would keep ovipositing on

the slightly suitable plant and put a severe selective pressure on the miners to develop the ability to overcome plant resistance. In a landscape setting this may not be very apparent, as most of the plants tend to be the susceptible 'Arborescens' variety or resistant 'Suffruticosa' variety. Gene frequency of boxwood leafminers surviving on 'Arborescens' and mating with those on resistant 'Suffruticosa' would dilute out the genes necessary to overcome 'Suffruticosa's' resistance (Karban 1992) In the wild, where plants may be farther apart and thus may reduce gene flow among boxwood leafminers utilizing resistant and susceptible cultivars, the boxwood leafminer could accumulate the genes necessary to improve performance on resistant plants. It may take a while as the boxwood leafminer is univoltine, but genetic isolation would help create the selective pressure to drive selection towards leafminers with better survival on formerly resistant cultivars.

Table 6: Analysis of Variance table for number of ovipositions in field evaluation.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	351.36	43.92	26.02	0.0001
Error	18	30.39	1.69		
Corrected Total	26	381.75			

Figure 13: Number of ovipositions in field survey of boxwood cultivars.  
Bars represent means and vertical lines represent standard errors.  
Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p = 0.05$ .



## Field Survey of Boxwood Cultivars

Table 7: Analysis of Variance table for number of surviving larvae in field analysis

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	59.89	7.49	21.08	0.0001
Error	18	6.39	.355		
Corrected Total	26	66.29			

Figure 14: Number of surviving larvae in field survey of boxwood cutivars.  
Bars represent means and vertical lines represent standard errors.  
Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p=0.05$ .



