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Growth and Development of Insecticide-Resistant and Susceptible Colorado Potato Beetle Larvae (Coleoptera: Chrysomelidae) on Different Solanaceous Hosts

presented by

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GROWTH AND DEVELOPMENT OF INSECTICIDE-RESISTANT AND SUSCEPTIBLE COLORADO POTATO BEETLE LARVAE (COLEOPTERA: CHRYSOMELIDAE) ON DIFFERENT SOLANACEOUS HOSTS

by

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ABSTRACT

GROWTH AND DEVELOPMENT OF INSECTICIDE-RESISTANT AND SUSCEPTIBLE COLORADO POTATO BEETLE LARVAE (COLEOPTERA: CHRYSOMELIDAE) ON DIFFERENT SOLANACEOUS HOSTS

by

Patti Lea Rattlingourd

The Colorado potato beetle, Leptinotarsa decemlineata (Say), is able to rapidly develop insecticide resistance and readily adapt to new hosts by combining several different resistance mechanisms. The same types of enzymes are probably responsible for insecticide resistance and allelochemical tolerance. The research was designed to measure larval growth on different hosts with varying allelochemical levels. Growth and development of insecticide-resistant and susceptible CPB larvae were measured on different potato cultivars, and on different solanaceous species. Leaf moisture content was recorded and glycoalkaloid and chlorogenic acid concentrations measured from each potato cultivar.

There were complex interactions with respect to larval growth parameters between CPB strains, host plants and allelochemicals. Susceptible strains were affected by allelochemicals more than resistant strains. Susceptible strains often consumed more than resistant strains, depending on the host. Significant interactions among growth rates occurred between strains. Susceptible strains grew as fast as resistant strains in some cases. Potatoes were the best host among the species tested. In most cases, S. chacoense and eggplant were not good hosts. Differences between potato cultivars exist as well. 'Superior' and 'Atlantic' were generally the best hosts.

'Superior' and 'Atlantic' cultivars were the cultivars fed to the CPB cultures. Resistant strains usually were not more efficient than susceptible strains.

There is no 'representative' resistant or susceptible CPB strain; they are all unique. Since the strains tested all came from different field sites, resistance status may be linked to other innate characteristics. Larval growth was affected by innate strain differences or resistance status (mechanisms of resistance and length of time resistance has been present). One of the susceptible strains grew slower after contamination by a resistant strain.

Abundant interactions between insect and plant characteristics exist. Interactions between plant characteristics and insect resistance status must be carefully considered before insecticide resistance management or IPM programs can be developed. The introduction of CPB resistant potato cultivars, in the future, may further complicate these interactions.

To my friends

Adam Peters and Tom Mowry:

your belief sustained me

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INTRODUCTION

The Colorado potato beetle (CPB) is an herbivore specialist restricted to about a dozen species of the family Solanaceae (Hsiao 1982) that vary greatly in their suitability as hosts (Hare 1983). Some of these hosts include eggplant (Solanum melongena L.), purple nightshade (S. dulcamara L.), silver leaf nightshade (S. eleagnifolium L.), potato (S. tuberosum L.), buffalo burr (S. rostratum Dunal), horsenettle (S. carolinense L.), pepper (Capsicum frutescens L.), tobacco (Nicotiana tabacum L.), tomato (Lycopersicon esculentum Mill.), and henbane (Hyoscyamus niger L.)(Gauthier et al. 1981). Each species varies in its suitability as a host (Hare 1983), and different CPB populations exhibit differences in host preference (Hare and Kennedy 1986).

The Colorado potato beetle (Leptinotarsa decemlineata Say) is thought to have originated in Southern Mexico feeding on buffalo burr (Solanum rostratum). It then moved northward and eventually began feeding on silver leaf nightshade (S. eleagnifolium) and potato (S. tuberosum) (Gauthier et al. 1981, Caprio 1987).

CPB larvae remain on the egg mass for at least 6 hours after hatching. Neonate larvae consume empty eggshells and cannibalize unhatched eggs if no other food source is available (Lashomb et al. 1987). Growth is exponential at first, but slows considerably around day 7, due to a decrease in food consumption. Larval development typically takes about 10 days. Prepupal fourth instars are pale orange, weigh ca. 150-200 mg, and are hard to the touch.

The Colorado potato beetle was one of the first highly destructive pests in the United States, and has had a greater effect on developments in crop protection methods and application equipment than any other pest. Hand picking of adults, larvae and egg masses was the only control method available to growers in the 1850's (Gauthier et al. 1981). The modern sprayer was developed in an attempt to combat the ever increasing damage done by the CPB. One of the first uses of Paris Green, the first arsenical insecticide, was against the CPB. Growers continued to use arsenical insecticides as late as the 1940's, even though they were phytotoxic, difficult to mix, and provided erratic control (Casagrande 1987). Of the botanical compounds tried, only rotenone had sufficient residual properties to be effective (Gauthier et al. 1982). Swiss entomologists discovered in 1939 that DDT effectively killed the CPB. Excellent control was also obtained with other chlorinated hydrocarbons such as dieldrin, chlordane, aldrin, heptachlor and methoxychlor during the mid to late 1940's and early 50's.

Insecticide resistance in the CPB has been present almost as long as pesticides have been. By the 1900's, rate differences were beginning to show with Paris Green (Gauthier et al. 1981). Various arsenicals were used and discarded due to increasing resistance in the CPB (Casagrande 1987). Resistance to DDT was reported in 1952, and resistance to dieldrin and other chlorinated hydrocarbons in 1958 (Gauthier et al. 1981). Research began in the mid 1950's to find soil treatments to supplement the failing foliar sprays.

The time period over which new insecticides are effective has become progressively shorter, although the use of synergists such as piperonyl butoxide has extended the life of some of these chemicals. In many potato growing regions in the eastern United States, organophosphates and

carbamates are completely ineffective, and there is now some resistance to the pyrethroids (Forgash 1985).

Along with other tactics such as biocontrol and crop rotation, host plant resistance may be a useful tool for CPB management. As early as 1861, it was known that some potato varieties were less "attractive" to the CPB than others. Riley recommended planting more preferred varieties around fields of less preferred varieties, placing small piles of potatoes in the field before the crop emerged, and killing the beetles attracted to them (Casagrande 1987). He believed that trap crops, less preferred varieties, crop rotations, early maturing varieties and isolated fields would be sufficient to control the CPB without any chemical input (Casagrande 1987).

Allelochemicals are thought to be important in the evolution of herbivore host plant preference (Barbosa 1988). Specialists generally have narrower host ranges than generalists (Pianka 1983), and presumably have developed a new detoxification system to allow them to make use of a new food source (Whittaker and Feeny 1971), while generalists remain unable to utilize it. Some authors argue that the importance of allelochemicals is being overemphasized, and that other factors such as generalist predators (Bernays and Graham 1988), plant apparency, nutritional factors and plant morphology determine the host range of an herbivore (Barbosa 1988). Regardless of which factor is most important in the evolution of host plant range, allelochemicals are also involved (Ehrlich and Murphy 1988).

The function of allelochemicals has been much debated. It has been proposed that they are nitrogenous waste products analogous to the nitrogenous waste products of animals. However, anabolic synthesis of glycoalkaloids, as opposed to catabolic formation of ammonia, urea, uric acid, etc. in animals, argues against a metabolic waste function (Rozenthal and

Janzen 1979). They are thought to aid the plant in its defense against herbivores (Tingey 1984, Rozenthal and Janzen 1979), as well as play a role in wound healing and disease resistance (Kuc 1984). For instance, since the synthesis of glycoalkaloids requires a major diversion of acetate out of the metabolic pathways into the steroid biosynthetic pathway, it would be logical to assume they must have an important function (Kuc 1984, Robinson 1974). They are known to have anticholinesterase activity (Bushway et al. 1987).

Glycoalkaloids are nitrogenous steroidal glycosides (Osman 1980) whose biosynthesis proceeds through the steroid pathway (Heftmann 1983). Most glycoalkaloids are present throughout the plant, but some are more restricted in their distribution (Roddick 1980). Leptines, found only in S. chacoense Bitter, are present only in the foliage (Stürckow and Low 1961). Because they are found only in the foliage, leptines are a possible source of CPB resistance.

Solanaceous plants may vary both in the total glycoalkaloid levels and in the types of glycoalkaloids present. Some have a wide range of different kinds of glycoalkaloids, while others have only a few types, but with higher total glycoalkaloid levels.

Glycoalkaloids are concentrated mainly in regions of high metabolic activity: the meristems and sprouts. They are synthesized primarily in the tops of plants and the roots (Osman 1980). As the plant matures, the glycoalkaloid concentration increases in the flowers, stolon, and tubers, while decreasing in other plant organs.

In addition to variation between species, almost any environmental factor can affect the glycoalkaloid levels. Climate, altitude, soil type and moisture, fertilization, air pollution, time of harvest, vine killing, pesticides and exposure of tubers to sunlight all can affect the total glycoalkaloid levels

of tubers at harvest (Sinden et al. 1984). Mechanical damage and length of storage can also affect the levels of glycoalkaloids in the tuber (Osman 1980). Most of these stresses do not cause whole-tuber total glycoalkaloid levels to increase above 20 mg/100 g fresh weight (the allowable limit for safe human consumption)(Sinden et al. 1984, Roddick 1980).

Allelochemicals can affect the levels of insecticide resistance in some insects (Kennedy and Farrar 1987, Yu et al. 1979, Brattsten et al. 1977). Strains of two-spotted spider mites, Tetranychus urticae (Koch), selected for survival on a resistant host plant had slightly higher tolerance levels to several pesticides than unselected mites. Yu et al. fed peppermint leaves to variegated cutworm larvae (Peridroma saucia Hübner) for two days and observed increased mixed function oxidase (MFO) levels. Larvae given peppermint leaves had 20% survivorship at .5% carbaryl compared to survivorship of larvae fed either bean leaves or an artificial diet (0% at 0.1% carbaryl). Brattsten et al. tested southern armyworm larvae Spodoptera eridania (Cramer) that had artificially induced MFO systems and concluded that they were better protected against nicotine poisoning than uninduced control larvae. 2-Tridecanone appears to be metabolized by and induces MFO's, the same enzymes that metabolize many insecticides (Kennedy and Farrar 1987). Recently, Carter and Ghidiu (1988) reared CPB larvae on plants with higher glycoalkaloid levels and found that the larvae were more resistant to fenvalerate than larvae reared on either potato or eggplant.

MFO systems are one of the most common insecticide resistance mechanisms (see appendix). Neal (1987) found no cost involved with MFO induction in Heliothis zea (Boddie) although he only compared induced MFO activity with uninduced activity. No studies I have found have compared the cost of MFO induction in insecticide-resistant populations with that of

susceptible populations. MFO systems ought to be constitutive rather than inducible if no cost is involved. The cost of MFO induction could be higher on more toxic hosts and in insecticide-resistant strains. Susceptible larvae should perform better on less toxic hosts than resistant larvae if a cost is involved. If a cost is not involved, resistant larvae should perform better than susceptible larvae.

It has been postulated that the early stages of insecticide resistance will carry fitness disadvantages, because genetic regulatory elements will not have had time to become established in the population (Argentine et al. 1989, Dobzhansky 1970). Resistance genes often have pleiotropic effects, such as reduced fitness, when insecticide selection is removed. If there were no negative effects, it is argued that resistance alleles would occur at greater frequencies even without selection (Argentine et al. 1989, Roush and Plapp 1982). An unselected organophosphate-resistant mosquito strain with a resistance ratio of 2.4 (LD_{50} resistant/LD_{50} susceptible) had significantly higher fecundity, shorter development times and higher viability than an organophosphate-resistant strain (resistance ratio=312) with no insecticide treatment. Diazinon-resistant homozygous offspring developed as fast as a susceptible strain (Ferrari and Georgiou 1981). A tetrachlorvinphos (tet) - resistant strain and diazinon-resistant/tet resistant hybrids developed significantly slower than a susceptible strain (Roush and Plapp 1982). No significant differences were found between susceptibles and heterozygotes, nor in a permethrin-resistant strain (Argentine et al. 1989). No significant differences in fecundity or fertility were found between resistant heterozygotes and susceptible mosquitoes, either (Ferrari and Georgiou 1981). Resistant heterozygotes developed as fast as susceptible house flies (Roush and Plapp 1982). An azinphosmethyl-resistant CPB strain produced

significantly fewer eggs and developed significantly slower than a susceptible strain.

Some insecticide resistant CPB strains have elevated MFO levels as a resistance mechanism (Ahammad-Sahib et al. 1990). Insecticide-resistant and susceptible CPB larvae may therefore differ in their abilities to utilize certain hosts. For example, insecticide-resistant CPB larvae might be more tolerant of plants with high glycoalkaloid levels than susceptible larvae. If mixed function oxidases are also involved in the breakdown of glycoalkaloids, then insecticide resistant larvae should be able to tolerate a higher level of toxicity in the host plant better than susceptible larvae. On a less toxic host, resistant larvae should perform better than susceptible larvae. If there is a cost to resistance, then susceptible strains should perform better than resistant strains.

Of the studies that have looked for a cost to resistance, most have either used a lab-selected culture or the homogeneous resistant strain used for genetic analysis of resistance. By that time, the genome may have been rearranged from when the insect was first brought in from the field. The insect needs to be tested for a fitness reduction before inbreeding to produce a homogeneous resistant strain occurs. The inbred strain will be genetically different than the one first collected, and fitness effects due to resistance will probably be less (genomic rearrangements will already have had time to occur).

If CPB larvae raised on plants with higher glycoalkaloids are more resistant to insecticides, then the reverse might also be true: insecticide-resistant CPB strains may be more tolerant of plants with higher glycoalkaloids than susceptible strains of larvae. Such interactions between insecticide resistance and glycoalkaloid tolerance need to be considered in

developing pest resistant varieties and insecticide resistance management programs. If there is an interaction between insecticide resistance and glycoalkaloid tolerance, this needs to be a consideration when developing pest management programs. Developing varieties with higher glycoalkaloid levels could increase the rate of development of insecticide resistance. The CPB develops resistance to new pesticides within a of couple years, and the rate of resistance development is ever increasing (Ioannidis 1990). The cost of trying to find pesticides that are still effective and discarding those that are ineffective is skyrocketing.

