POPULATION ECOLOGY OF TRICHOPPLUSIA NI IN GREENHOUSES

AND THE POTENTIAL OF AUTOGRAPHA CALIFORNICA NUCLEOPOLYHEDROVIRUS FOR THEIR CONTROL

by

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Abstract

The cabbage looper *Trichoplusia ni* is the major lepidopteran pest in tomato, cucumber and pepper greenhouses in British Columbia. *T. ni* has developed resistance to *Bacillus thuringiensis (Btk)* in some greenhouses and this compromises the effectiveness of this widely used bioinsecticide for the control of loopers. The production in greenhouses continues almost year round and loopers can be present in the crop very early in the season. Few other biological control options are available for this important pest, so new alternatives that are compatible with integrated pest management programs are required. In this work I explored the overwintering success of cabbage loopers and found that populations could survive winter in greenhouses but not outside. This poses a great risk for continued resistance of loopers to *Btk*. I then evaluated the potential of *Autographa californica* nucleopolyhedrovirus (AcMNPV) in controlling cabbage looper larvae that are resistant to *Btk*. I found that larvae resistant to *Btk* were twice as susceptible to AcMNPV as were those from a non-resistant colony. I also studied if host plants could alter the susceptibility of *T. ni* to AcMNPV and the speed of kill of the virus. I conducted bioassays with susceptible cabbage loopers on the three main crops cultivated in greenhouses as well as on artificial diet. Susceptibility did not vary among the hosts. Despite the fact that larval growth was highest on cucumber and lowest on pepper no biologically meaningful differences were found in speed of kill. These parameters could be further influenced by the behaviour and differential consumption of host plants by larvae. Observations in greenhouses showed that on tomato plants larvae tended to forage in the lower portion of the plants while on peppers they tended toward the top of the plants. This agrees with experimental results that showed larvae tended to go to the top of
pepper plants, the middle of cucumber plants and the bottom of tomato plants. Position was not affected by whether larvae were infected with AcMNPV but the behaviour of larvae could influence the distribution of virus on different crops. I conclude that AcMNPV has much potential as an alternative to Btk and that greenhouse level evaluations should proceed.
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Statement of Co-authorship

Papers from this manuscript have not been submitted yet although some of the chapters will be submitted in the near future.

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Chapter 1

Introduction

The cabbage looper, *Trichoplusia ni (T.ni)* Hübner (Lepidoptera, Noctuidae), is a generalist insect (Sutherland and Greene 1984) and it is the major lepidopteran pest in tomato, cucumber and pepper greenhouses in British Columbia (Manson 2003 and Janmaat 2004). This pest causes damage by larval feeding, mainly on the crop foliage. In tomato greenhouses, when *T. ni* is present at high density, the cabbage looper can damage the fruit. In cucumber greenhouses however, the insects feed on young fruits even when the density is low, making them unmarketable and causing considerable economic loss. The greenhouse industry in British Columbia contributes about 11% of the province’s total agriculture production value, with a farm gate value of $250 million \(^1\) and thus this pest can be of considerable importance.

Greenhouse growers in British Columbia control insect and disease pests mainly with the aid of biological control agents and occasionally with pesticides that have low impact to the environment. Regrettably, there are few other biological options available for the control of cabbage loopers inside greenhouses as alternatives to *Bacillus thuringiensis var kurstaki* (*Btk*). The cabbage looper can cycle monthly in greenhouses throughout the growing season (Janmaat 2004) and females can lay between 300 (McEwen and Hervey 1960) to over 1,000 eggs (Sutherland 1966). Therefore, several applications of *Bt* per year are needed to control the pest. The repeated use of this bioinsecticide had led to the development of resistance in cabbage loopers in greenhouses (Janmaat and Myers, 2003).

\(^{1}\) British Columbia Greenhouse Growers Association’s web page. www.bcgreenhouse.ca
This is aggravated by the almost year round production of vegetables, which is extended by the use of supplemental artificial light in some greenhouses. This long growing season can have a tremendous effect in continuing the resistance to *Btk*. Therefore, there is a need for the development of a new alternative for the control of *T. ni* that is compatible with the integrated pest management program that growers are currently carrying out.

Nucleopolyhedroviruses (NPVs: family Baculoviridae) are pathogens that mainly infect lepidopteran larvae. They are double stranded DNA viruses with enveloped, rod-shaped nucleocapsids incorporated into a protein matrix called the occlusion body (OB) (Granados and Federici 1986). They have a great potential as alternative for pest control (Moscardi 1999, Granados and Federici 1986). They occur in nature (Granados and Federici 1986, Podgwaite 1985), are safe for humans and environment (Granados and Federici 1986, Entwistle 1983), can be transmitted both horizontally (Evans 1986, Andreadis 1987) and vertically (Kukan 1999, Myers et al. 2000, Fuxa et al. 2002), and can co-evolve with the pest (Milks 1996, in review Cory and Myers 2003). These viruses infect the insects following the ingestion of the viral particles (Evans and Entwistle 1987). This means that the insect has to feed on the treated crop to become infected. Therefore, some level of damage has to be tolerated by the growers if they are to be used.

Two nucleopolyhedroviruses that infect the cabbage looper are the singly embedded nucleopolyhedrovirus of *Trichoplusia ni* (TnSNPV) and the multiply embedded nucleopolyhedrovirus of *Autographa californica* (AcMNPV). They vary in their host range, with TnSNPV being specific for cabbage looper and AcMNPV infecting many species in several families of Lepidoptera including *T. ni* (in review Cory and Myers 2003). For this thesis I worked with AcMNPV, the virus with the broader host spectrum.
The main objective of my thesis is to describe the population persistence, dynamics and behaviour of *T. ni* in vegetable greenhouses in British Columbia to explore the potential of AcMNPV as an alternative for the control of cabbage loopers. To accomplish my objective I focused my research to answer the following questions:

1- What is the source of cabbage loopers to greenhouses in British Columbia?

2- What are the population dynamics of *T. ni* inside greenhouses?

3- How effective might AcMNPV be for controlling *T. ni* populations that are resistant to *Btk*?

4- Does the crop type affect the susceptibility of cabbage loopers to the virus?

5- How might the consumption behaviour of larvae in each crop affect the performance of the viral agent?

These questions are addressed throughout the thesis. In Chapter 2, I discuss the population dynamics of *T. ni* inside commercial greenhouses. The main purpose of this chapter is to explore alternative sources to the immigration of cabbage loopers into the greenhouses that better help to explain the early presence of the pest and the appearance of resistance to *Btk* inside greenhouses.

In Chapter 3, I explore the susceptibility of *Btk*-resistant and non-resistant *T. ni* populations to AcMNPV. I conducted laboratory bioassays to test for cross-resistance between AcMNPV and *Btk*.

Chapter 4 includes a series of bioassays performed with AcMNPV on different crops. The main focus of this chapter is to find out if host plant can influence the performance of AcMNPV.
Chapter 5 addresses the differences in growth and behaviour of cabbage loopers when foraging on different crops. This information is valuable as a complement of bioassays and better predicts the potential of the virus in a more realistic scenario as well as the occurrence of horizontal transmission.

Chapter 6 discusses all the results and addresses future work.
1.2. References


Moscardi, F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. Annu. Rev. Entomol. 44:257-289


Chapter 2

The overwinter survival of cabbage loopers as a potential factor in their continued resistance to Btk

2.1 Introduction

The cabbage looper, *Trichoplusia ni*, is a migratory pest of subtropical origin and is currently of major concern in tomato, pepper and cucumber greenhouses in British Columbia where cabbage loopers are the major lepidopteran pest. Some *T. ni* greenhouse populations have developed resistance to *Bacillus thuringiensis* var *kurstaki* (*Btk*), the most widely used bioinsecticide inside greenhouses (Janmaat and Myers 2003). This resistance makes their management an increasing challenge.

The production of greenhouse crops runs almost year round with the exception of a 2–6 week clean-up period in which the greenhouse is unheated and cleaning procedures take place including the use of organophosphate insecticides (Dibrom® (active ingredient: Naled) and DDVP-10FS® (a.i.: Dichlorvos). The source of *T. ni* in greenhouses is uncertain. Roofs of greenhouses are vented and these could allow entrance of moths from the outside. However, cabbage loopers can be found as early as January-February in greenhouses while they normally do not occur outside until May.