# Field Survey of Boxwood Cultivars

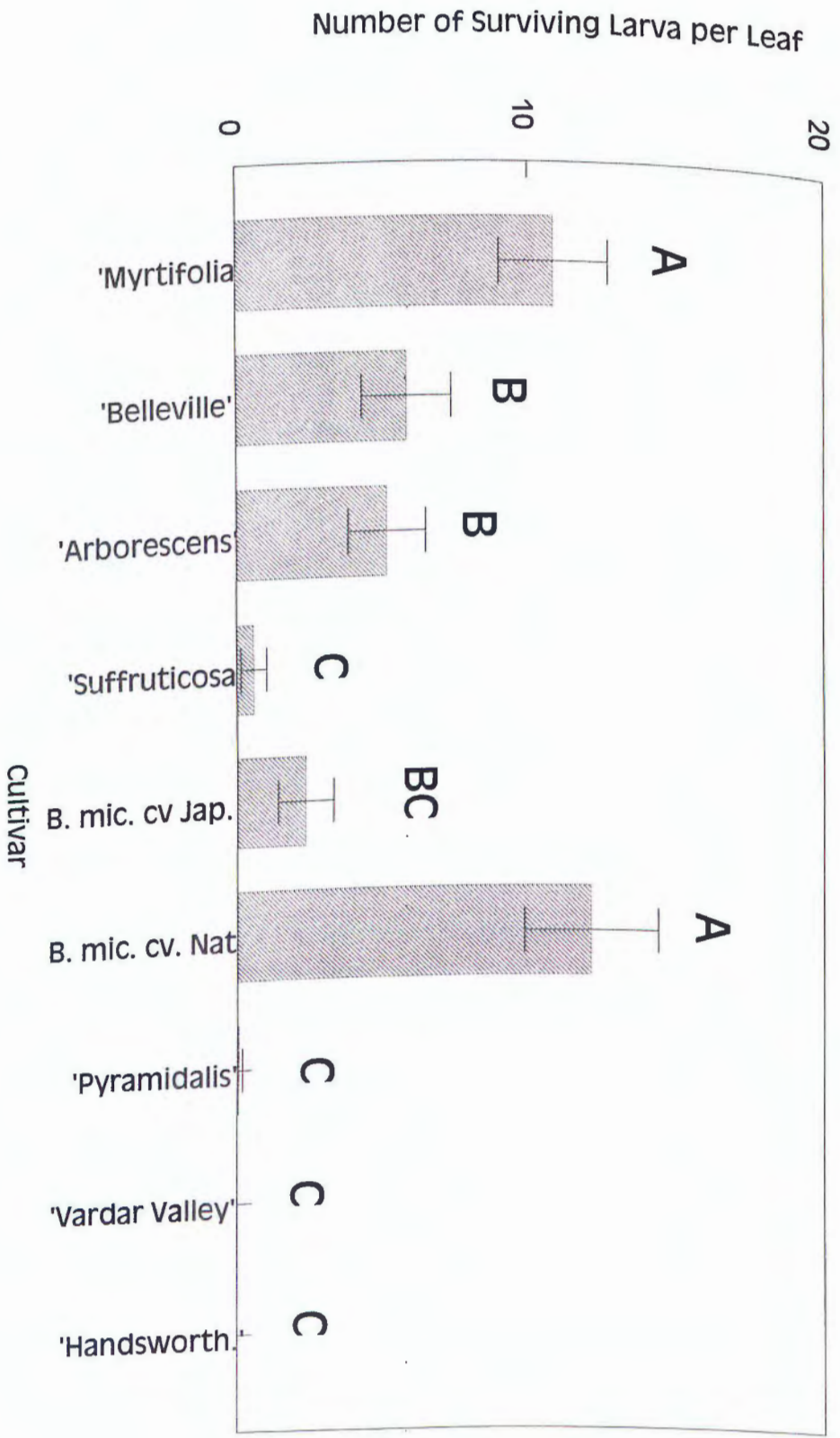


Table 8: General Linear Models table for number of ovipositions in no-choice aerial cage study.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	815.83	135.97	1.31	0.3506
Error	8	827.73	103.47		
Corrected Total	14	1643.55			

Figure 15: Number of ovipositions in no choice aerial cage study.  
Bars represent means and vertical lines represent standard errors.  
Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p = 0.05$ .