The goals of this thesis were to: 1. compare the growth and development of insecticide-resistant and susceptible CPB larvae on different solanaceous hosts; 2. measure glycoalkaloid and phenolic levels from the potato cultivars, and 3. assess any correlation between chemical levels and larval growth.

Materials and Methods

One insecticide resistant CPB strain (JP) was collected in Macomb County, MI, from a potato field that had been sprayed 10-20 times a year for the past 5-10 years with pyrethroid, organophosphate, carbamate and chlorinated hydrocarbon insecticides. This strain was also under selection in the lab with permethrin for about a year. The other resistant strain (LI) came from Long Island, NY, where there is a long history of insecticide resistance (Gauthier et al. 1981). This culture was in the lab for 8 generations. A susceptible strain (VE) was collected from volunteer potatoes near Vestaburg, Montcalm County, MI. A second susceptible culture (CR), was collected at the M.S.U. Collins Road Entomology Farm, where potatoes, tomatoes, eggplants

or nightshade spp. may also serve as hosts. All four strains had been maintained in the lab from one to five years on foliage of 'Superior' and 'Atlantic' potato cultivars.

The insecticide-resistant strains in this study have elevated MFO levels compared to the susceptible strains (Table 1). High levels of permethrin, azinphosmethyl and carbofuran resistance in the JP strain is due to the presence of elevated MFO levels (permethrin-specific and non-specific) and an altered acetylcholinesterase. Resistance in the LI strain is primarily due to higher MFO levels than the susceptible strains and JP, resulting in a higher LD_{50} to azinphosmethyl than JP (Ioannidis 1990).

The potato cultivar 'Superior' was used as a standard in all experiments because preliminary studies showed it to be a highly acceptable food. 'Superior', 'Onaway', 'Conestoga', 'Atlantic' and 'Russet Burbank' potato cultivars were used as hosts in experiments 1 and 2 and potato, S. chacoense, tomato and eggplant were used in experiments 3 and 4. The potatoes were grown from tubers in the greenhouse and S. chacoense, tomatoes, and eggplants were obtained as young plants about 30 cm high. All plants were grown in 20 cm diameter plastic pots in the greenhouse. Experiments were started when the plants were about 2 months old. Four to six plants of each type were used to reduce the possibility of induced glycoalkaloids from the removal of too much foliage (Osman 1980).

Leaflets were taken from the third to fifth leaves down from the terminal meristem and placed in a petri dish with the larvae in experiment 1. In experiments 2-4, leaves were again taken from the third to fifth nodes down from the terminal meristem, and placed in 0.5 dram vials with water and a cotton plug. Leaves and vials were placed in petri plates with the larvae and a piece of filter paper.

Table 1. LD_{50}'s of azinphosmethyl and carbofuran for Colorado potato beetle strains.[1]

BEETLE STRAIN	Azinphosmethyl(µg/g) LD_{50}	Carbofuran(µg/g) LD_{50}
LI	743.2	47.8
JP	272.9	>400.0
CR	1.3	0.2
VE	1.3	0.2

[1] Ioannidis 1990.

Experimental Design. 'Superior', 'Onaway' and 'Conestoga' were used in experiment 1, and 'Atlantic', 'Russet Burbank' and 'Superior' in experiment 2. Experiments 3 and 4 used four solanaceous species: potato cultivar 'Superior', S. chacoense accession no. 230580, tomato cultivar 'Mountain Pride', and eggplant cultivar 'Black Magic' in experiment 3, and 'Superior', S. chacoense accession no 230580, eggplant cultivar 'Black Magic' and tomato cultivar 'Better Boy' in experiment 4. The eggplants in experiment 4 were smaller and thornier than those in experiment 3, even though they were the same cultivar.

For experiment 1, one insecticide-resistant (JP) and one susceptible (VE) strain was used. For experiment 2-4, two resistant (JP and LI) and two susceptible strains were used (CR and VE). Six groups of 5 actively moving larvae (three days old) were randomly placed with a camel hair brush into 150 mm petri plates (30 total) and kept at 25° C with a 16:8 photoperiod (80-90% RH experiment 1, 40-50% experiment 2-4). RH inside the petri plates was highest in experiments 2-4 when vials were used. Two to three holes were cut in each lid in experiment 4, to help dissipate the humidity. Mortality was high in experiment 3, probably due to a pathogen from the high humidity inside the plate (dead larvae turned brown and disintegrated).

Experiments 1 and 2 were randomized complete block designs (replicated over time), and experiments 3 and 4 were completely randomized designs. All experiments were analyzed as factorials with treatments of host plant x CPB strain. The effect of host plant on larval development and performance of insecticide-resistant vs. susceptible CPB strain was tested with orthogonal contrasts using the General Linear Models procedure, and those treatment means separated by SNK test (SAS institute 1985). Glycoalkaloid and chlorogenic acid concentrations were also analyzed using the GLM

procedure and means separated with SNK (SAS institute 1985). Total development time, length of pupation, mean emergence and mean survival rates were analyzed by ANOVA (Statview, Feldmann et al. 1988).

Consumption Rates. Leaf material consumption was estimated by cutting and weighing two equivalent leaflets. One of each pair ("unfed") was dried in a drying oven at 45° C to calculate a dry/fresh weight ratio, and the other was fed to a group of larvae. Uneaten "fed" leaf material was removed after 24 h and dried in individual weigh boats also at 45°C. The leaflets were then placed in a desiccator at room temperature for at least 24 h, and weighed (Fisher-Scientific balance model no. XA-200DS) immediately after removal from the desiccator. Initial dry weight of "fed" leaflets was estimated from the mean dry/fresh wt ratio of "unfed" leaflets:

initial dry weight of "fed" leaflets= fresh weight x dry/fresh wt ratio

amount consumed= initial estimated dry weight - final dry weight

relative consumption rate= amt consumed/Σ weights

Growth and Development. Larvae were weighed every 3 days in experiment 1, and every day or every other day in experiment 2-4 until larvae entered the soil to pupate. Prepupal fourth instars were placed in plastic drinking cups filled with ca. 10 cm of potting soil. Food was provided until larvae dug into the soil to pupate.

Instantaneous growth rates were estimated using a linear regression of the natural logarithms of larval weights vs. time for the first 6 days (Statview,

Feldmann et al. 1988). Times that larvae entered the soil for pupation and emerged as adults and survivorship were recorded at each stage.

Leaf Moisture Content. Leaf moisture content was calculated as (1- dry/fresh wt ratio) x 100% from the leaves used to estimate consumption rates.

Glycoalkaloid Extraction. Freshly picked leaves from the third to the fifth node down from the terminal meristem of each cultivar were freeze-dried. Extraction of total glycoalkaloids was done using a modified version of Gull and Isenberg (1973). Three 500 mg dry wt samples of each cultivar were ground with a mortar and pestle, combined with 50 ml of 5% acetic acid and placed in a shaker for 30 min. Each solution was then vacuum filtered and placed in a hot water bath at 75° C for 30 min. Twenty ml of NH_4OH were added, and refrigerated overnight to precipitate the glycoalkaloids. The solutions were then centrifuged in a tabletop centrifuge (ca. 3g) for 10 min, and redissolved in five ml 5% acetic acid in methanol and divided into two 0.5 ml subsamples. Three ml of 50% ethanol: concentrated sulfuric acid (1:2) was added to each, and cooled on ice. One ml of 1% formaldehyde was added slowly, and mixed well. The absorbance at 562 nm was read on a spectrophotometer after 90 min. Concentration of glycoalkaloids (mole/l) following the method of Shih and Kuc (1973) was calculated as:

$$\frac{c}{x} = \frac{A}{E}$$

where c = concentration of glycoalkaloids (M)

A = absorbance at 562 nm

E = extinction coefficient

$(1.4 \times 10^4\ M^{-1}\ cm^{-1})$

ι = path length (1 cm)

x = amt leaf tissue

Phenolic Extraction. Three 200 mg dry wt samples of freeze dried leaf tissue of each cultivar of was ground in a mortar and pestle, combined with 10 ml 50% methanol, and heated in a 75° C water bath for 30 minutes. The precipitate from each sample was removed by centrifugation for 10 minutes in a tabletop centrifuge (ca. 3g), and the supernatant concentrated on a rotary evaporator and freeze dried. The samples were then redissolved in 0.2 ml of 50% methanol and separated on a TLC plate in an ethyl acetate/formic acid/water solvent (85:6:10) for 2 hrs. Chlorogenic acid standards were run alongside the samples. The chlorogenic acid band from each sample (including the standard) was scraped off the plate and redissolved in two ml .5N NaOH and allowed to settle. The absorbance was then read for each sample with a spectrophotometer at 750 nm. Chlorogenic acid concentrations were calculated by using standard curves from the standard samples.

Results and Discussion

Consumption Rates. In experiment 1, VE larvae generally consumed more than JP larvae (Table 2). There were significant differences in consumption

Table 2. Relative consumption rates (mg dry wt x mg^{-1} x day^{-1}) for resistant (JP) and susceptible (VE) Colorado potato beetle larvae fed three potato cultivars (experiment 1).

CPB		CONSUMPTION RATE (mg/mg/day)		
STRAIN	CULTIVAR	DAY 0[1]	DAY 3	DAY 6
JP	SUPERIOR	.2	.2	.1
	CONESTOGA	.2	.3	.1
	ONAWAY	.5	.3	.1
VE	SUPERIOR	1.1	.5	.1
	CONESTOGA	.9	.3	.2
	ONAWAY	1.9	.5	.2

[1] Consumption rates over the first 24 hrs.

DF		F	F
1	strains	72.81	8.45
	p<	(.0001)	(.0068)
2	cultivars	12.48	7.31
	p<	(.0001)	(.0026)
2	strain*cultivar		10.29
	p<		(.0004)

rates between CPB strains and cultivars on day 0, no significant differences on day 3, and significant differences between CPB strains, cultivars and interaction effects on day 6. VE larvae consumed significantly less on 'Superior' than on 'Onaway' and 'Conestoga', and there were no significant differences between JP consumption rates or any cultivar, causing significant interaction effects on day 6. Susceptible larvae consumed significantly more than resistant larvae on days 0 and 6 (Table 3).

In experiment 2, consumption rates were variable, but generally not significantly different with respect to strain or cultivar (Table 4). Susceptible larvae consumed significantly more than resistant larvae only on day 1 (Table 5).

In experiment 3, susceptible larvae generally consumed more on eggplant and tomato (Table 6). CPB strains, hosts, and interaction effects were significant on day 0, no significant differences on day 1, significant differences between CPB strain and hosts on days 2, 3, and 5, and significant differences between CPB strains on day 6. Susceptible larvae consumed significantly more than resistant larvae on days 3, 5, and 6 (Table 7). On day 2, resistant larvae consumed significantly more than susceptible larvae.

In exp 4, larvae generally consumed more on eggplant and tomato (Table 8). There were significant differences between host plants and interaction effects on day 0, significant differences between strains, hosts and interaction effects on day 2, significant differences between CPB strains and hosts on days 4 and 6. Susceptible larvae consumed significantly more than resistant larvae on days 4 and 6 (Table 9).

Table 3. Relative consumption rates (mg dry wt x mg $^{-1}$day $^{-1}$) for resistant (JP) and susceptible (VE) Colorado Potato Beetle larvae combined over all cultivars (experiment 1).

CPB STRAIN	CONSUMPTION RATES (mg/mg/day) DAY 0[1]	DAY 3	DAY 6[1]
JP	.30 b	.29 ns	.09 b
VE	1.36 a	.42 ns	.13 a

[1] Means within a column followed by the same letter are not significantly different from each other (SNK test; Day 0:df=1, F=72.53, p<.0001; Day 6: df=1, F=8.45, p<.0068).

Table 4. Relative consumption rates (mg dry wt x mg $^{-1}$day $^{-1}$) for resistant (JP and LI) and susceptible (CR and VE) Colorado potato beetle larvae (experiment 2).

CPB STRAIN	CULTIVAR	CONSUMPTION RATES (mg/mg/day) DAY 0[1]	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
LI	SUPERIOR	2.5	1.0	.7	.8	.5	.5	.4
	RUSSET	2.3	.6	.5	.7	.7	.4	.3
	ATLANTIC	1.9	1.9	.9	.6	.3	.5	.3
JP	SUPERIOR	1.5	1.5	.6	.8	.8	.4	.4
	RUSSET	1.5	1.5	.6	1.1	.7	.3	.3
	ATLANTIC	1.5	1.5	.7	.9	.5	.3	.3
CR	SUPERIOR	1.4	1.4	1.1	.7	.4	.4	.4
	RUSSET	1.1	1.1	1.0	1.2	.5	.3	.4
	ATLANTIC	2.0	1.7	.8	.8	.4	.4	.4
VE	SUPERIOR	1.4	1.5	.7	.6	.7	.3	.5
	RUSSET	1.6	1.2	.7	.6	.7	.3	.5
	ATLANTIC	1.9	1.1	.7	.7	.9	.4	.4

[1] Consumption rates over the first 24 hrs.

Table 5. Relative consumption rates (mg dry wt x mg $^{-1}$day $^{-1}$) for resistant (LI and JP) and susceptible (CR and VE) Colorado Potato Beetle larvae combined over all cultivars (experiment 2).

	CONSUMPTION RATE (mg/mg/day)[1]						
CPB STRAIN	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
LI	2.15	.71 b	.68	.72	.53	.46	.33
JP	1.40	.64 b	.65	.92	.62	.35	.32
CR	1.92	1.49 a	.98	.99	.43	.40	.39
VE	1.48 ns	1.28 a	.71 ns	.59 ns	.58 ns	.34 ns	.43 ns

[1] Means within a column followed by the same letter are not significantly different from each other (SNK test, df=1, F=18.56, p<.0001).

Table 6. Relative consumption rates (mg dry wt x mg $^{-1}$day $^{-1}$) for resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae fed 4 solanaceous hosts (experiment 3).