Adult cabbage loopers can fly long distances and this accounts for much of the spread of the pest northward into Canada in the spring and early summer, and southward late in the summer and fall (Mitchell and Chalfant 1984). A long distance movement experiment
carried out using black light traps on oil platforms located several kilometers off shore in the Gulf of Mexico found that large numbers of cabbage loopers were able to travel at least 161 km in a single flight (Debolt et al. 1984). In spite of this, mark-recapture experiments showed the maximum distance for moth recaptures to be 14.5 km (Debolt et al. 1984)

Adult flight, however, is not the only means by which cabbage loopers may be able to extend their northerly range each season (Poe and Workman 1984). For example, Lingren et al. (1979) proposed that other forms of dispersal could occur, such as the transportation of immature forms (eggs, larvae, and pupae) with plants from nurseries to agricultural production areas. Survival through the winter might also occur for larvae feeding on ornamental plants located near heated residences or greenhouses. These residual populations could contribute significant numbers of cabbage loopers much earlier in the spring than would dispersal from the south.

The cabbage looper is recorded as feeding on over 160 species of wild and cultivated plants, in 36 families (Sutherland and Greene 1984). The possibility exists for the persistence of cabbage loopers on various plants, such as weeds, in the vicinity of greenhouses (Lingren et al. 1979). For adjacent weeds to serve as refuges, cabbage loopers must be able to overwinter outside the greenhouses.

The northern extent of cabbage looper overwintering is unclear. Sirrine (1894) indicated that the cabbage looper could overwinter in the pupal stage in northern and central United States, but Sutherland (1966) reported that the insect could not overwinter near Long Island, NY. Elsey and Rabb (1970) stated that loopers probably did not often overwinter
in North Carolina. Furthermore, Poe and Workman (1984) claimed that loopers are likely to overwinter in greenhouses in northern areas.

Uncertainty exists about the potential sources of cabbage loopers to vegetable greenhouses in British Columbia other than immigration from the south. The aim of this study was to elucidate those sources. I asked the following questions: 1) can cabbage loopers survive over winter outside the greenhouses on weeds? 2) is *T. ni* able to persist through the unheated period inside greenhouses and survive the organophosphate applications used for the clean-up? and 3) could the reintroduction of moths occur with contaminated seedlings early in the season? The answers to these questions can yield important information that can be implemented as management practices to lessen the impact of this pest in commercial crops. By reducing and delaying the introduction of moths to greenhouses, the continued need for spraying cabbage loopers with *Bacillus thuringiensis* will be reduced and thus also the development of the resistance to *Bt*.

2.1.1 Growing season and greenhouse clean-up

Crops are established in greenhouses at the beginning of the production season with seedlings bought from nurseries.

I monitored plants for cabbage looper larvae weekly through all developmental stages, starting from seedlings with 3-4 true leaves (end of December- early January) until the end of the production season (late November – mid December). Between the end of the growing season and the beginning of the next season a clean-up process takes place.
During this period the greenhouses remain unheated and several sanitation procedures are carried out. This unheated period varies in length among the greenhouses ranging between 2-6 weeks. Although the type and extent of sanitation procedures also varies with each greenhouse, the clean-up generally involves removing all the plants, changing the plastic that covers the soil, replacing sawdust bags, rockwool blocks and strings, applying organophosphate insecticides, washing all irrigation system components, powerwashing walls and structures, and turning off the heating system. Not all of these procedures are necessarily carried out by all the growers and in some cases the activities overlap, which means that part of the greenhouse is being cleaned while the new seedlings are being introduced to the areas already cleaned.

2.2 Materials and methods

2.2.1 Monitoring of cabbage looper populations with pheromone traps

a. Outside greenhouses

To determine if *T. ni* were present outside the greenhouses before being detected inside, pheromone traps (Wing Trap II, containing cabbage looper lures supplied by Phero Tech Inc., Delta, BC. Canada) were placed close to greenhouses with 2 or 3 traps per greenhouse side to cover wind flow coming from all cardinal points. Lures were replaced every three weeks during the spring and summer and every 4 weeks in autumn and winter. Sticky trap inserts were replaced when more than 3 adults were trapped. Male captures were recorded almost every week.
b. Inside greenhouses

Pheromone traps and visual monitoring (explained below) were conducted inside a pepper (*Capsicum annuum* 'Triple 4') and two tomato (grafted *Lycopersicum esculentum* 'Rapshodie') greenhouses in Langley, British Columbia from June 2002 until June 2003. Only a portion (15,000 m²) of the pepper greenhouse was monitored with one pheromone trap/625 m². Two tomato greenhouse units (one of 7,000 m² and the other one of 12,000 m²) located in the same field plot were sampled with one pheromone trap/1000 m². Catches in traps were recorded weekly and the sticky trap inserts were replaced when more than 3 adults were trapped. The traps remained before, during and after the winter clean-up took place. The clean-up in the tomato greenhouses consisted of all the sanitation procedures previously described and the insecticide used was DIBROM®. In the pepper greenhouse only DDVP-10FS® was applied and none of the other sanitation practices except pulling out the crop were carried out.

2.2.2 Visual monitoring of cabbage loopers

a. On weeds outside greenhouses

In August 2002, I observed adult *T. ni* feeding on fireweed (*Epilobium angustifolium*) outside greenhouses. I then began to monitor the wild vegetation around the greenhouses for any developmental stages of cabbage loopers. This visual monitoring was done weekly on weed patches surrounding the greenhouses and near the pheromone traps. Most predominant weeds or turf components were observed. Plant patches were carefully
checked and the total time involved in this visual monitoring was approximately one hour per visit.

b. On crops inside greenhouses

In addition to the pheromone trapping, twenty plants were visually searched for cabbage looper stages every week. Six of those plants were in the rows near the glass walls because I observed a concentration of loopers in that area and this may have been associated with the vicinity of the primary heating pipes. The rest of the plants sampled were arbitrarily picked from other bays in the greenhouse. When the new seedlings were brought in from the nursery for planting, I conducted a more exhaustive sampling; 100 plants were completely checked and one row of plants from each bay was monitored by looking for visible signs of previous looper damage that could have occurred in the nurseries.

2.2.3 Overwintering survival of cabbage looper pupae inside greenhouses

a. Susceptibility of cabbage looper pupae to the organophosphate insecticides DIBROM® and DDVP-10FS®

To test the efficacy of insecticide treatment I placed pupae in the greenhouses and observed their survival after insecticide application. Of the two tomato greenhouses selected for this pupal survival experiment, one was treated with DDVP-10FS® as the clean-up insecticide and the other one with DIBROM®. Insecticide applications were carried out by greenhouse authorized personnel. At the latter greenhouse two DIBROM® applications a week apart, allowed two repetitions of the efficacy of this insecticide. The
product was applied at a rate of 10 ml/100 m$^3$ with spray equipment on to cold heating pipes. Following application, pipes were heated to 41°C for 4 hours. In the case of DDVP-10FS®, the product was fogged at 500 ml/1000 m$^3$ of space using electric fogging equipment.

Pupae used for these experiments were obtained from laboratory culture. Larvae of $T. ni$ were reared on artificial diet until pupation following the mass rearing procedure described by Ignoffo (1963) (see Appendices 1 and 2). Pupae were kept within their silken cocoons. Pupae were placed in muslin bags approximately 8 cm wide x 5 cm in height. Five pupae (~2 day-old) were placed inside each bag and were sealed with staples to prevent the escape of any emerging adults. These bags were placed in the commercial greenhouses the day prior to the insecticide application. The number of bags placed in each greenhouse varied with the experiment and the availability of pupae (see details in Tables 2.3 and 2.4). Bags were labeled with a colour tag from which they were hung on the greenhouse structure or a plant. Coloured tags reminded the greenhouse operators not to remove the bags during the clean-up. Bags were collected 48 hours later. The greenhouses remained closed for 24 hr during the insecticide treatment then they were ventilated for another day without heat. Control pupae were kept at the UBC experimental greenhouse at 20°C ± 2°C and were not exposed to the insecticides.