## Oviposition of Leafminers in No-Choice Aerial Cage Study

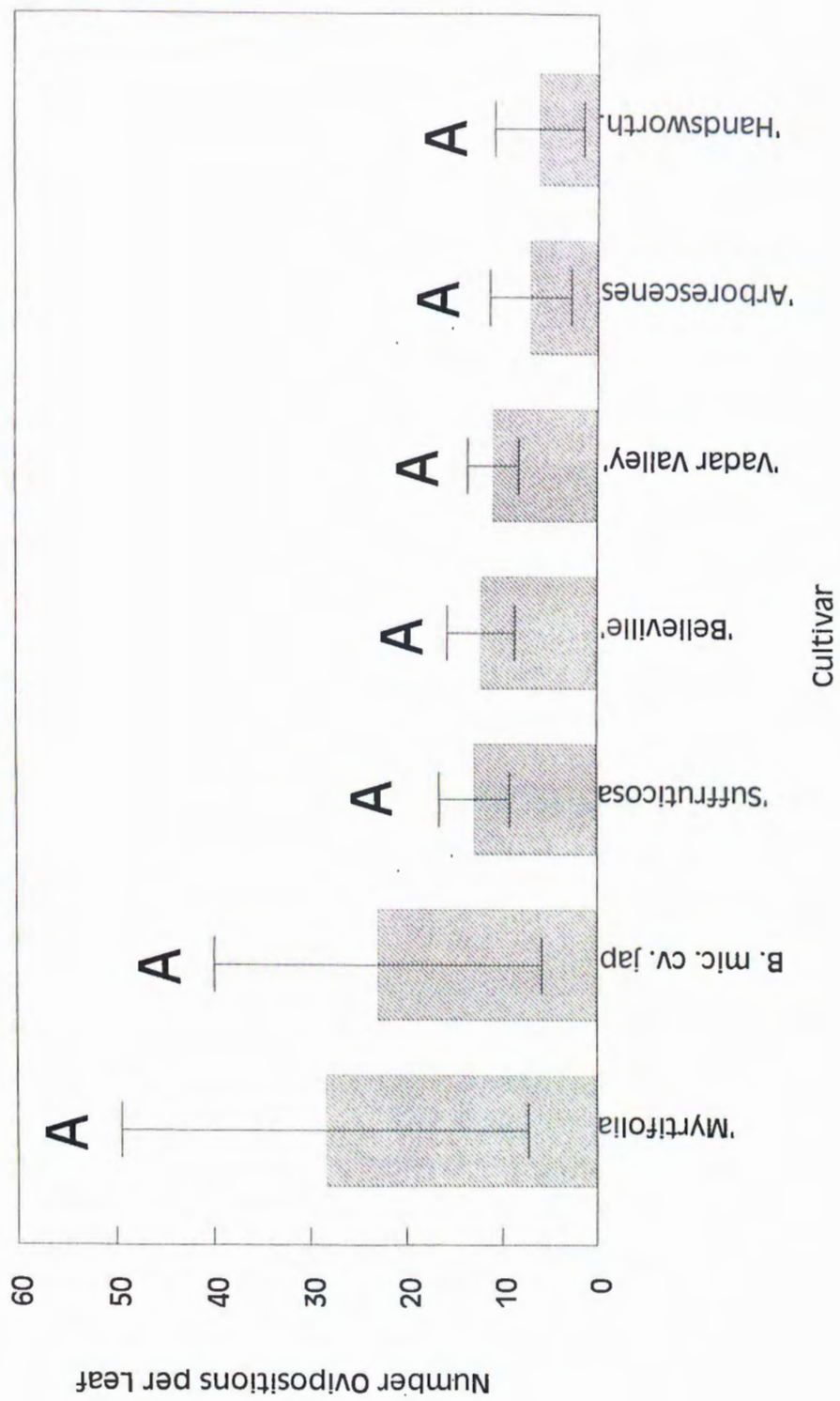


Table 9: General Linear Models Procedure for larval survival in no-choice aerial cage study.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	14.83	2.47	5.39	0.0164
Error	8	3.66	0.046		
Corrected Total	14	18.49			

Figure 16: Number of surviving larvae in aerial cage-no choice study. Bars represent means and vertical lines represent standard errors. Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p = 0.05$ .