		CONSUMPTION RATES (mg/mg/day)					
CPB STRAIN	HOST	DAY 0	DAY 1	DAY 2	DAY 3	DAY 5	DAY 6
LI	POTATO	2.04	2.81	1.05	.33	.41	.25
	CHACOENSE	1.65	2.38	1.23	.56	.06	.37
	EGGPLANT	2.48	2.31	2.21	.56	.03	.28
	TOMATO	2.46	2.32	1.49	.46	.08	.30
JP	POTATO	2.19	2.98	1.69	.33	.41	.30
	CHACOENSE	2.67	2.75	1.14	.44	.23	.26
	EGGPLANT	5.23	3.16	1.60	.51	.43	.24
	TOMATO	1.43	3.42	1.55	.55	.36	.23
CR	POTATO	2.87	2.99	.81	.47	.46	.33
	CHACOENSE	1.69	3.15	.90	.58	.43	.38
	EGGPLANT	2.77	3.71	1.57	.83	.66	.36
	TOMATO	1.75	2.12	1.06	.93	.55	.51
VE	POTATO	1.91	2.57	.71	.43	.46	.36
	CHACOENSE	1.86	3.87	.83	.56	.53	.35
	EGGPLANT	1.81	4.19	1.67	.69	.55	.54
	TOMATO	2.10	2.69	.73	.91	.68	.40

DF		F	F	F	F	F
3	strain	3.2	3.9	9.2	11.3	6.95
	p<	(.079)	(.0117)	(.0002)	(.0001)	(.0004)
3	host	5.1	6.2	10.1	4.1	
	p<	(.0031)	(.0006)	(.0001)	(.0015)	
9	strain*host	3.3				
	p<	(.001)				

Table 7. Relative consumption rates (mg dry wt x mg $^{-1}$day $^{-1}$) for resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae combined over host plant (experiment 3).

CPB STRAIN	CONSUMPTION RATES (mg/mg/day) [1]					
	DAY 0[2]	DAY 1	DAY 2	DAY 3	DAY 5	DAY 6
LI	2.11 b	2.43	1.49 a	.48 b	.35 b	.29 b
JP	2.88 a	3.09	1.46 a	.43 b	.35 b	.27 b
CR	2.29 ab	2.99	1.09 ab	.71 a	.52 a	.40 a
VE	1.92 b	3.33 ns	.98 b	.65 a	.56 a	.40 a

DF		F	F	F	F
1	res. vs. susc.	10.95	19.96	27.76	19.28
	p<	(.0013)	(.0001)	(.0001)	(.0001)

1 Means within a column followed by the same letter are not significantly different from each other (SNK test, p<.05).

2 Consumption rate over the first 24 hours.

Table 8. Relative consumption rates (mg dry wt x mg $^{-1}$day $^{-1}$) for insecticide resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae fed four different solanaceous hosts (experiment 4).

CPB STRAIN	HOST	CONSUMPTION RATES (mg/mg/day) DAY0[1]	DAY 2	DAY 4	DAY 6[2]
LI	POTATO	1.14	.19	.36	.22
	CHACOENSE	3.22	.23	.34	.22
	EGGPLANT	1.33	.47	.38	.38
	TOMATO	2.26	1.20	.94	.20
JP	POTATO	2.45	.21	.31	.28
	CHACOENSE	.99	.39	.36	.34
	EGGPLANT	1.85	.56	.43	.35
	TOMATO	1.78	1.04	.69	.31
CR	POTATO	2.37	.31	.66	.32
	CHACOENSE	1.03	.54	.59	.48
	EGGPLANT	5.49	2.90	1.30	.50
	TOMATO	3.2	1.25	1.20	.74
VE	POTATO	2.95	.33	.44	.41
	CHACOENSE	1.11	.48	.91	.49
	EGGPLANT	6.02	.89	.63	.83
	TOMATO	3.25	1.78	1.85	.79

[1] Consumption rates over the first 24 hours.
[2] LI larvae died on tomato.

DF		F	F	F	F
3	strain	3.5	3.9	6.5	20.9
	p<	(.0184)	(.0113)	(.0001)	(.0001)
3	host		9.7	8.3	9.8
	p<		(.0001)	(.0001)	(.0002)
9	strain*host	2.3	2.4		
	p<	(.0258)	(.0017)		

Table 9. Relative consumption rates (mg dry wt x mg $^{-1}$day $^{-1}$) for resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae combined over host plant (experiment 4).

CPB STRAIN	CONSUMPTION RATE (mg/mg/day)[1]			
	DAY 0[2]	DAY 2	DAY 4	DAY 6
LI	1.99	.52 b	.47 b	.42 ab
JP	1.77	.56 b	.45 b	.51 a
CR	3.05	1.18 a	.95 a	.28 b
VE	3.33 ns	.89 ab	.96 a	.25 b

[1] Means within a column followed by the same letter are not significantly different from each other (Day 4: SNK test, df=1, F=21.18, p<.00001).

[2] Consumption rate over the first 24 hours.

Growth and Development. In experiment 1, JP larvae weighed more than VE larvae from the beginning and remained so to the end (Table 10). As larvae grew, small differences were compounded, resulting in large differences by day 6. There were significant differences between CPB strains on day 0, between strains and significant interaction effects on day 3, and between strains on day 6. The significant interaction effects on day 3 were caused by JP larvae weighing the most on 'Onaway' and least on 'Conestoga', while VE larvae weighed most on 'Conestoga' and least on 'Onaway'. Resistant larvae weighed significantly more than susceptible larvae on day 6 (Table 11).

Growth rates were not significantly different between JP and VE larvae on any cultivar (Table 12). JP larvae on 'Conestoga' had the highest survival and the pupal stage was significantly shorter than for the other larvae. Perhaps 'Conestoga' is higher in nutrients or moisture content than 'Superior' and 'Onaway'.

Mean survival and emergence were not significantly different between resistant and susceptible larvae (Table 13). Resistant larvae developed significantly faster than susceptible larvae, and the length of pupation was also significantly shorter for JP larvae (Table 13).

In experiment 2, larvae of all other strains weighed less than CR larvae at the beginning and CR larvae always weighed less on 'Russet Burbank' than on 'Superior' or 'Atlantic' (Table 14). There were significant differences between CPB strains on day 0, day 2, between CPB strains and cultivars on day 3, between strains and cultivars on day 4, between strains, cultivars and significant interaction effects on day 5 and between strains, cultivars and interaction effects on day 6. Resistant larvae weighed significantly more than susceptible larvae on day 6 (Table 15).

Table 10. Mean weights of insecticide-resistant (JP) and susceptible (VE) Colorado potato beetle larvae (experiment 1).

CPB STRAIN	CULTIVAR	LARVAL WEIGHTS (mg) DAY 0	DAY 3	DAY 6
JP	SUPERIOR	.98	7.06	58.59
	CONESTOGA	.82	6.96	51.80
	ONAWAY	.91	7.97	57.10
VE	SUPERIOR	.74	5.24	44.71
	CONESTOGA	.72	5.30	39.73
	ONAWAY	.69	3.44	36.37

DF		F	F	F
1	strains	24.1	51.2	31.5
	p<	(.0001)	(.0001)	(.0001)
2	cultivars			
	p<			
2	strains*hosts		4.3	
			(.012)	

Table 11. Day 6 mean weights of resistant (JP) and susceptible (VE) Colorado potato beetle larvae combined over all cultivars (experiment 1).

CPB STRAIN	LARVAL WEIGHTS (mg)[1]
JP	51.42 a
VE	42.79 b

1 Means within a column followed by the same letter are not significantly different from each other (SNK test, df=1, F=13.87, p<.0003).

Table 12. Growth, survival, emergence rates and total development times for resistant (JP) and susceptible (VE) Colorado potato beetle larvae fed 'Superior', 'Onaway', and 'Conestoga' potato cultivars (experiment 1).

CPB STRAIN	CULTIVAR	GROWTH RATES (mg/mg/day)	95% C.L.	% LARVAL SURVIVAL[1]	% ADULT EMERGENCE[2]	TOTAL DEV.TIME (days ± SE)[3]	LENGTH OF PUPATION (days ± SE)[4]
JP	SUPERIOR	.69 a	.65-.73	88	100	19.2 ± .2	10.3 ± .2
	CONESTOGA	.63 a	.59-.68	95	97	18.7 ± .2	9.7 ± .2
	ONAWAY	.69 a	.69-.73	97	87	18.9 ± .2	10.1 ± .2
VE	SUPERIOR	.67 a	.63-.70	83	-5	-5	-5
	CONESTOGA	.67 a	.63-.71	90	89	19.6 ± .3	12.7 ± .4
	ONAWAY	.66 a	.63-.68	78	100	19.2 ± .3	11.8 ± .3

[1] Larvae surviving from hatching up through pupation.
[2] Number of adults emerging vs. number entering the soil for pupation.
[3] Days from hatching to adult emergence.
[4] Days from entering the soil to adult emergence.
[5] Superior plants were mistakenly sprayed for aphids with endosulfan-contaminated pirimor, killing all the larvae.
[6] Confidence limits followed by the same letter overlap each other.

Table 13. Mean survival, emergence, and total development time of resistant (JP) and susceptible (VE) Colorado potato beetle larvae (experiment 1).

CPB STRAIN	% MEAN SURVIVAL	% MEAN EMERGENCE	TOTAL DEV. TIME[1]
JP	93	95	18.95 b
VE	84 ns	94 ns	19.45 a

1 Means within a column followed by the same letter are not significantly different from each other (SNK test, df=1, F=2.1, $p<.05$).

Table 14. Mean weights of resistant (JP and LI) and susceptible (CR and VE) Colorado potato beetle larvae fed three different cultivars (experiment 2).

CPB STRAIN	CULTIVAR	LARVAL WEIGHT (mg) DAY 0	DAY 1	DAY 2	DAY 3	DAY 5	DAY 6
LI	SUPERIOR	0.51	2.4	4.7	9.7	36.2	77.2
	RUSSET	0.52	2.2	4.1	7.9	37.9	70.1
	ATLANTIC	0.58	2.4	4.9	8.5	26.5	61.8
JP	SUPERIOR	0.61	1.6	3.4	8.3	39.5	71.9
	RUSSET	0.57	1.3	2.9	6.9	31.1	52.5
	ATLANTIC	0.59	1.3	2.8	5.8	27.4	61.6
CR	SUPERIOR	0.84	2.5	6.1	11.3	53.4	97.3
	RUSSET	0.87	2.4	5.8	6.9	27.5	61.4
	ATLANTIC	0.84	2.2	6.3	9.9	45.8	99.3
VE	SUPERIOR	0.81	1.5	3.7	8.0	32.2	54.6
	RUSSET	0.77	1.8	3.1	8.1	27.9	54.2
	ATLANTIC	0.72	1.6	3.1	7.7	28.4	46.3

DF		F	F	F	F	F	F
3	strains	45.4	29.2	65.9	3.1	8.3	18.5
	p<	(.0001)	(.0001)	(.0001)	(.0275)	(.0001)	(.0001)
2	cultivars				4.2	7.7	6.5
	p<				(.0161)	(.0005)	(.0017)
6	strains*cult					2.9	3.8
	p<					(.0081)	(.0012)

Table 15. Day 6 mean weights of resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae combined over all cultivars (experiment 2).

CPB STRAIN	LARVAL WEIGHT (mg)[1]
LI	69.63 b
JP	61.64 b
CR	85.56 a
VE	51.80 c

1 Means within a column followed by the same letter are not significantly different from each other (SNK test, df=1, F=4.33, p<.0382).

LI and CR larvae grew fastest, except for CR larvae on Russet Burbank (Table 16). Growth rates within a strain varied but generally were not significantly different (except for CR on Russet). Although CR larvae weighed more at the beginning than LI larvae, LI larvae grew faster than CR larvae, so mature weights were similar.

Mean survival of resistant larvae did not differ significantly from susceptible larvae. Adult emergence ranged from 38-91% (Table 16). Emergence was generally high on 'Superior' except for CR (50%), and generally low on 'Atlantic'. Although mean emergence was not significantly different between resistant and susceptible strains (Table 17), JP and VE had significantly higher mean emergence than LI and CR. LI and CR strains grew the fastest, had the lowest mean emergence, but did not consume significantly more than the other strains. There may be a trade off between survival and rapid growth. Larvae developed fastest on 'Superior' (Table 16). Susceptible strains developed significantly slower than resistant larvae Table 17). The length of the larval stage was not significantly different between strains, while the length of pupation was significantly longer for CR strain than the other strains.

Experiments 3 and 4 were analyzed separately due to significant differences between larval weights in the two experiments. In experiment 3, strains, hosts (except day 0), and sometimes interactions were significant factors. Larvae generally weighed more on potato than eggplant (Table 18). Starting with day 3, VE larvae weighed much less on eggplant than on potato. By day 7, larvae on eggplant weighed 68% less than larvae on potato. On day 0, there were significant differences between CPB strains, between strains, hosts and significant interaction effects on day 1, between strains and hosts on days 2, and 3, and between strains, hosts, and interaction effects on days 5 and

Table 16. Growth, survival, emergence rates and total development times for resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae fed 'Superior', 'Russet Burbank' and 'Atlantic' potato cultivars (experiment 2).

CPB STRAIN	CULTIVAR	GROWTH RATES (mg/mg/day)	95% C.L.[1,4]	% LARVAL SURVIVAL[1]	% ADULT EMERGENCE[2]	TOTAL DEV. TIME (days ± SE)[3]	LENGTH OF PUPATION (days ± SE)[4]
LI	SUPERIOR	.87 a	.82-.92	65	65	18.8 ± .6	10.3 ± .5
	RUSSET	.84 ab	.79-.89	68	36	18.2 ± .9	10.0 ± 1.3
	ATLANTIC	.81 abc	.76-.85	69	38	18.1 ± .5	9.8 ± .5
JP	SUPERIOR	.77 bc	.74-.81	75	91	18.5 ± .3	10.4 ± .3
	RUSSET	.72 cd	.69-.76	78	79	18.8 ± .1	10.8 ± .1
	ATLANTIC	.74 cd	.71-.78	65	82	19.0 ± .2	11.0 ± .2
CR	SUPERIOR	.79 abc	.75-.83	78	50	19.6 ± 2.1	11.7 ± 2.4
	RUSSET	.69 d	.65-.73	68	50	20.1 ± .8	13.0 ± .9
	ATLANTIC	.77 bc	.74-.81	68	56	23.1 ± .6	13.5 ± .6
VE	SUPERIOR	.75 cd	.71-.78	67	77	19.1 ± .3	10.5 ± .3
	RUSSET	.76 cd	.72-.78	76	63	19.9 ± .9	11.3 ± .5
	ATLANTIC	.73 cd	.70-.76	60	76	22.6 ± 1.3	10.6 ± .6

1 Larvae surviving from hatching up through pupation.
2 Number of adults emerging vs. number entering the soil for pupation.
3 Day after hatching to adult emergence.
4 Days from entering the soil for pupation and emergence as adults.
5 Confidence limits within a column followed by the same letter overlap each other.