After collection from the commercial greenhouse, pupae were placed in a controlled environment chamber at 26°C and the emergence of adults was recorded daily. Pupae were left in their cocoons to determine whether insecticide deposits could alter the survivorship of the adults. Emerged adults were caged in the same type cages used for adult rearing (see Appendix 1) to determine egg production of survivors and to test if
reduced fertility occurred following exposure to the insecticides. A 10% sucrose solution was provided as a food source for adults inside the cages and paper towelling was used as an oviposition substrate. This paper towel was changed every other day, and two egg sheets in the peak of the oviposition period were collected to test for egg hatchability. Subsets of ca. 25 eggs were separated and placed inside 170 ml Styrofoam cups and kept at 26°C for 2 days. The number of neonates hatched was recorded. For survivors of DIBROM® a total of 158 eggs in 6 subsets were tested and for DDVP-10FS® survivors 287 eggs were checked in 10 subsets.

b. Survival of cabbage looper pupae to winter temperatures

This experiment was carried out in the same greenhouses as the previous experiment and pupae were reared in the laboratory using the same protocol. Five plastic 21 ml cups with one 2 day-old pupa each were placed in 455 ml paper cups that had a metal screen on one side and were covered with a plastic lid. These cups were distributed inside the greenhouses following the powerwashing and were kept there for the entire period in which the greenhouse remained unheated (2 weeks in one greenhouse and 5 weeks and 1 day in another greenhouse). Cups were collected the day prior to reheating. Pupae with their respective cocoons were kept in the individual plastic cups inside the control environment chamber at 22°C. Adult emergence was recorded.

2.2.4 Overwintering survival of cabbage looper pupae outside greenhouses

During the winter 2002-2003 pupae were placed outside, in the vicinity of the tomato and pepper greenhouses, in Langley. On November 2nd 2002, 25 pupae were collected from
laboratory colonies and cocoons removed. These were placed individually in 29 ml cups in the periphery of the pepper greenhouse both in open sites as well as in some more protected ones (i.e. under tree canopy, close to walls). During the monitoring routine inside the tomato greenhouse, 32 pupae were collected on November 13th 2002 and were kept inside their cocoons with part of the leaf material attached. Those pupae were also placed inside individual plastic 29 ml cups and distributed in the vicinity of the greenhouses to check for survivorship at outside temperatures. These were left outside until May 2003 to determine if they would emerge at the same time that the first adults were trapped in the outside pheromone traps. After that time, non-emerged pupae (all of them in this case) were brought to the laboratory and were kept at 26°C to check for adult emergence. During the winter 2003-2004, 36 pupae were again kept outside the tomato greenhouses in Langley. Pupae from larvae reared in the laboratory were kept with their cocoons. They were placed outside the greenhouses in individual 29 ml cups on December 6th 2003. Pupae were collected on January 10th 2004 and were kept in the laboratory at 22°C until adult emergence.

2.2.5 Overwintering survival of cabbage looper pupae in the laboratory at 9°C

I carried out this experiment to corroborate my previous findings of overwinter survival inside greenhouses. A temperature of 9°C was chosen from the mean average temperature observed inside greenhouses during the winter of 2002-2003 and 2003-2004. A total of 189 pupae of *T. ni* with their silky cocoons were obtained from laboratory cultures and
were individually placed in 28.4 ml plastic cups. From those, 27 pupae were kept as controls and the remaining 162 were labeled in subsets of 27 as 1, 2, 3, 4, 5 or 6 weeks. All pupae were randomly assigned to 4 trays that were maintained in the refrigerator at 9°C. After one week, the 27 pupae designated as 1 week were removed from the trays and exposed to an acclimation period, which consisted of keeping the pupae at 16°C for 3 days, then at 20°C for one day. The following day the temperature was raised to 22°C for one day, and finally pupae were maintained at 26°C until adult emergence. This procedure was repeated every week for each subset for six weeks.

2.3. Statistical Analyses

Statistical analyses were done with JMPin 4.04. (Student Version of SAS Institute) and the tests used are indicated for each specific case in the results section. The level of significance (α-value) in all tests was 0.05.

2.4. Results

*T. ni* were already established outside and inside both greenhouses in June 2002, when the pheromone trapping study started (Fig. 2.1a and 2.1b). For the pepper greenhouse, no cabbage looper males were collected outside from late October (28th) until late April (29th). This pepper greenhouse was located close to a nursery that produced vegetables (tomato, pepper, cucumber and crucifers) as well as ornamental seedlings and plants. Early in the season it is a common practice for the nursery to put the seedlings of crucifer
crops outside to acclimate. The first male moth catches outside coincided with the presence of the cole crop seedlings in the adjacent nursery field.

The tomato greenhouse had no field cole crops in the vicinity, and the main agricultural activities were hay, corn, and berry production. The greenhouse was surrounded by trees, which could interfere with the immigration of cabbage looper moths from outside this plot. Even though the main peak of moth catches for this location was in September and October, possibly associated with a great flux of moths coming from inside the greenhouse, no males were captured in the winter (Mid November until May 29th). The visual monitoring of weeds started late in August 2002 and from September 4th a variety of cabbage looper stages were found on different weed species. All instars, including eggs, were collected and reared on artificial diet in the laboratory until adult emergence for identification. The alfalfa looper, *Autographa californica* is very similar to the cabbage looper at immature stages, but adults from both species are easily distinguishable. All collected individuals were cabbage loopers, and no *A. californica* were found. A summary of all instars recorded and their host weeds is presented in Table 2.1. After November 27th 2002, no loopers were observed on weeds outside. The pepper greenhouse was surrounded by mowed grass that eliminated possible weed refuges for cabbage loopers.

The results of pheromone trapping at the end of the season inside the greenhouses are shown in Table 2.2. *T. ni* captures were higher in the second tomato greenhouse (Tomato 2) than in Tomato 1 and Pepper greenhouses. During the unheated period no looper adults were caught in the greenhouses but several days after the temperature was increased for the new growing season, adults were trapped inside the Tomato 2 and Pepper
greenhouses, indicating that the density of loopers before the clean-up and the quality of the clean-up are potentially influencing the presence of the pest early in the season.

The pepper greenhouse only received a DDVP-10FS® application and even though it had the lowest density of loopers before the clean-up, they were still present at the beginning of the season. This suggests that the treatment was not effective. The fact that no loopers were caught in the traps during the clean-up could indicate that they are able to survive in the pupal stage and that they emerge after heating is reestablished or that adults do not fly at cool temperatures.

The insecticides used in the clean-up procedure were ineffective in reducing the survival of pupae of the cabbage looper (Table 2.3). They did not kill the pupae and they also did not suppress adult fertility. The mean hatchability of eggs laid by moths that survived DIBROM® and DDVP® was 91.5% ± 2.5 and 90.1% ± 1.6 respectively.

Pupal survival declined with an increase in the length of exposure to 9°C in the laboratory (Fig. 2.2). A positive and significant linear relationship was found between the percentage mortality and time of exposure ($r^2 = 0.97$, $F = 186.8$; $df = 5.6$; $p<0.0001$). Moreover, wing abnormality of emerging adults also increased with the length of exposure to cold temperatures but in this case, a negative and significant quadratic relationship was found between the percentage of normal winged adults and the length of exposure ($r^2 = 0.92$, $F = 21.6$; $df = 4.6$; $p = 0.007$). In addition, while survival of the pupae exposed for two weeks to winter temperatures inside greenhouses was similar to controls, 82% vs 90% (Table 2.4, Wilcoxon-Test $\chi^2 = 0.053$; $df = 1$; $p = 0.82$), only 42.5% survived after 5 weeks (Wilcoxon-Test $\chi^2 = 18.8$; $df = 1$; $p<0.0001$) of which 62% were
deformed adults (Table 2.5). Finally, none of the pupae placed outside survived the winter in either 2002-2003 or 2003-2004. Temperature records outside during those winters are given in Table 2.6 and Appendix 3 (Fig. A.3.1 and A.3.2). It is very unlikely that *T. ni* is able to survive outside and to migrate to the greenhouses early in the season.