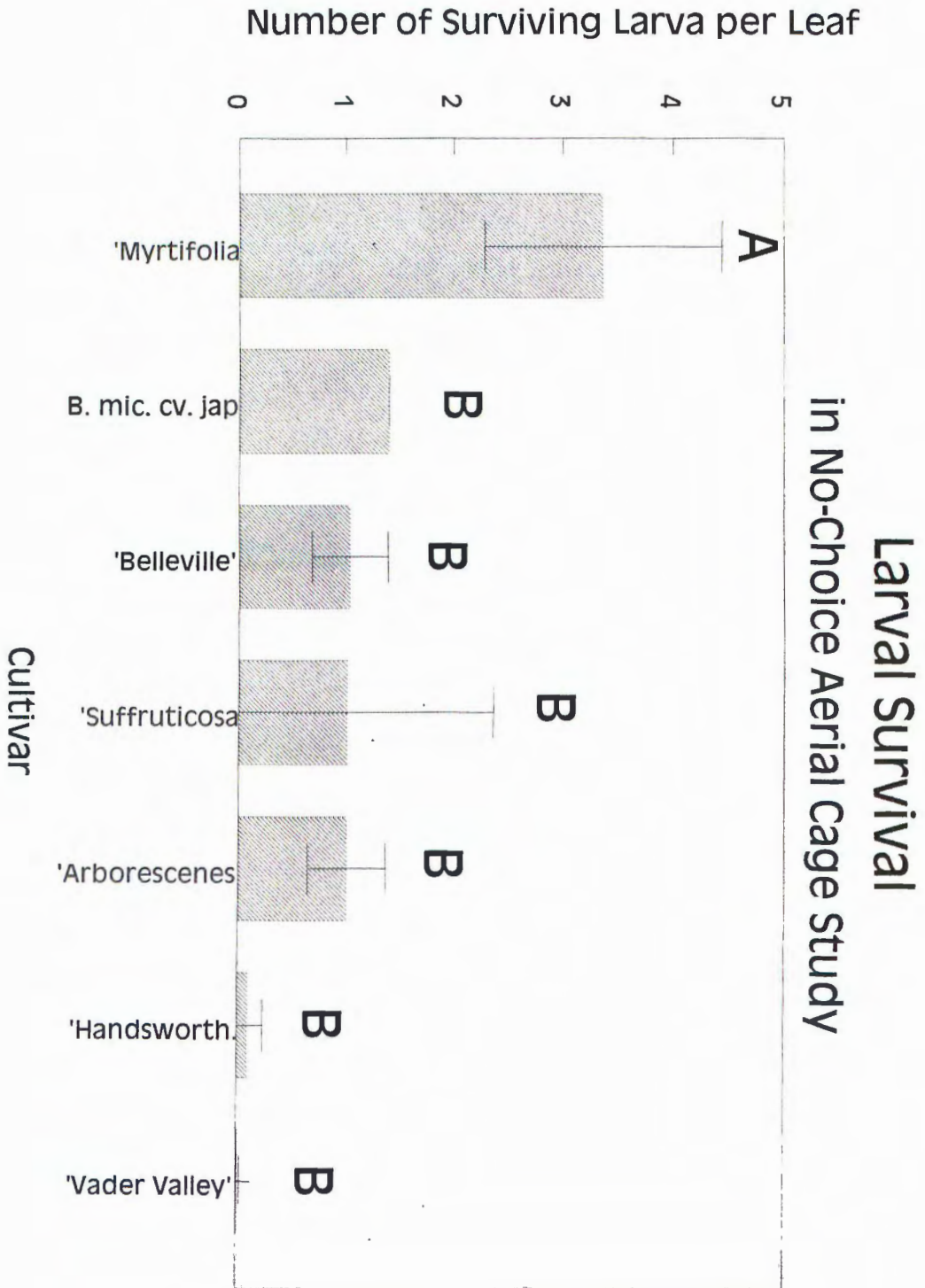


Table 10: Analysis of Variance Table for effect of heliotropism on oviposition.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	756.61	252.20	2.24	0.093
Error	59	6643.23	112.60		
Corrected Total	62	7399.84			



Figure 17: The effect of heliotropism on oviposition. Both choice and control cages are shown. Bars represent means and vertical lines represent standard errors. Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p = 0.05$ .