Table 17. Mean survival, emergence and total development time for resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae combined over all cultivars (experiment 2).

CPB STRAIN	SURVIVAL	EMERGENCE[1]	TOTAL DEV. TIME[1]
LI	67	46 b	18.86 b
JP	73	84 a	18.78 b
CR	71	52 b	21.31 a
VE	70 n.s.	72 a	20.58 ab

1 Means within a column followed by the same letter are not significantly different from each other (total development time: SNK test, df=1, F=9.98, p<.0026)

Table 18. Mean weights of insecticide-resistant (JP and LI) and susceptible (CR and VE) Colorado Potato Beetle larvae on four solanceous hosts (experiment 3).

CPB STRAIN	HOST	LARVAL WEIGHTS (mg) DAY 0	DAY 1	DAY 2	DAY 3	DAY 5	DAY 6	DAY 7
LI	POTATO	.72	1.87	5.37	10.99	29.56	56.72	73.44
	CHACOENSE	.62	1.32	4.14	6.25	8.86	15.08	35.49
	EGGPLANT	.98	1.91	5.77	9.47	27.93	54.58	71.03
	TOMATO	.78	1.21	1.65	2.15	1.10	-1	-1
JP	POTATO	.85	2.61	5.80	10.34	23.20	44.80	74.55
	CHACOENSE	.84	1.80	3.13	4.60	8.01	13.40	20.59
	EGGPLANT	.80	2.48	5.84	10.05	22.50	39.54	64.24
	TOMATO	1.00	1.07	1.77	2.06	4.27	4.92	18.60
CR	POTATO	.87	2.17	4.94	10.48	44.97	103.5	108.09
	CHACOENSE	1.01	3.43	3.13	7.58	20.14	57.76	61.44
	EGGPLANT	.98	4.42	4.39	10.02	42.20	63.43	-1
	TOMATO	.91	2.21	1.85	6.62	7.70	11.67	19.24
VE	POTATO	1.20	2.15	4.11	10.13	22.71	44.72	68.52
	CHACOENSE	1.11	1.68	2.21	6.47	12.37	22.82	32.68
	EGGPLANT	1.06	1.91	3.67	5.24	12.09	21.70	21.70
	TOMATO	.97	1.14	1.53	2.56	5.17	9.88	12.82

[1] larvae died on tomato.

DF		F	F	F	F	F	F
3	strains	9.6	44.8	7.5	3.5	17.2	27.9
	p<	(.0001)	(.0001)	(.0001)	(.03)	(.0001)	(.0001)
3	hosts		28.6	34.6	28.6	43.3	52.5
	p<		(.0001)	(.0001)	(.0001)	(.0001)	(.0001)
9	strains*hosts		7.0			4.8	4.5
	p<		(.0001)			(.0002)	(.0005)

6. CR larvae weighed significantly more than LI larvae, which weighed significantly more than JP and VE larvae on day 6 (Table 19).

Growth rates within a strain were not generally significantly different between potato, eggplant, or S. chacoense (Table 20). The susceptible strains grew faster on tomato than LI larvae.

Percent adult emergence was highest on potato and S. chacoense, and poorest on eggplant and tomato. Mean survival was not significantly different between strains, but host plants and interaction effects were highly significant (Table 21). Larval survival was also highest on potato and S. chacoense, and generally much lower on eggplant and tomato. Mean adult emergence was not significantly different between resistant and susceptible larvae. All strains developed fastest on potato and slowest on tomato, with significant strain and host effects (Table 21). Resistant larvae did not develop significantly faster than susceptible larvae. CR and LI larvae developed significantly faster than JP and VE larvae although the length of pupation was not significantly different between any strains.

In experiment 4, except for day 8, the weights of CR larvae were similar to those of JP larvae (Table 22). There were significant differences between CPB strains on day 0, between strains, hosts, and interaction effects on days 2,4, and 6, significant differences between strains, hosts and interaction effects on day 6 (Table 22). Resistant larvae did not weigh more than susceptible larvae (Table 23). LI larvae weighed significantly more than the other strains, and VE larvae weighed significantly more than JP.

LI and VE larvae grew significantly faster on potato than JP and CR (Table 24). CR and LI larvae grew significantly faster on potato than on S. chacoense, eggplant and tomato. The resistant strains grew significantly

Table 19. Day 6 mean weights of resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae combined over host plant (experiment 3).

CPB STRAIN	LARVAL WEIGHTS (mg)[1]
LI	35.63 b
JP	27.54 c
CR	45.28 a
VE	26.55 c

[1] Means within a column followed by the same letter are not significantly different from each other (SNK test, df=1, F=12.23, p<0.0006).

Table 20. Instantaneous growth rates, survival, emergence and total development times for resistant (JP and LI) and susceptible (CR and VE) Colorado potato beetle larvae on four solanaceous hosts (experiment 3).

CPB STRAIN	HOST	GROWTH RATES[1] (mg/mg/day)	95% CL	% LARVAL SURVIVAL[2]	% ADULT EMERGENCE[3]	TOTAL DEV. TIME[4] (days ± SE)
LI	POTATO	.89 a	.80-.98	77	67	19.0 ± .3
	CHACOENSE	.74 ab	.63-.84	73	57	20.5 ± 1.1
	EGGPLANT	.91 a	.81-1.00	77	60	20.3 ± .5
	TOMATO	.19 g	.09-.28	10	0	-
JP	POTATO	.64 c	.58-.69	60	52	20.5 ± .4
	CHACOENSE	.40 e	.34-.45	77	67	21.2 ± .4
	EGGPLANT	.67 b	.61-.73	32	20	22.0 ± .5
	TOMATO	.26 fg	.19-.33	32	10	25.7 ± .6
CR	POTATO	.79 ab	.74-.84	57	43	18.2 ± .3
	CHACOENSE	.61 cd	.55-.66	72	63	19.8 ± .4
	EGGPLANT	.69 b	.63-.76	17	10	18.0 ± .8
	TOMATO	.45 de	.35-.55	68	38	22.3 ± .7
VE	POTATO	.58 cd	.54-.63	67	57	19.6 ± .3
	CHACOENSE	.48 de	.41-.55	67	53	19.1 ± .5
	EGGPLANT	.47 de	.40-.55	40	23	20.4 ± .4
	TOMATO	.36 ef	.29-.43	50	33	22.7 ± 1.1

[1] Confidence limits followed by the same letter overlap each other.
[2] Larvae surviving from hatching up to prepupation.
[3] Number of adults emerging vs. number entering the soil for pupation.
[4] Days from hatching to adult emergence.

Table 21. Survival, emergence, length of pupation and total development time for resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae combined over host plant (experiment 3).

CPB STRAIN	MEAN SURVIVAL[1]	MEAN EMERGENCE	LENGTH OF PUPATION	TOTAL DEV. TIME
	58	46	11.7	19.8
JP	50	37	12.9	21.9
CR	62	38	13.1	20.0
VE	56 ns	42 ns	11.9 ns	20.8 ns

1 Means within a column followed by the same letter are not significantly different from each other (SNK test; df = 3, F = 3.92, p < .01; F = 13.92, p < .0001).

DF		F	F
3	strain		3.92
	p<		(.01)
3	hosts	11.96	13.92
	p<	(.0001)	(.0001)
9	strains*hosts	4.35	
	p<	(.0001)	

Table 22. Mean weights of resistant (JP and LI) and susceptible (CR and VE) Colorado potato beetle larvae on four solanaceous hosts (experiment 4).

CPB		LARVAL WEIGHTS (mg)			
STRAIN	HOST	DAY 0	DAY 2	DAY 4	DAY 6
LI	POTATO	.42	2.82	15.06	49.18
	CHACOENSE	.40	3.71	11.71	34.71
	EGGPLANT	.44	2.07	7.31	23.88
	TOMATO	.44	.60	.52	-1
JP	POTATO	.57	2.88	16.68	38.70
	CHACOENSE	.58	2.70	12.61	27.69
	EGGPLANT	.56	2.09	6.36	15.35
	TOMATO	.56	2.01	4.14	10.06
CR	POTATO	.53	2.68	10.51	32.93
	CHACOENSE	.53	2.84	9.73	24.50
	EGGPLANT	.48	1.77	4.95	24.30
	TOMATO	.52	1.73	3.79	22.15
VE	POTATO	.50	2.45	12.70	48.95
	CHACOENSE	.76	2.9	7.33	26.98
	EGGPLANT	.53	2.10	5.00	16.80
	TOMATO	.51	1.64	3.80	24.00

DF		F	F	F	F
	strains	21.1	4.4	13.1	9.86
	p<	(.0001)	(.0045)	(.0001)	(.0001)
3	hosts		46.3	147.8	38.95
	p<		(.0001)	(.0001)	(.0001)
9	strains*hosts		5.4	4.82	2.92
	p<		(.0001)	(.0001)	(.0038)

Table 23. Day 6 mean weights of resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae combined over host plant (experiment 4).

CPB STRAIN	LARVAL WEIGHT(mg)[1]
LI	36.29 a
JP	23.79 c
CR	25.86 bc
VE	30.11 b

1 Means within a column followed by the same letter are not significantly different from each other (SNK test, $p<.05$).

Table 24. Instantaneous growth rates, survival rates emergence and total development times for resistant (JP and LI) and susceptible (CR and VE) Colorado potato beetle larvae on four solanaceous species (experiment 4).

CPB STRAIN	HOST	GROWTH RATES (mg/mg/day)	95% CL	% LARVAL SURVIVAL[1]	% ADULT EMERGENCE[2]	TOTAL DEV. TIME[3] (days ± SE)
LI	POTATO	.80 a	.77-.83	87	74	21.7 ± .3
	CHACOENSE	.74 bcd	.71-.77	87	36	22.5 ± .4
	EGGPLANT	.64 efg	.59-.69	73	53	32.5 ± 2.0
	TOMATO	.10 j	.02-.18	-	-	-
JP	POTATO	.70 cde	.67-.74	100	77	19.5 ± .2
	CHACOENSE	.65 ef	.62-.69	80	34	20.5 ± 3.3
	EGGPLANT	.53 hi	.49-.57	83	53	20.4 ± .2
	TOMATO	.41 i	.36-.47	80	17	24.4 ± .7
CR	POTATO	.69 de	.67-.72	77	50	22.7 ± .4
	CHACOENSE	.65 ef	.62-.67	77	20	32.5 ± 2.0
	EGGPLANT	.59 fgh	.55-.64	87	16	25.3 ± 3.9
	TOMATO	.57 fgh	.50-.64	63	25	22.9 ± 1.1
VE	POTATO	.78 ab	.75-.81	92	68	23.7 ± .9
	CHACOENSE	.64 efg	.60-.68	80	12	28.0 ± 3.0
	EGGPLANT	.54 ghi	.49-.60	72	19	26.0 ± 1.1
	TOMATO	.50 hi	.42-.58	80	12	22.0 ± .9

[1] Larvae surviving from hatching up to prepupation.

[2] Number of adults emerging vs. number entering the soil for pupation.

[3] Days from hatching to adult emergence.

[4] Confidence limits followed by the same letter overlap each other.

faster on S. chacoense than on eggplant or tomato. Within a strain, larvae always grew significantly faster on potato than on S. chacoense (except for JP), eggplant and tomato.

Survival of JP and VE strains was significantly higher than LI, while not significantly different from CR. Larval survival and survival to adulthood on eggplant was fairly high for resistant strains, but poor for susceptible strains (Table 25). Larval survival on S. chacoense was also high, but survival to adulthood was poor. Adult emergence for all strains was generally quite high on potato and low on S. chacoense (Table 24). There were significant differences between CPB strains and hosts for mean emergence. Resistant strains had significantly higher mean emergence than susceptible strains (Table 25). Resistant strains developed slowest on tomato, and susceptible strains developed slowest on S. chacoense and eggplant (Table 24). Resistant strains developed significantly faster than the susceptible strains. The susceptible strains developed slower on S. chacoense than on potato, while the resistant strains developed as fast on S. chacoense as on potato. CR larvae had similar growth rates and development times as LI, but CR consumed more than LI larvae. Although a susceptible strain, CR may perform so well because it may have become preadapted to feeding on more than one host (see Materials and Methods).

In experiment 3, JP larvae grew significantly slower than LI and CR. JP did not differ significantly in length of pupation or total development time from LI. CR had the highest growth rates and the shortest development time.

In experiment 4, JP larvae grew slower than LI but were not significantly different in length of pupation or total development time. Unlike experiment 3, CR larvae in experiment 4 grew slower than LI, and no longer significantly faster than JP. This decrease in the growth rates could be

Table 25. Mean survival, emergence, length of pupation and total development time for resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae combined over host plant (experiment 4).

CPB STRAIN	MEAN SURVIVAL[1]	MEAN EMERGENCE	LENGTH OF PUPATION[1]	TOTAL DEV. TIME[1]
LI	88 a	40 a	12.3 b	22.3 b
JP	86 a	46 a	11.7 b	20.7 b
CR	76 ab	28 b	17.0 a	25.9 a
VE	82 a	28 b	15.6 a	24.6 a

DF		F	F	F
1	res. vs. susc.	26.79	20.36	24.95
	p<	(.0001)	(.0001)	(.0001)

[1] Means within a column followed by the same letter are not significantly different from each other (SNK test, p<.05).

caused by the introduction of insecticide resistance, since consumption rates, mean survival, emergence and total development times now resembled those of the VE strain. The sudden development of insecticide-resistance in CR could be energetically more costly, lowering growth rates, etc. The CR strain was apparently contaminated by JP between experiments 3 and 4; LD_{50}s were similar and both strains were resistant to the same chemicals.