### 2.5. Discussion

Cabbage looper pupae were unable to survive winter conditions in the vicinity of Vancouver, British Columbia during two consecutive years, even though the winter in 2002-2003 was mild compared to the conditions registered for the winter in 2003-2004. This agrees with the results of Sutherland (1966) and Elsey and Rabb (1970), but differs from those of Sirrine (1894, 1899), Dustan (1932), and Walker and Anderson (1936) who found that *T. ni* overwinters in northern and central United States in the pupal stage. The field season of cole crops started later in 2003 compared to 2002. This delay in planting could explain the lower number of *T. ni* that were trapped adjacent to the pepper greenhouse in June 2003 compared to 2002. That no loopers were trapped outside during the winter and that no pupae survived to adulthood after exposure to outside winter temperatures, strongly suggests that cabbage loopers are not migrating to the greenhouses from outside in British Columbia in early spring. However the efficiency of pheromone lures could be lowered at cold temperatures and may reduce the probability of capturing looper males (Gaston et al. 1971, Sower et al. 1973). Inside greenhouses, however, temperatures can reach 21°C in the daytime during the unheated period (unpublished temperature records from greenhouses).
Moths were not apparently introduced with plants. The visual monitoring of seedlings at the beginning of the season showed no signs of previous damage. The fact that Tomato 1 and Tomato 2 greenhouses received the seedlings from the same propagator, and that Tomato 1 had no loopers until late May while Tomato 2 had loopers present almost immediately after planting, also strongly suggests that loopers were not imported with the seedlings that year but persisted through the clean-up.

The insecticides used during the clean-up procedure are apparently ineffective for controlling the early pupal stage of cabbage loopers. More than 93% of deployed pupae survived the DDVP® application and almost 85% survived the DIBROM® application (Table 2.3). These insecticides did not affect the fecundity and fertility of the adults. While these insecticides may still be effective for other stages of the loopers or other greenhouse pests (Smith et al. 1970), they are not effective against cabbage looper pupae. Henneberry and Kishaba (1967) reported that moths of the cabbage looper held at a constant 10°C were able to live at least 30 days in the presence of food. Therefore adults, if not controlled with an insecticide, could be another source of carry-over in those greenhouses with shorter unheated clean-up periods. Although winter temperatures inside greenhouses after 5 weeks were detrimental to pupal survival, in some cases they were not sufficient to eliminate the pest completely (Table 2.4).

Exposure of pupae to 9°C in the laboratory increased the frequency of adults with abnormal wings. Similar effects were observed in the greenhouses. For the 2 week unheated period inside greenhouses, the percentage of wing deformity of moths was not significantly higher than for controls and was half that of individuals maintained at 9°C
constant in the refrigerator for 2 weeks. After 5 weeks, the percentage of wing deformity of moths emerging from pupae stored inside greenhouses was 62% compared to 100% in the refrigerator experiment. Toba et al. (1973) found increased adult deformity at cold temperatures (7.3°C – 12 °C constant) and Grau and Terriere (1967) observed deformed adults after exposure of pupae to high temperature (30°C). Interestingly, they found that deformity only occurred in larvae reared on artificial diet while those reared on bean plants produced normal adults after pupae were exposed to 30°C. These results indicate that complete extrapolation from laboratory experiments to greenhouses are not necessarily valid. Shorey and Farkas (1973) showed that T. ni males, that had had their wings removed, were capable of finding mates by walking trails on foot. This shows that even deformed adults can be capable of reaching females to mate. However, the success of finding mates will certainly depend on the pest density.

These findings are important for understanding the potential risk that a carry-over from one season to another has in the continued resistance to Bt inside greenhouses. The development of resistance is strongly associated with the number of Bt applications the population has previously received and fitness costs of Bt resistance results in a loss of resistance in about 2-8 generations once the selection pressure is removed (Janmaat and Myers 2003). The more the T. ni population density can be reduced at the end of the season the higher the chances to delay the first Bt application the following season, giving the population a chance to lose the resistance acquired during the previous season. This shows the importance that a good quality clean-up can have in reducing the risk of continuing or developing resistance to this broadly-used biopesticide.
2.6. Conclusions

This study demonstrates that: 1- winter conditions in British Columbia do not prevent *T. ni* from overwintering inside greenhouses, 2- the efficacy of the insecticides used during the clean-up of greenhouses in British Columbia are insufficient to kill the early pupal stage of *T. ni* which can consequently remain inside the greenhouse to the following season, 3- the population density of the cabbage looper and the clean-up quality may be related to the overwintering success, 4- the carry-over from one season to another is one source of cabbage loopers early in the season and it is therefore a threat for continued resistance to *Bacillus thuringiensis*. As a consequence, growers can improve and implement several other tools in their management plan for the control of this pest such as: 1- use other insecticides rather than *Bt* at the beginning of the season when other biological agents have not yet been released into the greenhouse or at the end of the season when the biocontrol agents are not of primary importance. This will lead to a reduction of the population density before the clean-up takes place, 2- intensify clean-up strategies, do not skip any step in the clean-up procedure, 3- keep the unheated period as long as possible, or keep the greenhouse heated for a longer period with no plants before the insecticide application in order to facilitate adult emergence since they are susceptible to the insecticides, 4- use light or pheromone traps at the beginning of the season and monitor the seedlings before planting for the early detection of the pest in order to adopt appropriate measures of control at the right time.

Further investigations could involve studies of the inside overwintering success of cabbage looper populations resistant to *Bt*, the combined effect of organophosphate
insecticides and winter temperatures, as well as more exhaustive research on the susceptibility of different stages of the cabbage loopers to these products.
Figure 2.1a. Pheromone trap catches outside (grey bars) and inside (black bars) a pepper greenhouse in the vicinity of Langley, B.C. in 2002-2003. The months of the year from June 2002 until June 2003 are indicated on the X-axis. The black dotted horizontal bar indicates the months in which no cabbage looper males were collected outside. The black plain horizontal bar shows the period in which the clean-up took place inside the greenhouse. Small arrows indicate when Bt applications were made inside the greenhouse.
Figure 2.1b. Pheromone trap catches outside a tomato (grey bars) and inside two tomato greenhouse units (grey dashed and black bars) in the vicinity of Langley, B.C. in 2002-2003. The months of the year from June 2002 until June 2003 are indicated on the X-axis. Black dashed horizontal bar indicates the months in which no cabbage looper males were collected outside. Black plain horizontal bar shows the period in which the clean-up took place inside the GH. Small arrows indicate the Bt applications made inside GHs.
Table 2.1. Records of cabbage looper found on weeds during visual monitoring in the proximity of the tomato greenhouses (unless indicated) in Langley, B.C.

<table>
<thead>
<tr>
<th>Weed</th>
<th>T. ni Instar(^1)</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fireweed</td>
<td>Adults and eggs</td>
<td>04/09/2002</td>
</tr>
<tr>
<td><em>Epilobium angustifolium</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Clover</td>
<td>Adults, eggs and L2</td>
<td>27/09/2002</td>
</tr>
<tr>
<td><em>Trifolium pratense</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dandelion</td>
<td>Adults</td>
<td>27/09/2002</td>
</tr>
<tr>
<td><em>Taraxacum officinale</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild lettuce</td>
<td>L2 and L5</td>
<td>18/09/2002</td>
</tr>
<tr>
<td><em>Lactuca sp.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plantain</td>
<td>L3 to L5</td>
<td>18/09/2002</td>
</tr>
<tr>
<td><em>Plantago sp.</em></td>
<td>L3, L5 and Pupae</td>
<td>12/11/2002</td>
</tr>
<tr>
<td>Volunteer tomato</td>
<td>L3 to L5</td>
<td>27/09/2002</td>
</tr>
<tr>
<td><em>Lycopersicum esculentum</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) L2= Second Instar Larva; L3=Third Instar Larva; L5=Fifth Instar Larva

\(^2\) Collected in the vicinity of the Pepper greenhouse.
Table 2.2. Number of males / 1000m² captured in pheromone traps inside 3 greenhouses in Langley, B.C. before (heated), during (unheated) and after (heated) clean-up.