## Effect of Heliotropism on Oviposition

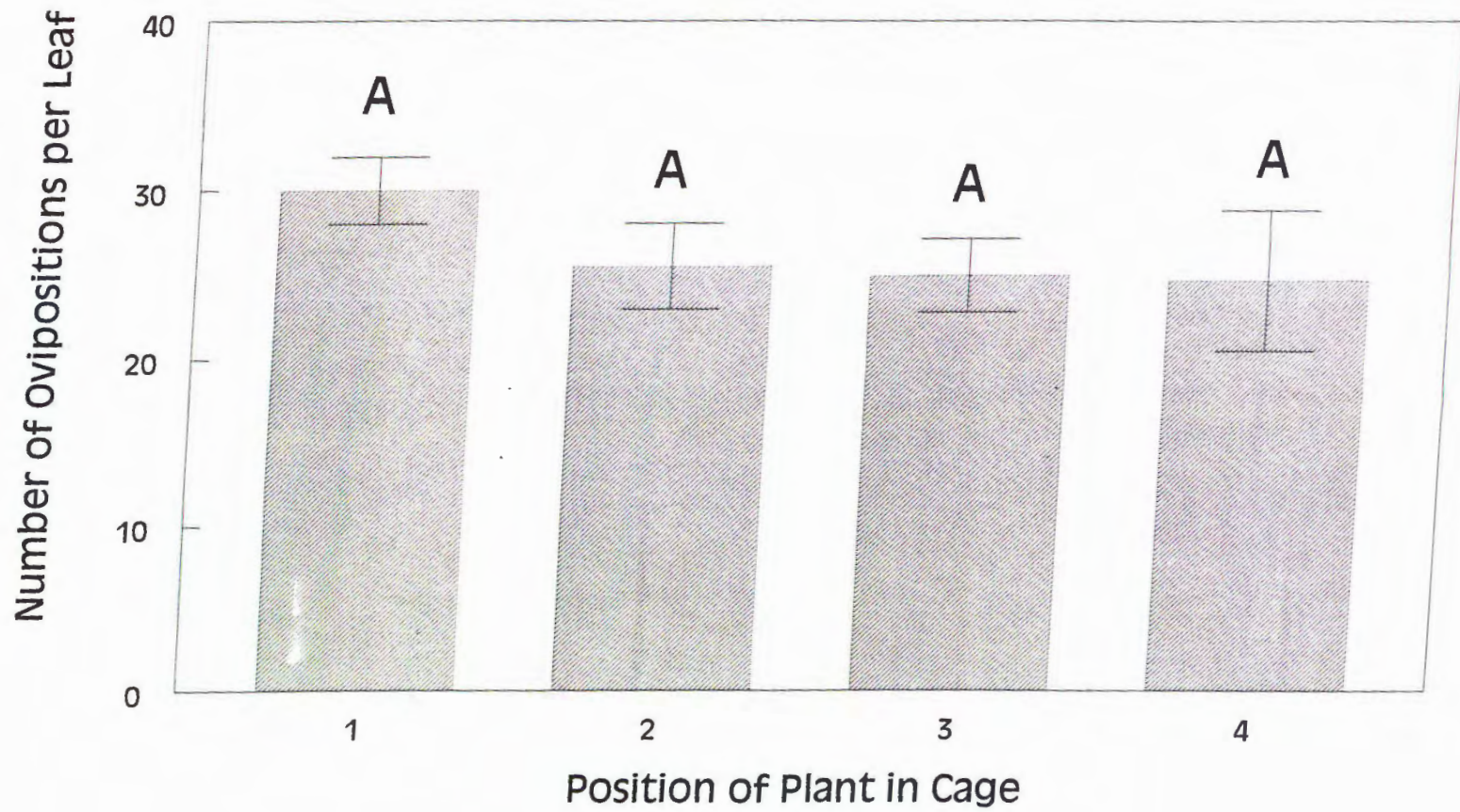


Table 11: Analysis of Variance Table for number of ovipositions by cultivar in no-choice cage study.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1538.07	512.69	6.76	0.0008
Error	44	3334.792	75.79		
Corrected Total	47	4872.87			

Figure 18: Average total ovipositions for each cultivar in no-choice cage study. Bars represent means and vertical lines represent standard errors. Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p=0.05$ .