Insecticide-resistance on Long Island has been present for >25 yrs. Resistance in the LI strain is extremely stable (Ioannidis pers. comm.). The cost associated with such resistance may be negligible. JP's resistance is newer than LI's, and not as stable (Ioannidis, pers. comm.). LI has primarily elevated MFO levels as a resistance mechanism, and JP has two types of elevated MFO's (permethrin specific and nonspecific), and an altered acetylcholinesterase (Ioannidis 1990). The difference was probably because resistance in the LI strain is older than resistance in the JP strain.

Leaf Moisture Content. In experiment 1, there was very little difference in leaf moisture content between cultivars except on day 0 (Table 26). On day 0 'Conestoga' had significantly higher moisture content than 'Superior', and both were significantly higher than 'Onaway'.

In experiment 2, there was also little difference between cultivars (Table 27). 'Atlantic' consistently had the highest moisture content. On days 3, 5, and 6, Russet Burbank had significantly lower moisture content than 'Atlantic'. On days 5 and 6, 'Superior' also had significantly lower moisture content that 'Atlantic'.

In experiment 3, S. chacoense and tomato were consistently low in moisture content. Potato generally had the highest moisture content, and larvae grew fastest on it (Table 28).

Table 26. Leaf moisture content in Superior, Conestoga, and Onaway potato cultivars on each day of the experiment (experiment 1).

	% LEAF MOISTURE ± SE		
CULTIVAR	DAY 0	DAY 3	DAY 6
SUPERIOR	84.0 ± .35 b	88.2 ± .32	86.3 ± .68
CONESTOGA	88.9 ± .58 a	88.4 ± .17	88.6 ± .54
ONAWAY	80.5 ± .56 c	88.7 ± .13 ns	87.9 ± .62 ns

[1] Means followed by the same letter are not significantly different from each other (SNK test, $p<.05$).

Table 27. Leaf moisture content of 'Superior', 'Russet Burbank' and 'Atlantic' potato cultivars on each day of the experiment (experiment 2).

	% MOISTURE CONTENT ± SE						
CULTIVAR	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
SUPERIOR	88.7 ± .2	88.7 ± .5	89.1 ± .4	89.5 ± .2ab	88.9 ± .3	90.3 ± .3a	91.2 ± .3 b
RUSSET	87.8 ± .3	89. ± .4	88.8 ± .3	88.5 ± .3b	89.3 ± .3	88.2 ± .6b	89.8 ± .4 a
ATLANTIC	90.3 ± .3 ns	89.6 ± .4 ns	89.6 ± .2 ns	90.9 ± .3 a	89.4 ± .3 ns	90.5 ± .2 a	91.4 ± .3 b

[1] Means followed by the same letter are not significantly different from each other (SNK test, $p<.05$).

Table 28. Leaf moisture content of each host on each day of the experiment (experiment 3).

	% MOISTURE CONTENT ± SE[1]					
HOST	DAY 0	DAY 1	DAY 2	DAY 3	DAY 5	DAY 6
POTATO	88.3 + .6 a	83.8 + .6 a	90.1 + .7 a	88.8 +1.1 a	84.0 + .5 a	82.8 + 1.4 a
CHACOENSE	82.0 + .5 b	83.7 +1.1 a	82.7 +1.7 c	83.3 + .6 b	79.5 + 1.6 b	78.5 + .6 a
EGGPLANT	87.4 + .5 a	84.3 + .4 a	86.7 +1.3 ab	82.5 + .7 b	85.3 + .7 a	81.7 + .9 b
TOMATO	85.8 + .7 ab	81.5 + .3 b	82.3 + .4 c	82.3 +1.0 b	81.2 + 1.5 ab	81.7 + 1.5 a

[1] Means within a column followed by the same letter are not significantly different from each other (SNK test, $p < .05$).

[illegible]

In experiment 4, potato and S. chacoense were consistently the highest in moisture content (Table 29). Eggplant was consistently lower than potato and S. chacoense, but not always lower than tomato. Lower moisture content may be enough to reduce larval growth.

Glycoalkaloid Concentrations. Glycoalkaloid concentration was a significant factor between potato cultivars ($df = 4$, $F = 9.12$, $p<.0001$). 'Atlantic' had significantly higher glycoalkaloid concentrations than 'Superior' and 'Russet Burbank' (Figure 1). 'Onaway' and 'Conestoga' also had significantly higher concentrations than 'Russet Burbank'.

In experiment 1, glycoalkaloid concentration had no relationship to growth rates of either resistant or susceptible larvae. Susceptible larvae consumed more on day 0 as the glycoalkaloid concentration increased. Consumption rates of resistant larvae remained constant despite increases in glycoalkaloid concentration.

In experiment 2, larval growth rates decreased as the glycoalkaloid concentration increased. CR larvae grew slower on 'Russet Burbank' than did the other three strains, although on 'Superior' and 'Atlantic', CR was only lower than LI. A similar trend could be seen with the consumption rates. As the glycoalkaloid concentration increased, consumption rates decreased more rapidly than the growth rates.

Chlorogenic Acid Concentration. There were significant differences between potato cultivars in chlorogenic acid concentrations ($df = 4$, $F = 4.26$, $p<.046$). 'Conestoga' and 'Russet Burbank' had significantly higher chlorogenic acid levels than 'Superior' and 'Atlantic', and 'Onaway' had the highest levels

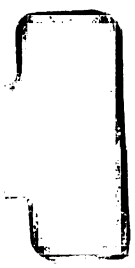

Table 29. Leaf moisture content of each solanaceous host on each day of the experiment (experiment 4).

	% MOISTURE CONTENT ± SE			
CULTIVAR	DAY 0	DAY 2	DAY 4	DAY 6
POTATO	89.3 + .32 a	88.8 + .24 b	89.9 + .27 a	90.0 + .38 a
CHACOENSE	89.9 + .19 a	89.7 + .44 a	90.9 + .29 a	90.8 + .37 a
EGGPLANT	83.2 + .37 c	83.9 + .49 d	84.3 + .78 b	84.4 + .76 b
TOMATO	87.0 + .32 b	86.9 + .29 c	86.5 + .21 c	86.5 + .55 b

[1] Means within a column followed by the same letter are not significantly different from each other (SNK test, $p < .05$).

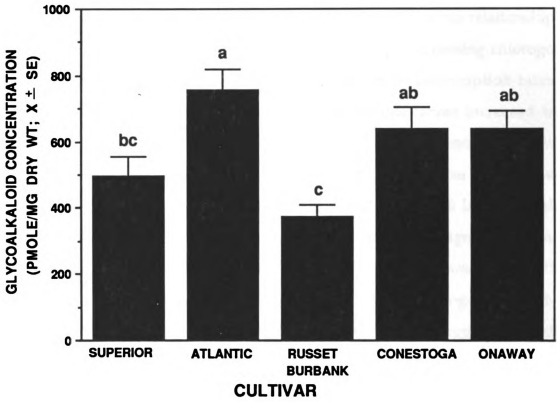

Figure 1. Concentration of glycoalkaloids (pmole/mg dry wt) in five potato cultivars.

(Figure 2). Chlorogenic acid is known to be a feeding stimulant (Hsiao 1982), but may interfere with growth at high concentrations.

In experiment 1, chlorogenic acid concentrations had no relationship to growth rates of either resistant or susceptible larvae. Increasing chlorogenic acid concentrations had no significant relationship to consumption rates of resistant larvae, but consumption rates of susceptible larvae increased with increasing chlorogenic acid levels. 'Onaway' had high concentrations of both glycoalkaloids and chlorogenic acid, and susceptible larvae fed 'Onaway' consumed the most, but weighed less than other larvae. VE larvae weighed significantly less than JP larvae, and VE larvae consumed significantly more on 'Onaway' than on 'Superior' or 'Conestoga'. ('Superior' was intermediate in glycoalkaloids and low in chlorogenic acid, and 'Conestoga' was high in glycoalkaloids and intermediate in chlorogenic acid). This suggests a possible interaction effect by high concentrations of both glycoalkaloids and chlorogenic acid. When the concentration of only one is high, it might not reduce feeding and growth. VE larvae might also have difficulties metabolizing allelochemicals. However, VE larvae on 'Onaway' consumed significantly more than on 'Superior' and 'Conestoga'. This suggests either an allelochemical effect or a nutrient deficiency, reducing growth and raising the consumption rate. VE larvae might also have difficulties metabolizing allelochemicals.

In experiment 2, all larvae grew well on 'Atlantic' despite high clycoalkaloid concentrations (and low chlorogenic acid concentrations), so maybe high concentration of glycoalkaloids alone was not sufficient to retard growth. Since CR larvae on 'Russet Burbank' grew slowly, it probably is deficient in nutrients. 'Russet Burbank' did not have high concentrations of either glycoalkaloids or chlorogenic acid, but it was slightly lower in moisture

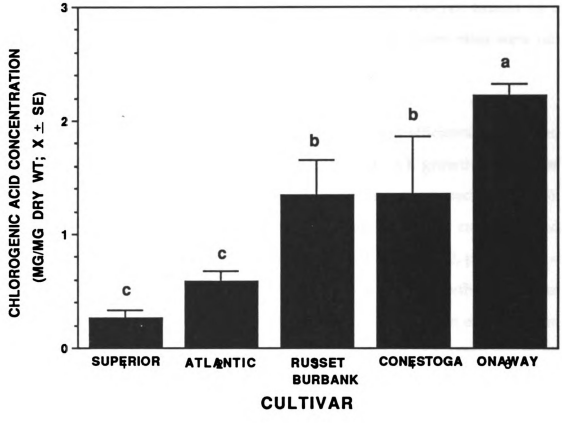

Figure 2. Chlorogenic acid concentration (mg/mg dry wt) in five potato cultivars.

content. CR seemed to have the most variable growth rates, with the lowest and one of the highest. The low growth rate probably was not caused by a repellent or lack of a feeding stimulant since the consumption rates were not significantly different from the other strains.

Larval Growth Efficiencies. In experiment 1, JP growth efficiencies increased slightly as the glycoalkaloid concentration increased. VE growth efficiencies decreased slightly as the glycoalkaloid concentration increased (Figure 3). There were significant differences in efficiencies between cultivars and significant strain x cultivar interactions (ANOVA, df = 2, F = 4.0, $p<.0288$; df = 2, F = 6.14, $p<.0058$). 'Superior' had significantly higher growth efficiencies than the other two cultivars. Susceptible larvae were not more efficient than resistant larvae. Chlorogenic acid concentrations had the same effect on growth efficiencies as glycoalkaloid concentrations (Figure 4).

In experiment 2, glycoalkaloid concentration had no significant effect on larval efficiencies (Figure 5). There were no significant differences between strains, cultivars or interaction effects. Chlorogenic acid concentrations had no significant effect on larval efficiencies, either (Figure 6).

In experiment 3, all strains improved slightly in efficiency on S. chacoense over potato, then decreased on eggplant and tomato (Figure 7). VE was consistently low on all hosts, and the lowest in efficiency except on eggplant. There were significant differences between strains and hosts (df = 3, F = 4.8, $p<.0046$; df = 3, F = 4.3, $p<.0082$). LI and JP larvae were significantly more efficient than VE larvae. Resistant larvae were more efficient than susceptible larvae (df = 1, F = 12.21, $p<.0009$). Larval efficiencies were significantly higher on 'Superior' than on eggplant or tomato.

In experiment 4, all strains except LI and VE were lowest in efficiency on S. chacoense, and improved again on eggplant (Figure 8). There were no significant differences between strains, hosts, or between resistant and susceptible strains.

The differences in overall larval performance between experiments 3 and 4 were probably due primarily to differences between the plants. The eggplants in experiment 3 generally had moisture contents similar to that of potato, whereas in experiment 4, the eggplants were consistently significantly lower in moisture content than potato. The eggplants in experiment 4 were also smaller and thornier than those in experiment 3; experiment 4 was carried out in November, and experiment 3 was done in August. All of these factors may have had some impact on larval efficiencies.

Conclusions

Results indicate a multitude of interactions between insecticide resistance, larval development and plant characteristics. In general, growth rates increased as resistance levels increased (Figure 9). CR larvae were an exception-they grew significantly faster than JP or VE, yet they had the same LD_{50} as VE. The CR strain may be adapted to feeding on more than one host, since it was collected from an area where hosts other than potatoes are often present. Although JP larvae have higher MFO levels than CR, JP larvae grew slower. Since JP's permethrin resistance is newer than LI's, resistance (i.e. MFO induction) could be more costly for JP. LI grew faster than the other strains, (although not significantly higher than CR), and it is also known to have the highest MFO levels (Ahammad-Sahib et al. 1990).

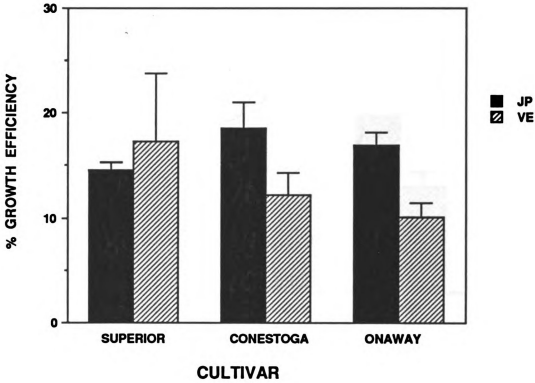

Figure 3. Growth efficiencies of resistant (JP) and susceptible (VE) CPB larvae on each of three potato cultivars arranged according to increasing glycoalkaloid concentration (Superior=498 pmole/mg dry wt; Conestoga=636 pmole/mg dry wt; Onway=639 pmole/mg dry wt) (experiment 1).

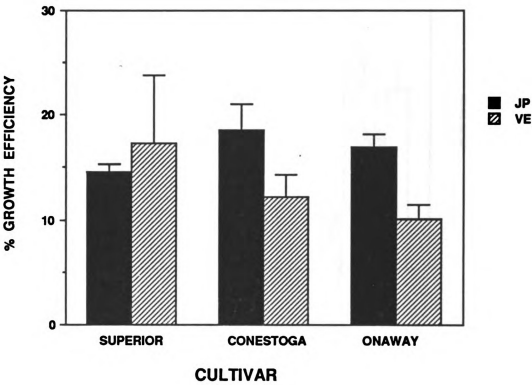

Figure 4. Growth efficiencies of resistant (JP) and susceptible (VE) CPB larvae on three different cultivars arranged according to increasing chlorogenic acid concentrations (Superior=.27 mg/mg dry wt; Conestoga=1.36 mg/mg dry wt; Onaway=2.22 mg/mg dry wt)(experiment 1).