<table>
<thead>
<tr>
<th>Greenhouse</th>
<th>Before</th>
<th>During</th>
<th>After</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepper</td>
<td>1.1</td>
<td>0</td>
<td>0.13</td>
<td>0.2</td>
</tr>
<tr>
<td>Tomato 1</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tomato 2</td>
<td>3.3</td>
<td>0</td>
<td>0.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* Male captures obtained 5 and 12 days after seedlings were planted.
Table 2.3. Efficacy of the organophosphate insecticide applications on the *T. ni* pupal stage inside commercial greenhouses. Mean percentage of adults emerged from pupae following treatment. Statistical results from Wilcoxon Test. Same letters indicate treatments that are not statistically different.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Mean % of Adults alive ± (SE)</th>
<th>N**</th>
<th>Wilcoxon test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIBROM®</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C*</td>
<td>83.3 ± (3.33) a</td>
<td>6</td>
<td>χ² = 0.17; p = 0.69</td>
</tr>
<tr>
<td>T*</td>
<td>84.8 ± (2.89) a</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>DDVP®</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C*</td>
<td>95.0 ± (5.00) a</td>
<td>4</td>
<td>χ² = 0.14; p = 0.70</td>
</tr>
<tr>
<td>T*</td>
<td>93.0 ± (2.03) a</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

* T = treated; C = control
** N = Total number of cups with 5 pupae inside
Fig. 2.2. Relationship between *T. ni* pupal mortality and adult deformity and the length of exposure to a constant 9°C temperature in the laboratory.

Week 0 represents the control group that were not exposed to 9°C.

% Mortality = -6 + 2.30 * days; $r^2=0.97$; $F = 186.8$; $df = 5,6$; $p < 0.0001$.

% Normal adults = 59.64-1.98 * days + 0.05 (days-21)$^2$; $r^2 = 0.92$; $F = 21.6$; $df = 4,6$; $p = 0.007$.

\[
r = 0.97 \quad r^2 = 0.92
\]
Table 2.4. Mean percentage of *T. ni* adults emerged from pupae after exposure to winter temperatures inside greenhouses. Statistical results from Wilcoxon Test. Different letters indicate treatments are statistically different.

<table>
<thead>
<tr>
<th>Length of Exposure</th>
<th>Mean % of Adults alive ± (SE)</th>
<th>N**</th>
<th>Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C*</td>
<td>90.0 ± (4.5) a</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>T*</td>
<td>81.7 ± (8.3) a</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>5 Weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C*</td>
<td>90.0 ± (4.1) a</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>T*</td>
<td>42.5 ± (6.6) b</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

* T = treated; C = control

**N = Total number of cups with 5 pupae inside

\[ \chi^2 = 0.05; p = 0.82 \]

\[ \chi^2 = 18.8; p < 0.0001 \]
Table 2.5. Mean percentage of adults emerged with deformed wings after exposure to winter temperatures inside greenhouses. Statistical results from Wilcoxon Test. Different letters indicate treatments are statistically different.

<table>
<thead>
<tr>
<th>Length of Exposure</th>
<th>Mean % of Deformity ± (SE)</th>
<th>N**</th>
<th>Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C* 2 Weeks</td>
<td>22 ± (5.6) a</td>
<td>10</td>
<td>$\chi^2 = 0.39; p =0.53$</td>
</tr>
<tr>
<td>T* 2 Weeks</td>
<td>27 ± (5.1) a</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>5 Weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C* 5 Weeks</td>
<td>33 ± (3.8) a</td>
<td>14</td>
<td>$\chi^2 = 10.8; p = 0.001$</td>
</tr>
<tr>
<td>T* 5 Weeks</td>
<td>62 ± (5.2) b</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

* T = treated; C = control

** N = Total number of cups with 5 pupae inside
Table 2.6. Comparison of temperatures recorded by Environment Canada at Vancouver Airport between Dec 1\textsuperscript{st} and Jan 10\textsuperscript{th} of two consecutive winters (2002/2003 and 2003/2004).

<table>
<thead>
<tr>
<th></th>
<th>Maximum Temperature</th>
<th>Minimum Temperature</th>
<th>Mean minimum Temperature</th>
<th>Mean Average Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter 2002/03</td>
<td>13.5 °C</td>
<td>-2.9 °C</td>
<td>2.67 °C</td>
<td>5.5 °C</td>
</tr>
<tr>
<td>Winter 2003/04</td>
<td>11.9 °C</td>
<td>-12.2 °C</td>
<td>0.37 °C</td>
<td>3.28 °C</td>
</tr>
</tbody>
</table>
2.7 References


Potential of AcMNPV as an alternative for control of cabbage loopers that are resistant to Btk

3.1 Introduction

Resistance to insecticides has a great impact on the control of insect pests. It leads to economic loss, increased environmental risk as a consequence of the use of repeated and high doses, and uncertainty about the performance of new products (Wood 1999). Insect resistance to a specific synthetic or biological insecticide can lead to positive or negative consequences. First, fitness costs associated with resistance might make the pest more susceptible to other causes of mortality and pathogens (Milks and Myers 2003) and this could suppress the maintenance of resistance. Development of cross-resistance to other insecticides with a similar mode of action however could negatively reduce the number of alternatives available for pest resistance management.

The use of Bacillus thuringiensis (Bt) has greatly increased since its commercial release in 1938. It is the most widely used bioinsecticide in organic and integrated pest management production accounting for 90% of the bioinsecticide market (Chattopadhyay et al. 2004). During recent years, its use has been expanded even more with the incorporation of the Bt toxin gene into genetically modified crops. Tabashnik et al. (1990) reported the appearance of resistance to Bt by the diamondback moth, Plutella xylostella
in the field in Hawaii, and Janmaat and Myers (2003) reported the evolution of resistance
to Bt by the cabbage looper, *Trichoplusia ni*, in commercial greenhouses of the Fraser
Valley, B.C. Canada. Other species have been selected for resistance to Bt in the laborator
y(In review Ferre & Van Rie, 2002).

Greenhouse growers in British Columbia deal with high levels of resistance to Bt in
cabbage loopers, the major lepidopteran pest in that system. Therefore, the need is great
to develop new strategies to maintain *T. ni* population levels below economic damage
thresholds. If Bt-based products become ineffective due to resistance, organic farmers
will have lost this valuable resource (McGaughey et al. 1998)

Greenhouses represent an ideal environment for the selection for insecticide resistance
because insect populations can continuously reproduce during most of the year. Thus they
require repeated and higher applications of insecticides. Overwintering of cabbage
loopers in greenhouses, as shown in Chapter 2, makes the management of resistance even
more difficult. These factors appear to contribute to the rapid increase of resistant
individuals.

The aim of "resistance management" is not to stop resistance entirely, but to slow its
development and extend an insecticide's useful lifespan as long as possible (Comins
1977). According to Croft (1990) there are three goals of resistance management:
avoiding resistance when and if possible, delaying resistance as long as possible, and
making resistant populations revert to susceptibility. Management strategies could
involve the use of more than one biocontrol agent in order to reduce the selection
pressure of each agent and to slow or avoid the development of resistance. Studies
conducted by Janmaat and Myers (2003) showed that the resistance of cabbage loopers to *Bacillus thuringiensis* increases with an increase in the number of *Bt* applications. In addition, resistant individuals reverted to susceptibility in several generations when growers stop spraying *Bt*. These results greatly support the idea that using an alternative bioinsecticide to break down the resistance to *Bt* is a viable strategy.

Although the use of baculoviruses seems to fit within the proposed strategy, the goal of this chapter is to test for cross-resistance in *T. ni* between *Bacillus thuringiensis var kurstaki* and a potential alternative bioinsecticide: a nucleopolyhedrovirus of the alfalfa looper *Autographa californica* (AcMNPV). I investigated if individuals resistant to *Bt* are as susceptible to virus as individuals that lack *Bt* resistance. Resistance to one pathogen could possibly increase the susceptibility of *T. ni* to other pathogens. Demonstration of a lack of cross-resistance or even better, increased susceptibility of resistant individuals to AcMNPV would support the recommendation for future development of AcMNPV as a component of a *Bt* resistance management plan.