## Preference for Each Cultivar in No-Choice Cages

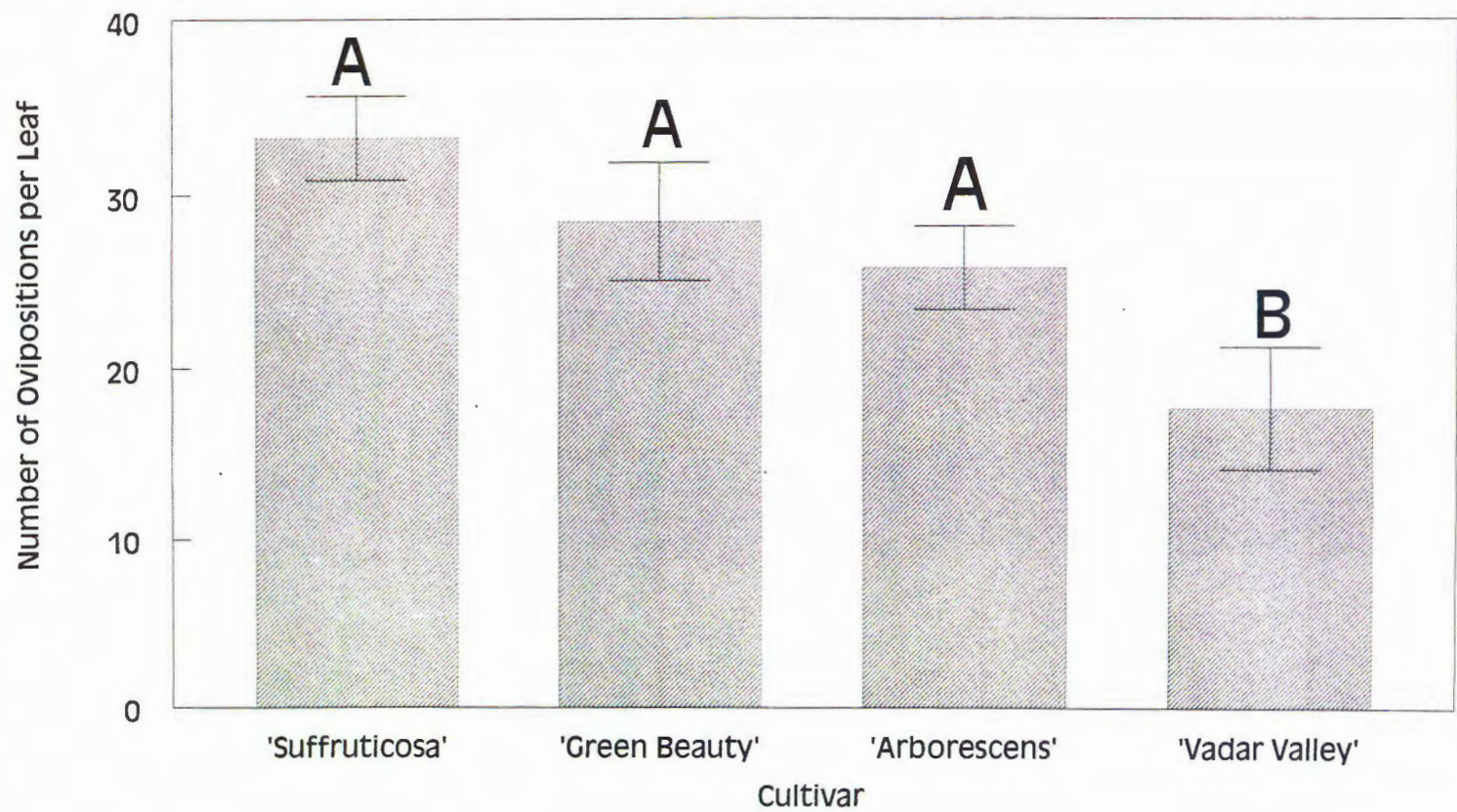
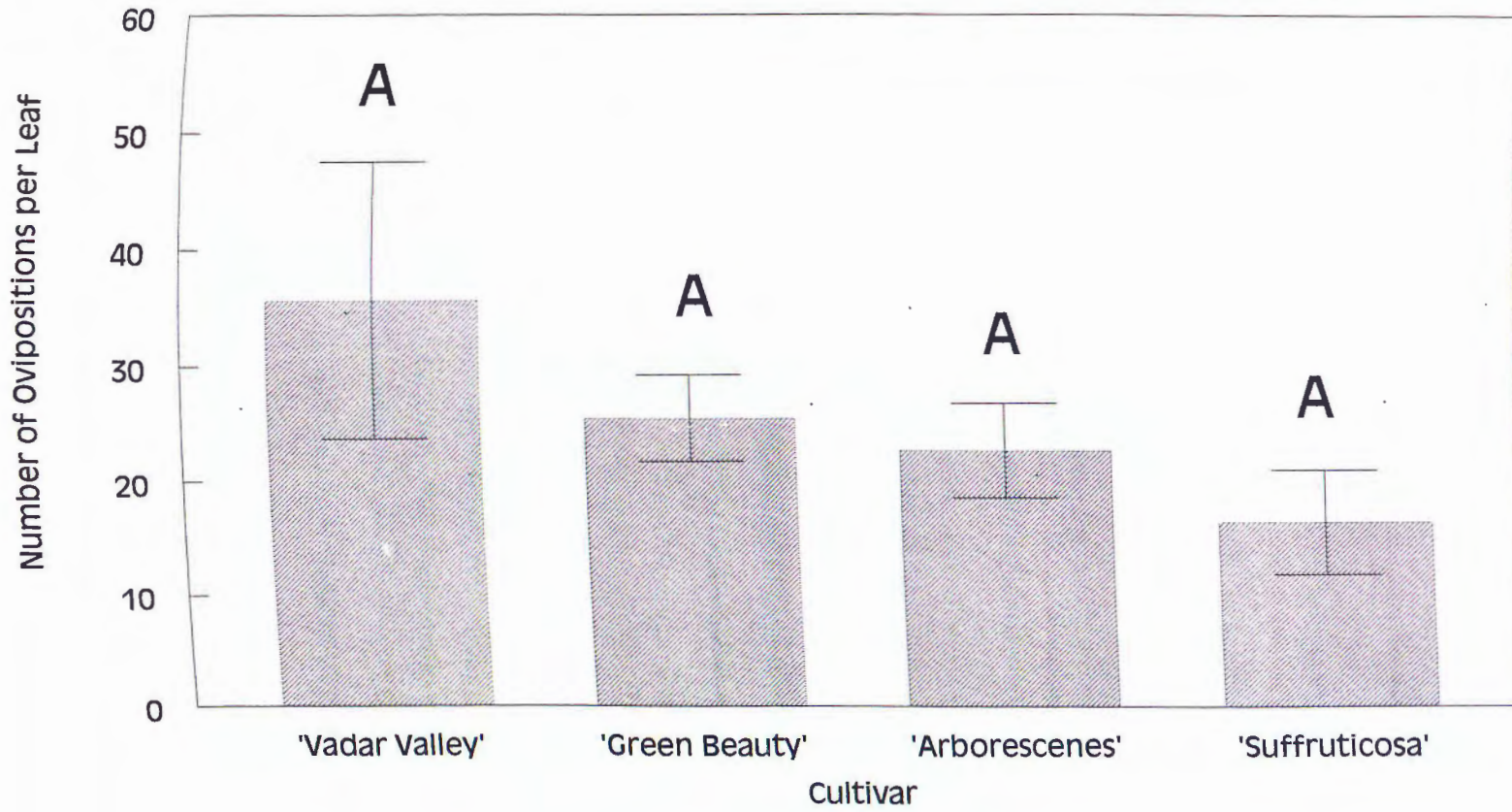