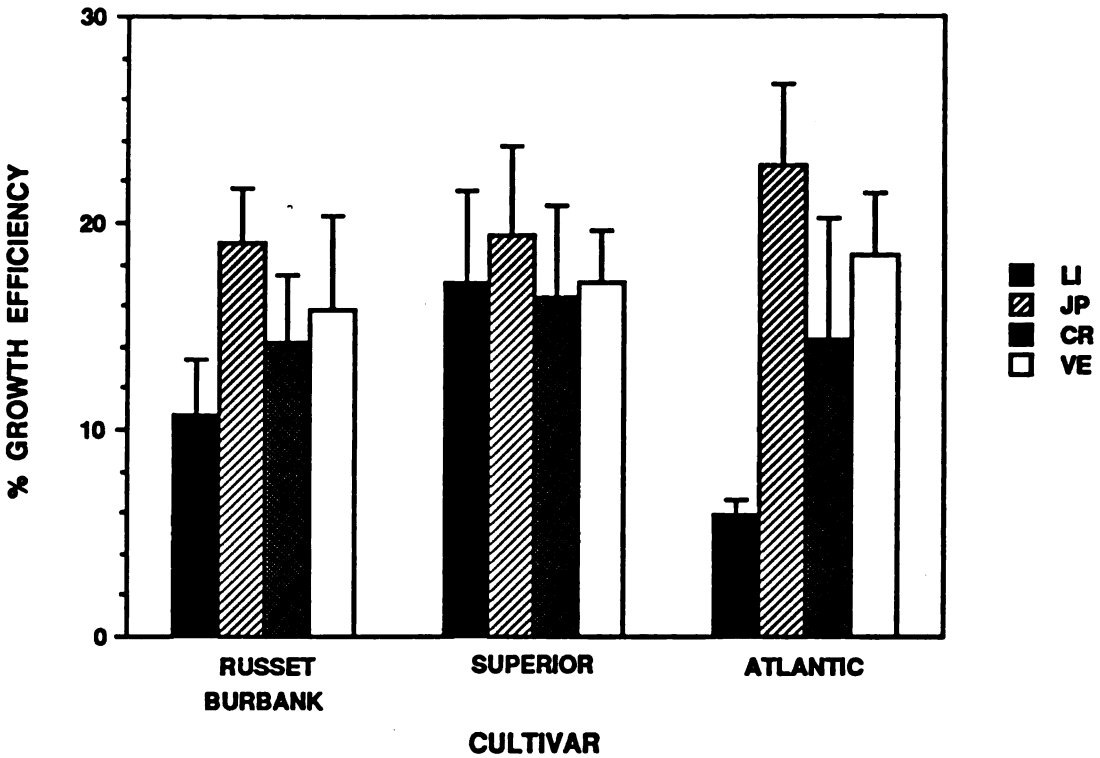

Figure 5. Growth efficiencies of resistant (LI and JP) and susceptible CPB larvae on three different cultivars, arranged according to increasing glycoalkaloid concentration (Russet Burbank=378; Superior=498 pmole/dry wt; Atlantic=756 pmole/mg dry wt)(experiment 2).

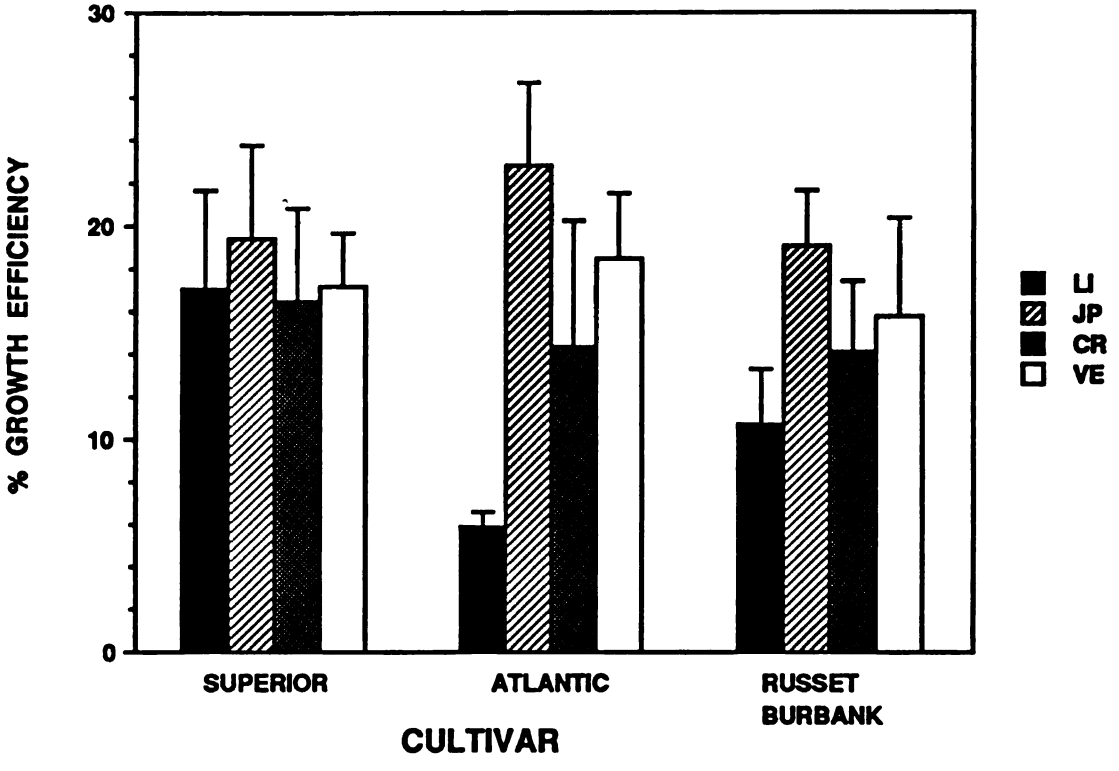

Figure 6. Growth efficiencies of resistant (LI and JP) and susceptible (CR and VE) CPB larvae on three different potato cultivars, arranged according to increasing chlorogenic acid concentration (Superior=.27 mg/mg dry wt; Atlantic=.59 mg/mg dry wt; Russet Burbank=1.35 mg/mg dry wt)(experiment 2).

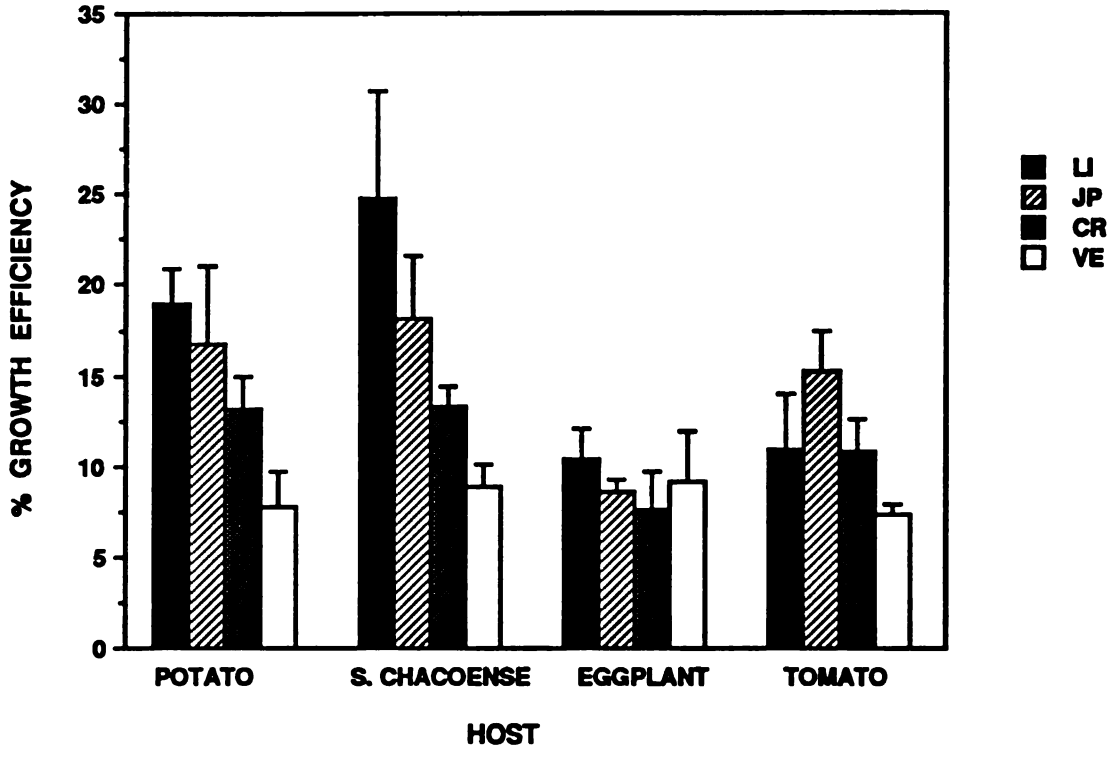

Figure 7. Growth efficiencies of resistant (LI and JP) and susceptible (CR and VE) CPB larvae on four solanaceous species (experiment 3).

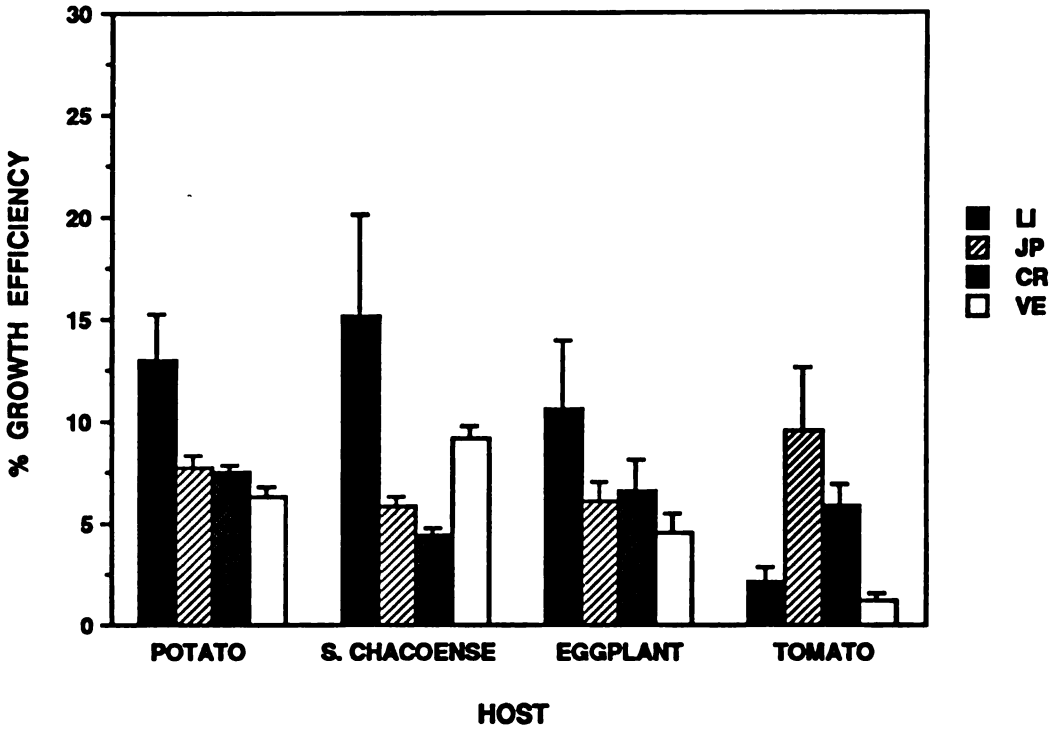

Figure 8. Growth efficiencies of resistant (LI and JP) and susceptible (CR and VE) CPB larvae on four different solanaceous species (experiment 4).

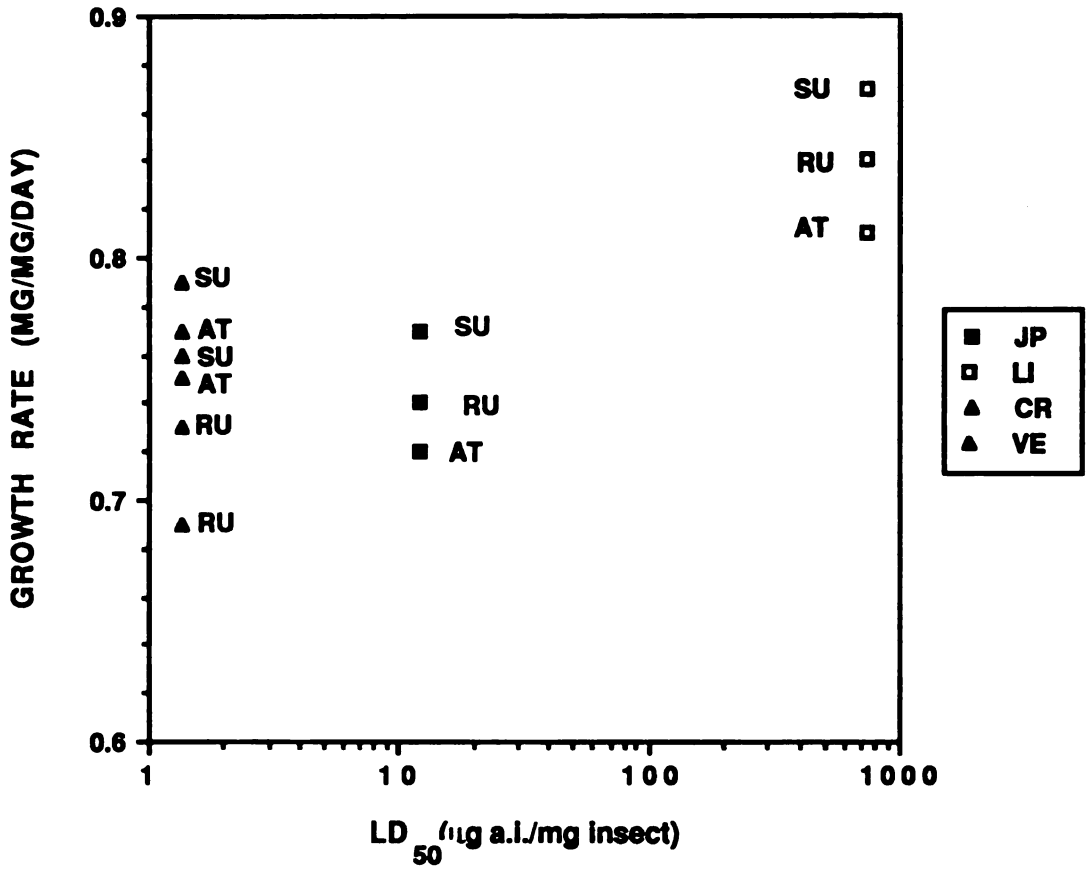

Figure 9. LD_{50} for azinphosmethyl and growth rate for each treatment combination of resistant (LI and JP) and susceptible (CR and VE) Colorado potato beetle larvae on each cultivar (SU=Superior; AT=Atlantic; RU=Russet Burbank).