**Objectives**

1) To determine if cross-resistance between AcMNPV and *Btk* occurs in *T. ni* populations.

2) To determine if the resistance to *Btk* confers a higher susceptibility of *T. ni* to AcMNPV.
3.2 Materials and Methods

Three bioassays were conducted using two *T. ni* colonies: both derived from descendants of insects collected from the Gipanda (GIP) greenhouse in 2001 by Alida Janmaat and collaborators. In the first generation, this colony was found to be extremely resistant to *Bt* compared to the laboratory colony (RC). Subsequently a GIP – resistant population (GIP-R) was established by continuous selection with *Bt* almost every generation (See selection method described in Janmaat 2004 p. 62). A GIP – susceptible population (GIP-S) was established by maintaining the colony without any further selection with *Bt*. This colony became susceptible to *Bt* after several generations. By the time of the experiment, the level of resistance to *Bt* of GIP-R and GIP-S, measured as LC$_{50}$s, was found to be 35,486 IU/ml and 2,180 IU/ml respectively. Further details about the method used to estimate the level of resistance of the GIP colonies are given in Appendix 4.

Bioassays

Susceptible and resistant colonies were reared following the procedure described in Appendix 1. A description of the *Bt* selection protocol is given in Janmaat (2004) page 62. Egg sheets were collected and put out to hatch in 4 L plastic buckets after 2-4 days of collection. After 2 days at 26°C, neonates were transferred to 175 ml cups containing artificial diet (25 neonates/cup), prepared following the recipe shown in Appendix 2. After 5 days, mid to late second instar larvae (L2) were starved in individual 21 ml plastic cups for 3 h and were then randomly assigned to each of the treatments.

$^1$ IU= International Units
The multiple nucleopolyhedrovirus of *Autographa californica* (AcMNPV) was provided by Dr. Martin Erlandson (Saskatoon Research Centre), and originated from samples isolated from infected *T. ni* larvae collected in Fraser Valley greenhouses. The viral concentration in the stock was rechecked by viral occlusion body counting with a Neubauer hemocytometer (see Appendix 5). The final viral doses used for the three replicates were 2.7, 6.6, 13.4, 27 and 67 PIBs/larva. The viral doses were presented to larvae on cucumber (cv. Natika ez) leaf discs. After consuming the leaf disc completely individuals were kept on artificial diet for the rest of the experiment (Forschler et al. 1992; Manson 2003; Cook et al. 2003).

**Procedure**

Viral dilutions of AcMNPV, prepared following the protocol explained in Appendix 6, were removed from the freezer several hours before dosing and thawed at room temperature inside a 455 ml paper cup with lid to avoid the incidence of light and therefore degradation of the viral dilutions.

Cucumber leaves, cv. Natika ez, were collected from plants cultivated at the UBC Horticultural greenhouse. Plants had approximately 6-8 leaves at the time of the experiment. Middle leaves were used for the experiment. Leaves were collected in a tray covered with a damp paper towel on the bottom and a transparent plastic lid to prevent desiccation.

Six trays per colony were labeled to individualize each treatment (Control, D1, D2,...,D5) and each tray was provided with a damp paper towel and 30 pieces of damp
filter paper (Whatman N°1), of 1.8 cm$^2$. Using a cork borer # 2 ($\varnothing = 4$ mm), cucumber leaf discs were cut and placed on top of the filter paper pieces, one leaf disc per piece. Once the dilutions were thawed, 2 µl of viral dilution was added to each leaf disc using a micropipette. In the case of the control treatment, 2 µl of distilled water was added to each leaf disc. The doses were added systematically from lowest to highest to avoid contamination. The drop of virus solution was allowed to dry at room temperature. This required between 3-4 hours. The paper towel was kept moist to avoid leaf water loss. After drying, each leaf disc with its filter paper was transferred into individual plastic 21 ml cups. One starved larva was placed inside each plastic container. Thirty larvae were used for each dose. Larvae were allowed to feed on the leaf disc for 24 h at 26°C in the growth room.

After 24 h, all larvae that finished eating the leaf disc were included in the experiment and were transferred to individual 30 ml cups filled with 2.5 ml of artificial diet and kept at 24°C inside a growth chamber. Mortality was recorded for the first time 48 h after the day of the infection, and after that, twice a day. Survivors were transferred to new diet every 5-7 days, until pupation. Date of death, pupation date, date of adult emergence and pupal weight were recorded.

The experiment was repeated 3 times.
3.3 Statistical Analysis

The analysis of LD$_{50}$ was done using GENSTAT 5 Release 4.1 (1998) by fitting the results to the Probit Analysis outlined by Finney (1971).

Before obtaining the LD$_{50}$ for each of the colonies, data were corrected for natural mortality in the control treatment as necessary (see Table 3.1).

A t-test was conducted for the analysis of pupal weight by sex using JMPin 4.04 (2002).

For the analysis of the time to pupation I performed a Kruskal-Wallis test for non-parametric data using JMPin 4.04 (2002).

Finally, time to death was analyzed with a survival analysis performed with JMPin 4.04 (2002) where Log Rank and Wilcoxon tests were used to look for differences between treatments.

The alpha level was set at 0.05 for all tests unless otherwise specified.

3.4 Results

a. Analysis of Mortality

The first step in the analysis of mortality consisted of correcting for natural mortality that occurred in the control groups. As shown in Table 3.1, the natural mortality in control groups was less than 5% for each colony in repetitions 1 and 2, and therefore, there was no need for mortality correction. However, in repetition 3 mortality exceeded 5% and Abbot’s formula was used to correct those values (Abbot 1925).

After mortality corrections were applied in repetition 3, a Probit Analysis was carried out with Genstat 5 to check for differences among replicates. There were no differences
among replicates for both colonies. For the susceptible colony, the deviance ratio was 1.54 with a \( p = 0.214 \) while for the resistant colony the deviance ratio was 0.68 with a \( p = 0.505 \). Therefore, the results of the three replicates for each colony were combined.

The last step was to look for significant differences between the resistant and the susceptible colonies. Results after statistical analysis showed that there are significant differences between the colonies (deviance ratio: 16.13, \( p < 0.0001 \)). Table 3.2 shows the values of LD\(_{50}\) and their confidence intervals expressed as the number of polyhedra inclusion bodies (PIBs)/larva for each colony.

The confidence intervals derived from Genstat 5 and shown in Table 3.2 do not overlap for the two colonies. The individuals resistant to \( Bt \) are twice as susceptible to AcMNPV as individuals susceptible to \( Bt \).

b. Time to Death

The time to death was analyzed using a survival curve obtained with JMPin for the dose that caused more than 80% of mortality in both colonies (dose 5: 67 PIBs/larva). This non-parametric statistical analysis was chosen because data were not normally distributed. Time to death decreased with increased dose from 9.0 days with dose 1 to 5.3 days with dose 5 for the resistant colony (\( \chi^2 \) Log Rank Resistant = 31.38, df = 4, \( p < 0.0001 \)) and from 9.1 days with dose 1 to 5.0 days with dose 5 for the susceptible colony (\( \chi^2 \) Log Rank Susceptible = 28.9, df = 4, \( p < 0.0001 \)). No differences were detected by dose between colonies (\( \chi^2 \) Log Rank Dose 1 = 0.160, \( p = 0.69 \); \( \chi^2 \) Log Rank Dose 2 = 0.024, \( p = 0.88 \); \( \chi^2 \) Log Rank Dose 3 = 0.016, \( p = 0.97 \); \( \chi^2 \) Log Rank Dose 4 = 1.67, \( p = 0.20 \); \( \chi^2 \) Log Rank Dose 5 = 0.73, \( p = 0.39 \)). The reason for
choosing the dose that killed more than 80% of the individuals is based on potential commercial applications. JMPin performs both a Wilcoxon and a Log-Rank test to detect differences in survivorship between treatments. No differences in time to death were detected between the susceptible and the resistant colonies with $5.0 \pm 0.17$ days and $5.3 \pm 0.22$ days respectively ($\chi^2_{\text{Log Rank}} = 0.73, \ p = 0.39$).

c. Time to Pupation

The time from second instar to the pupal stage for untreated controls is shown in Fig. 3.1. A non-parametric test, Wilcoxon-Kruskal Wallis, was performed with JMPin 4.04 because data were not normally distributed.