Table 12: Analysis of Variance table for number of ovipositions by cultivar in choice cage study cultivar effect on ovipositional preference

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	764.89	254.96	1.75	0.21
Error	12	1746.10	145.51		
Corrected Total	15	2510.98			

Figure 19: Average ovipositions for each cultivar in choice cage study. Bars represent means and vertical lines represent standard errors. Means that share the same letter do not differ by a Student-Newman-Keuls test,  $p = 0.05$ .

## Cultivar Preference in Choice Cage Study





## CHAPTER 6

### CONCLUSIONS AND CONTROL RECOMMENDATIONS

The boxwood leafminer is a serious pest in landscape settings. It can be difficult to manage, but proper techniques and timing will provide effective control. Several cultivars show varying levels of susceptibility to boxwood leafminer attack. This finding can be used by landscape managers to plan landscapes that are both resistant to leafminer attack and will not need chemical control for leafminer.

For existing landscapes where leafminers are a problem; Avid or Merit, sprayed at the first sign of adult emergence, will give effective control of leafminer populations.

There are numerous resistant cultivars, including 'Vardar Valley', 'Suffruticosa', and 'Handsworthiensis'. There are also varieties that are highly susceptible to leafminer attack such as 'Arborescens', 'Myrtifolia' and 'Belleville'.

An unusual behavior of the boxwood leafminer is that despite the vast difference in cultivar susceptibility, the adult leafminers fail to discriminate between susceptible and resistant plants. This may be due to

the ephemeral nature of boxwood leafminer adults. Adults have little time to select hosts. This may constrain adults to lay as many eggs as possible in a short period of time. However, it appears that there may be selective pressure to colonize marginally suitable host plants.

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