THESIS

Susceptible CPB strains were affected by cultivars with high concentrations of both glycoalkaloids and chlorogenic acid. Susceptible larvae were not affected by high glycoalkaloid concentration alone. Although no cost effects can be clearly seen here, glycoalkaloid concentrations in these cultivars may not be high enough to adversely affect larval development. A definite interaction between insecticide resistance, plant chemistry and larval development exists.

The contaminated strain may provide an interesting technique for looking for a cost to insecticide resistance. Because of the reduced growth between the two experiments, and its similarity in growth and LD_{50} to the JP strain, there may be a slight energetic cost in the initial development of resistance.

No apparent cost to high MFO levels in LI could be detected. Since insecticide-resistance has been present in New York for >25 yrs, the resistance may be well established and very stable by now, and thus not carry any significant cost. Although the JP strain may have been resistant for ten years or more, it has an altered target site and comparatively new permethrin resistance. Not enough time may have passed for its resistance to become as stable as LI's. Although the JP strain performed as well as the LI strain on the different cultivars, it did not perform as well on different species. The different species may have several factors interacting, in addition to allelochemicals.

Insecticide resistance due to MFO's may not carry much of a metabolic load (Neal 1987), although newly developed resistance could be more costly. The allelochemicals in this study had no effect on the resistant strains. Glycoalkaloids are known to have anticholinesterase activity (Bushway et al. 1987). The JP strain has an altered acetylcholinesterase (Ahammad-Sahib et

al. 1990, Ioannidis 1990), so there may be an interaction between the two. The LI strain has extremely high MFO levels, so glycoalkaloids may be detoxified before they have any effect.

There is no 'representative' resistant or susceptible strain, they are all unique. Each of the four strains tested has its own unique history of exposure to alternate hosts, preferred hosts, breeding pool, and so on. The resistance status or history of each strain will also affect the overall strain performance. The number of chemicals a population has been exposed to and how long, the interval between exposures, the number of resistance mechanisms developed; all these factors will affect the overall performance of a strain on a particular host.

Developing potato varieties with novel types glycoalkaloid types and high glycoalkaloid levels would have no effect on already resistant CPB populations, but they may increase the rate of insecticide resistance development. These new varieties might lengthen the generation time of susceptible populations, allowing natural enemies more time to affect the population. Pest management programs need to consider the interaction between allelochemicals and resistant populations. If allelochemicals do increase the resistance development rate, alternative control strategies such as diversionary crops, crop rotations and biocontrol may be more effective.

Future research should include a large number of resistant and susceptible strains with a range or resistance mechanisms (i.e. a range of MFO levels, different types of altered target sites, etc.). Data on allelochemical and nutrient levels, developmental data, moisture content, CPB strain history and leaf architecture also need to be included. LD_{50}s before and after rearing strains on high and low allelochemical plants need to be taken. Population studies should be done on different strains with different types of resistance

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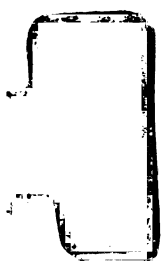
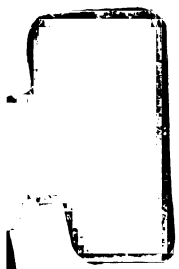


mechanisms and LD_{50}s checked for increases in the rates of resistance development to pesticides.

The future of CPB resistance management looks bleak if it continues on its present course. Many complex factors are involved in the understanding of interactions between insect and plant characteristics, and a better understanding of the interactions involved is needed in order to avoid making the same mistakes with host plant resistance that were made with pesticides.

TMBB:S



APPENDIX 1

Record of Deposition of Voucher Specimens*

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

Voucher No.: 1990-2

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

Growth and Development of Insecticide Resistant and Susceptible Colorado Potato Beetle Larvae (Coleoptera: Chrysomelidae) on Different Solanaceous Hosts*

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

Entomology Museum, Michigan State University (MSU)

*Only three of the four strains used could be deposited as voucher specimens, because the CR strain was destroyed before voucher specimens could be collected.

Investigator's Name(s) (typed)

Patti Lea Rattlingourd

Date: June 1, 1991

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

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum files.
Research project files.

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

APPENDIX 1.1
Voucher Specimen Data
Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Leptinotarso decemlineata Colorado potato beetle Strains: Vestaburg susceptible	MI Montcalm Co. Vestaburg Aug. 1987					8			
(JP) Macomb multiple resistant	MI Macomb Co. near Macomb July 1987					8			
LI azinphosmethyl resistant	Lab Entom. MSU					8			

(Use additional sheets if necessary)

Investigator's Name(s)

Patti Lea Rattlingourd

Date July 1, 1991

Voucher No. 1990-2
Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

F.W. Stehr July 1, 1991
Curator Date

THESIS

Appendix 2:

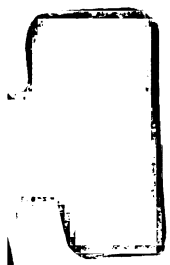
EFFECT OF PIPERONYL BUTOXIDE (PBO) ON THE GROWTH AND DEVELOPMENT OF INSECTICIDE-RESISTANT AND SUSCEPTIBLE CPB LARVAE

The synergist PBO (piperonyl butoxide) is an inhibitor of mixed-function oxidase (MFO) systems in insects. It is used extensively to elucidate the mechanisms of insecticide resistance (Ioannidis 1990). For insects that use elevated MFO levels as a resistance mechanism, PBO intake should reduce growth and possibly consumption rates.

MFO's function as a generalized detoxification system by utilizing molecular oxygen and energy from NADPH to convert lipophilic substrates into more easily excreted polar metabolites. MFOs are involved in the primary metabolism of many plant toxins and endogenous chemicals such as hormones. It is widely assumed that the primary function of MFO's in herbivorous insects is the detoxification of allelochemicals (Neal 1987). MFOs are inducible by both secondary plant compounds and insecticides (Neal 1987, Yu et al. 1979, Yu 1982).

The purpose of this study was to feed PBO to a resistant and a susceptible CPB strain with high MFO levels (from text, exp 1) to test the effect on larval growth, and if it would reduce growth to a similar level as the susceptible strain.

THESIS



Materials and Methods

The insecticide resistant CPB strain JP was collected in Macomb County MI, from a potato field that had been sprayed 10-20 times a year for the past 5-10 years with pyrethroid, organophosphate, carbamate and chlorinated hydrocarbon insecticides. This strain has also been under selection in the lab with permethrin for about a year. High levels of permethrin, azinphosmethyl and carbofuran resistance in the JP strain is due to the presence of elevated MFO levels (permethrin-specific and non-specific) and an altered acetylcholinesterase. A susceptible strain, VE, was collected from volunteer potatoes near Vestaburg, Montcalm County, MI. These two strains were used in a previous experiment with different types of potato cultivars (see experiment 1).

Larvae were fed a range of PBO dipped potato leaflets every day or every other day at .01x, .05x, .1x, 1x, and 2x for VE larvae, and .1x, 1x, 2x, 4x, and 8x the recommended field rate for JP larvae (.3ml PBO/500 ml water). A control group was fed water dipped leaves. Larvae vary in their MFO levels (Ioannidis pers. comm.). Neonate larvae have almost no MFO levels, therefore tests were run on second instars. Five actively moving second instars were randomly placed with a camel hair brush into 50 mm petri plates with filter paper and predipped leaflets. Leaflets taken from the second or third nodes down from the meristem were placed in a petri dish with five second instars. Larvae were reared at 25°C with 80-90% RH. 10-15 larvae (2-3 petri plates) were used at each PBO concentration.

Results and Discussion

JP larvae

PBO daily - 4x and 8x the recommended dose were toxic (Figure 1). Larvae on the other PBO concentrations (.1x, 1x, and 2x) gained weight until day 4, and then gained weight more slowly than the control larvae. Concentrations of PBO of 2x or less have similar effects.

PBO every two days - .05x was added to find a dose with no effect on larvae. There was no apparent effect at .05x on larval weight gain (Figure 2). Larvae continued to increase in weight for 24 hours after receiving PBO dipped leaves, but decreased in weight between 24 and 48 hours after. This is probably due to larvae having to ingest a certain critical amount before any effects can be seen. .1x-4x all had similar effects until day 7, but 8x gained weight very slowly. On day 8, only 2x and 8x showed any decrease in weight from the application of PBO on day 6. These larvae may not have consumed enough leaf tissue to show any effects from the PBO, or maybe they were too large to be affected by concentrations less than 2x any more.

VE larvae

PBO daily - .01x had no effect on larvae until day 7 (Figure 3). The 2x was toxic to larvae on a daily basis, while the .05x, .1x and 1x reduced growth to a very low level.

PBO every two days - .01x had no effect on larval growth (Figure 4). .05x-2x again reduced growth to a low level, although not as low as with PBO every day. The one day lag effect that was seen for the JP larvae receiving PBO every other day was not evident here. The larvae were younger starting out this experiment than the JP larvae were. Perhaps the MFO's were not as induced as they are when larvae are 2 days older, making them easier to inhibit. Or it

could be because this is a susceptible strain, with lower MFO levels to start with.

While not enough numbers to assign statistical significance to, it was enough larvae to see that PBO has an effect. Even low concentrations such as 1x or .1x the recommended field rate might be enough to control a susceptible CPB population without any insecticide. With a resistant population, spraying PBO every few days and reducing pesticide application might be a more effective method for control.

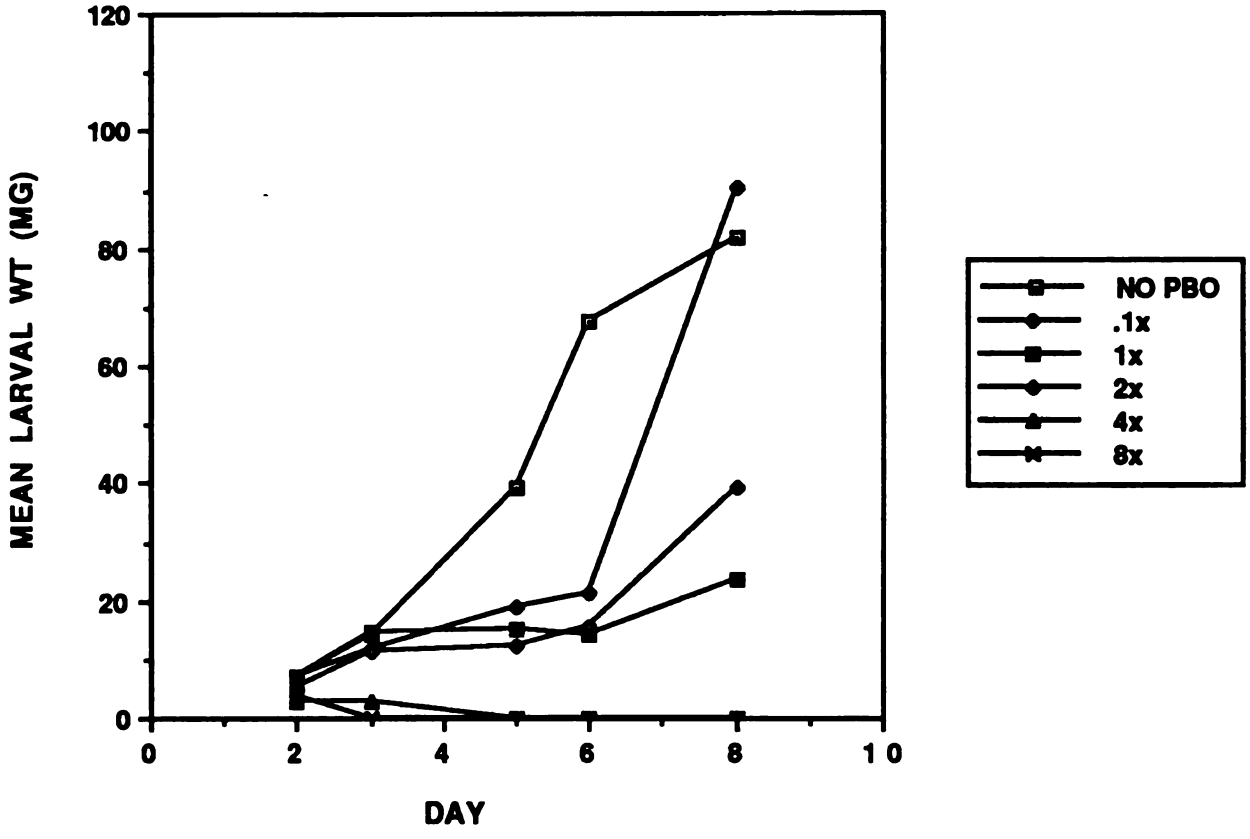


Figure 1. Mean weights of resistant (JP) Colorado potato beetle larvae fed PBO dipped potato leaves daily.