Individuals susceptible to Bt reached pupation at $15.8$ days $\pm 0.2$ days while resistant individuals required $20.9$ days $\pm 0.4$ days. These differences are statistically significant ($\chi^2 = 69.48; \ df = 1; \ p < 0.0001$) and suggest a fitness cost associated with resistance.

d. Pupal Weight

A t-test was performed to look for differences in pupal weights. Pupal weights of males and females were analyzed separately and were ln-transformed to make data normal before the t-test analysis. Despite the differences in the time to pupation, the pupal weights of the control groups for the resistant and susceptible colonies were the same (weight-Resistant $\text{females} = 225.4 \pm 5.5$ mg; weight-Susceptible $\text{females} = 227.9 \pm 6.4$ mg; weight-Resistant $\text{males} = 250.1 \pm 7.7$ mg; weight-Susceptible $\text{males} = 254.2 \pm 4.9$ mg). Even though resistant individuals had slightly smaller pupal weights, those differences were not statistically significant ($t_{\text{females}} = -0.202; \ df = 66; \ p \ |t| = 0.8406; \ t_{\text{males}} = -0.467; \ df =$
3.5 Discussion

Both, AcMNPV and Bt are ingestion, non-systemic bioinsecticides. Midgut cells and the peritrophic membrane (PM) may play a key role in the infection process of both biocontrol agents. In spite of these similarities, there are important differences in the in vivo pathway necessary for these agents to kill the host. The lack of cross-resistance can be attributed to differences in the mechanism of infection of Bt and AcMNPV. The experimental results show that the 16X resistance to Bacillus thuringiensis was not associated with an increased resistance of T. ni to AcMNPV but resistant individuals were slightly (2X) more susceptible to the virus. These findings are in accordance with other examples in which cross-resistance was evaluated but the other way around, for populations resistant to viruses and not to Bt. For example Fuxa and Richter (1990) reported that the resistance to the Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV) did not affect the susceptibility of Spodoptera frugiperda to either Bt or the chemical methyl parathion, and Milks and Myers (2003) showed that there was no cross-resistance between the singly embedded nucleopolyhedrovirus of the cabbage looper (TnSNPV) and Bacillus thuringiensis in populations of T. ni resistant to TnSNPV. One possible explanation is that midgut proteases are essential for toxin formation in the Bt in vivo pathway, while in the case of baculoviruses, proteases are responsible for the inactivation of occlusion derived viruses (ODVs). After liberation, ODVs must rapidly pass through the peritrophic membrane to avoid degradation by proteases (Elam et al. 1990). According to Oppert et al. (2000), midgut extracts from Indian meal-moth, Plodia
*interpunctella* resistant to *Bt* had lower proteolytic activity than extracts from susceptible insects and these had a reduced capacity to activate Cry1Ac protoxin. These findings may indicate that if lower proteolytic activity confers resistance to *Bt*, this could reduce the availability of enzymes capable of degrading the ODVs and facilitate their passage through the peritrophic membrane.

Another possible explanation is that the susceptibility of lepidopteran larvae to NPV is influenced by the rate at which the midgut cells are sloughed off (Keddie et al. 1989; Washburn et al. 1995; Washburn et al. 1998; Hoover et al. 2000). Individuals that develop more rapidly may slough off these cells before the virus spreads to other tissues thus reducing infection (Milks and Myers 2002). Originally, the greater resistance of rapidly growing larvae was thought to be related to the increasing weight of the larva (review in Cory and Myers 2003). However, comparisons of oral with intra-hemocoelic inoculation demonstrated that this resistance did not occur when virus was injected but was related to infection through the midgut (Teakle et al. 1986).

I did not record the larval weight of controls at the time of the infection. However, the time to pupation could be used as a measure of developmental rate. Janmaat and Myers (2003) showed that one indicator of reduced fitness of *T. ni* resistant to *Bt* is the slower larval growth of resistant individuals compared to susceptible ones. Although larvae of both colonies were the same age at the time of infection, there were differences in the time to pupation of larvae in the control group (Fig. 3.1) indicating that resistant individuals developed more slowly compared to susceptible individuals. Furthermore, only the larvae that finished eating the leaf disc in 24 h were included in the experiments.
For the susceptible colony the final number of larvae included was 30% higher than for the resistant colony, supporting the possibility that resistant individuals could have been smaller at the moment of dosing. However, the fact that the smaller individuals, that had not finished eating, were not included in the experiment is likely to have compensated for differences in initial larval size. Secondly, to assess the level of resistance to *Bt*, larvae of the same age were used for the experiment. Resistant individuals, even though they may have been smaller, were sixteen times more resistant to *Bt* than the susceptible individuals. If cross-resistance had been present in the bioassays with AcMNPV, then a similar level of resistance to the virus might have been expressed, but this was not the case. Ultimately, in a greenhouse that has both susceptible and resistant individuals, susceptible individuals will grow more rapidly than resistant ones. For instance, this could be another advantage in the management of resistance to *Bt* with AcMNPV as an alternative agent, because individuals susceptible to *Bt* will be more likely to survive the application of the virus and to spread the susceptible genes, and the resulting population will then be killed by the next *Bt* application following the virus.

Another parameter of primary importance is the time it takes the virus to kill the pest. Implementation of Baculoviruses for pest control has been slow because they are slow to kill insects compared to synthetic insecticides (Granados and Federici 1986). In spite of the slower developmental time exhibited by the resistant individuals, the time to death for susceptible versus resistant populations was much the same. The higher susceptibility of the resistant colony seems to compensate for slower viral replication, associated with longer development time. Thus, susceptible and resistant individuals have the same time to death.
3.6 Conclusions

Given that no cross-resistance occur and that resistant individuals to Bt are twice as susceptible to AcMNPV these biocontrol agents might complement each other and might be compatible for their use in a rotation strategy in greenhouses. Further investigations should focus on experimental applications in greenhouses, with both agents alone in a rotational schedule as well as in mixtures.
Table 3.1. Values of control mortality (%), LD$_{50}$s (PIBs/larva) and slopes for each replicate of susceptible and resistant colonies obtained with PROBIT ANALYSIS (Genstat 5).

<table>
<thead>
<tr>
<th>Colony</th>
<th>Replicate</th>
<th>LD$_{50}$ (95 % CI)</th>
<th>Slope (s.e)</th>
<th>Control Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIP-S</td>
<td>1</td>
<td>13 (9-19)</td>
<td>0.68 (0.13)</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14 (10-20)</td>
<td>0.72 (0.14)</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>19 (14-23)</td>
<td>0.65 (0.15)</td>
<td>7.2*</td>
</tr>
<tr>
<td>GIP-R</td>
<td>1</td>
<td>6 (4-10)</td>
<td>0.52 (0.17)</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7 (4-10)</td>
<td>0.67 (0.19)</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8 (5-12)</td>
<td>0.86 (0.21)</td>
<td>9.4*</td>
</tr>
</tbody>
</table>

* Mortality in these repetitions had to be adjusted by Abbott’s correction formula before further analysis.
Table 3.2. LD$_{50}$ values and confidence intervals obtained with Probit Analysis for each colony, with repetitions combined.

<table>
<thead>
<tr>
<th>PIBs/ larva</th>
<th>Lower 95%</th>
<th>LD$_{50}$</th>
<th>Upper 95%</th>
<th>Slope ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>12</td>
<td>15</td>
<td>19</td>
<td>0.66 ± 0.06</td>
</tr>
<tr>
<td>Resistant</td>
<td>5</td>
<td>7</td>
<td>10</td>
<td>0.68 ± 0.07</td>
</tr>
</tbody>
</table>
**Fig. 3.1.** Development time to pupation from second instar of larvae resistant (R) and susceptible (S) to *Bt*. Data obtained from untreated group and analyzed by Wilcoxon / Kruskall–Wallis in JMPin 4.04. Asterisk indicates a statistical difference. $\chi^2 = 69.4$; p = <0.0001; $\alpha = 0.05$
3.7. References


GENSTAT. 1998. Release 4.1. in Rothamsted Experimental Station, Lawes, Agricultural Trust: Harpenden, UK.