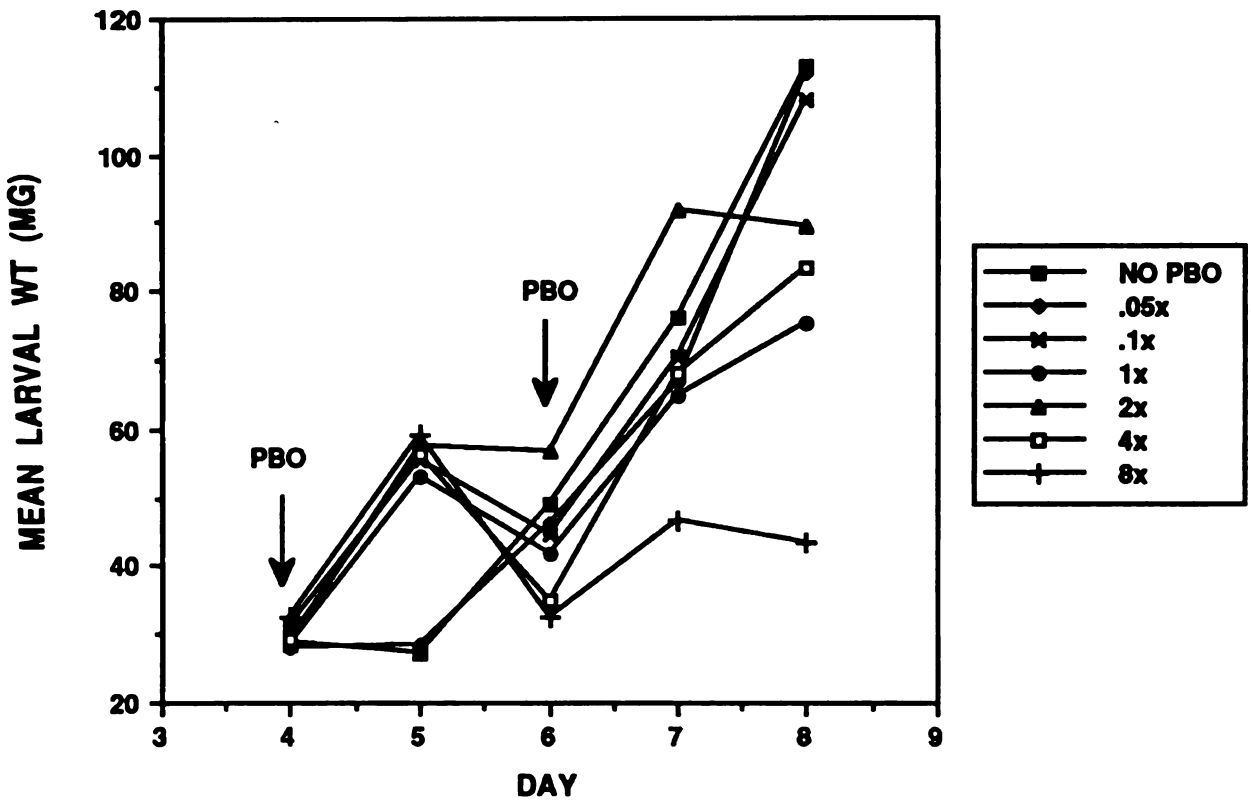


Figure 2. Mean weights of resistant (JP) Colorado potato beetle larvae fed piperonyl butoxide-dipped potato leaflets every other day.

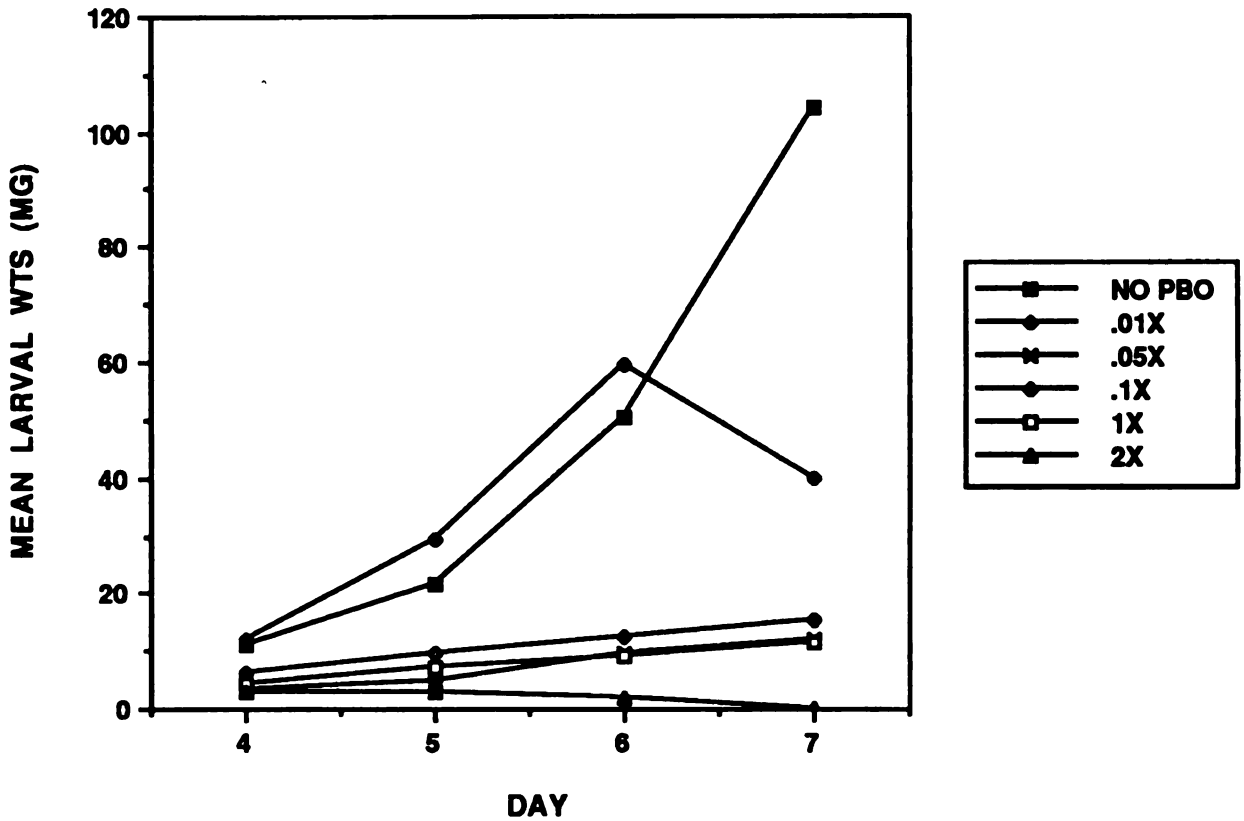


Figure 3. Mean weights of susceptible (VE) Colorado potato beetle larvae fed piperonyl butoxide-dipped potato leaflets daily.

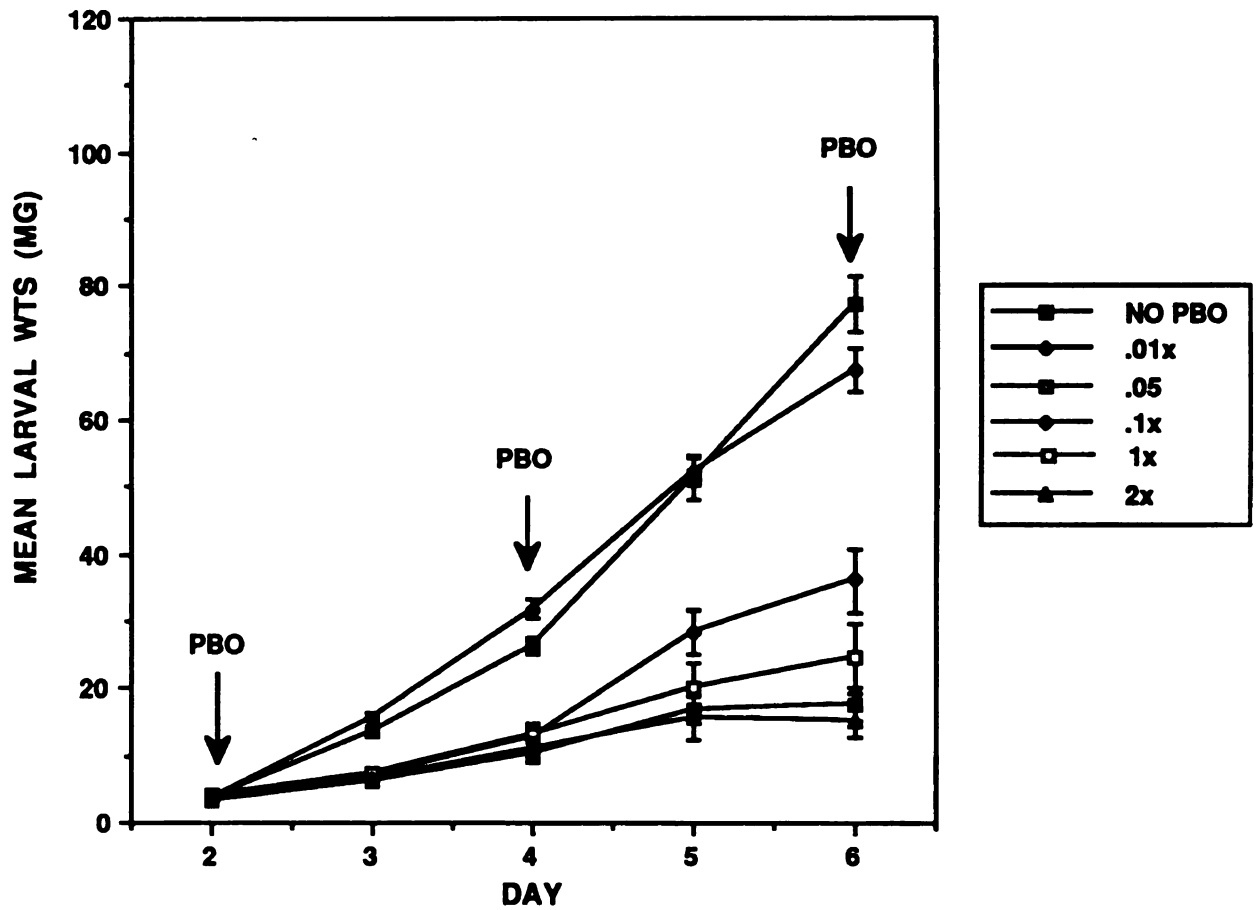


Figure 4. Mean weights of susceptible (VE) Colorado potato beetle larvae fed piperonyl butoxide-dipped potato leaflets every other day.

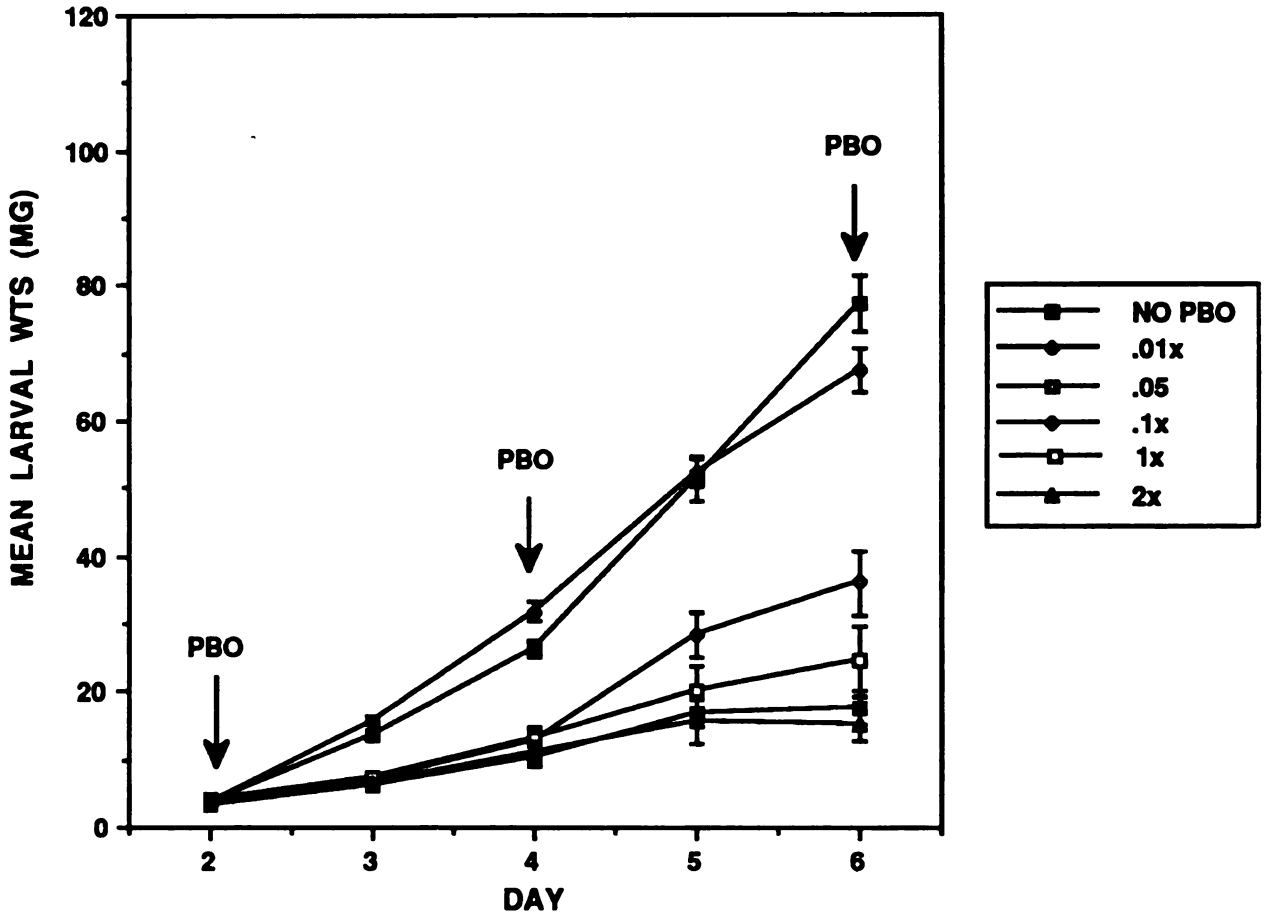


Figure 4. Mean weights of susceptible (VE) Colorado potato beetle larvae fed piperonyl butoxide-dipped potato leaflets every other day.

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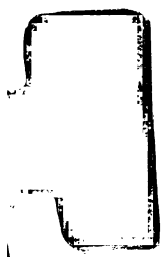

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