Chapter 4

*Does host plant affect the susceptibility of *Trichoplusia ni* to *AcMNPV?*

4.1 Introduction

Baculoviruses are entomopathogens that have been used as microbial pesticides to control pests in many crops. Nucleopolyhedroviruses are one of the two genera in the family Baculoviridae that have been successfully implemented in the biological control of pests in forestry and agriculture (Fuxa 1987; Moscardi 1999). A number of factors can affect the performance of these natural pathogens for the control of pests. Temperature, for example, plays a key role in regulating the time to kill but not host mortality. Higher temperatures cause faster deaths (van Beek et al. 2000; Ignoffo 1966; Frid 2002). Other factors such as the age at which larvae become infected (Milks 1996; Bianchi et al. 2001), the quantity of virus consumed, and virus-food plant interactions (Forschler et al. 1992; Hoover et al. 1998; Farrar and Ridgway 2000; Bianchi et al. 2001) can alter the susceptibility of the insect pest to the viruses.

Because the route of entry of baculoviruses is oral, it is assumed that factors affecting virulence must act in the insect’s midgut environment within a short time (Hoover et al. 1998). The effects of host plants could result from direct antagonism (Kushner and Harvey. 1962) or synergy between leaf compounds and microbes. Plant chemistry can
modulate infection in the gut and nutrient content can determine host survival (review in Cory and Myers 2003). Plants can also influence virus interactions in other ways: plant architecture affects virus persistence, and the palatability of the plant can modify the mobility of insect hosts and virus acquisition through levels of consumption (review in Cory and Myers 2003). Virus-plant chemical interactions can reduce larval susceptibility to baculoviruses (Richter and Abdel-Fattah 1987; Keating et al. 1988; Forschler et al. 1992; Santiago-Alvarez and Ortiz-Garcia 1992; Hoover et al. 1998a), or increase the susceptibility of larvae (Forschler et al. 1992; Hoover et al. 1998a). For example, larvae of *Spodoptera exigua* were more susceptible to SeMNPV when feeding on plants (tomato and chrysanthemum) in comparison to artificial diet (Bianchi et al. 2001). Manson (2003) found that the susceptibility of *T. ni* to TnSNPV varied with the crop on which the larvae were infected. If the efficacy of baculoviruses in a greenhouse situation is consistent with laboratory bioassays, virulence and speed of action determined in the laboratory may be used to indicate baculovirus efficacy at the crop level (Bianchi et al. 2000).

Cabbage loopers are susceptible to AcMNPV, a virus first isolated in *Autographa californica*, the alfalfa looper. Here I analyzed the susceptibility of cabbage loopers to AcMNPV on the three major crops cultivated in greenhouses in British Columbia (tomato, cucumber and bell pepper), as well as on artificial diet, in order to be able to predict the potential of AcMNPV if used in larger scale greenhouse experiments.
Objectives

1- To find the doses of AcMNPV that could effectively control T. ni on tomato, cucumber and pepper.

2- To investigate if there are interactions between AcMNPV and the host plant that changes the insect’s susceptibility to the virus.

3- To find the virus doses that minimize the time to death on each crop.

4- To test if the susceptibility of virus to larvae fed on artificial diet can be extrapolated to crop plants.

4.2 Materials and Methods

Bioassays were conducted using Trichoplusia ni that originated from three different colonies (TOM F3, GLEN, GIP). All colonies were reared following the procedure explained in Appendix 1. These colonies were established in the laboratory after collection from commercial greenhouses.

TOMF3 was collected from a tomato greenhouse in Langley, BC and was kept on tomato leaves for 7 generations and then transferred to artificial diet for 3 generations prior to the experiments.

GLEN colony came from a pepper greenhouse and GIP from a tomato greenhouse in the Fraser Valley of British Columbia. These colonies were originally established by Alida Janmaat (Janmaat and Myers 2003) and have been kept in the laboratory on artificial diet for several years.
The three colonies were tested for susceptibility to NPV at different times. In the first trial I used TOMF3 and cucumber leaves because larvae were less susceptible to TnSNPV on cucumber (Manson 2003). This colony was lost due to very low fecundity and failed egg hatch before it was possible to repeat the experiment and include the other crops. Further bioassays were conducted with GLEN. This colony was very robust, however, only two repetitions could be carried out before the colony crashed due to a virus outbreak. Two further replicates were then performed with the GIP colony.

Bioassays

For most of the bioassays I used four different food sources: tomato, cucumber, pepper and artificial diet discs on which I offered the virus to the host. All crops were grown from seeds inside the UBC Horticulture greenhouse. Tomato, *Lycopersicum esculentum* "Maribel ez TmC5VF2Fr wi", and pepper, *Capsicum annuum* L. "Triple 4 ez", seedlings were obtained by sowing seeds on Redi-earth (35-45% peat + 55-65% vermiculite) inside individual cells of seedling trays (72 cells per tray). Cucumber, *Cucumis sativus* "Natika ez", seeds were directly sown in four inch, disposable, plastic pots filled with potting mix (regular peat 75%, regular perlite 25%, N-P-K- starter; supplied by West Creek Farm- Fort Langley). All containers were drenched with a 1% fungicidal solution of No-Damp® (Oxine Benzoate 2.5%) to prevent damping-off and other early fungal diseases. All pots were placed in a hot and humid bedding bench inside the greenhouse until the emergence of the seedlings. They were then moved to a regular bench until they had approximately 2 true leaves. At that time, plants were transplanted to five inch plastic pots filled with potting mix. Plants were kept at 24°C ± 2°C without any supplementary lighting and a regular flooding fertirrigation with 120 ppm of 15-5-15 Cal-Mg Scotts
Petters Excel® fertilizer provided daily in the fall and winter and twice a day during spring and summer.

Pepper plants were used after 2-3 months of being sown. Tomato plants were used after 1.5-2 months of being sown and cucumber plants were used after 1-1.5 months, depending on the season. In the case of peppers, plants had 9-13 leaves, but only new completely expanded leaves were used to dose the virus. Cucumber leaves were collected from the middle position from plants having between 5-8 leaves. Tomato leaves were collected from the bottom of the plants, however senescent leaves were avoided. The decision to pick leaves from different parts of the plant depending on the crop comes from the observation of the pest’s foraging behaviour on each crop in both, commercial and experimental greenhouses (see Chapter 5 for more details). Artificial diet was prepared following the recipe described in Appendix 2.

Egg sheets of each colony were collected and allowed to hatch in 4 L plastic buckets after 2-4 days of collection. After 2 days at 26°C, neonates were transferred to 170 ml cups containing artificial diet (25 neonates/cup). After 5 days larvae were starved in individual 21 ml plastic cups for 3 h and then randomly assigned to treatments.

The multiply embedded nucleopolyhedrovirus of *Autographa californica* (AcMNPV) was provided by Dr. Martin Erlandson (Saskatoon Research Centre, AAFC), and originated from samples isolated from infected caterpillars collected from Fraser Valley greenhouses. The viral concentration in the stock was rechecked by viral occlusion body counting using a Neubauer hemocytometer (see Appendix 5).
The virus doses were offered to larvae on discs of artificial diet and leaves cut with a cork borer #2 (Ø = 4 mm). One control and four to five viral doses per food source were used. The doses changed with the colony. For GLEN and TOMF3 they were 1, 2.7, 27 and 268 PIBs/larva after viral recounting, while for GIP they were 2.7, 6.6, 13.4, 27 and 67 PIBs/larva after viral recounting. The doses used for GLEN and TOMF3 before recounting were suggested by Martin Erlandson according to previous experiments he had done, however a great variation within LD50 and confidence intervals were detected so doses were adjusted for the bioassays conducted with GIP colony.

After completely consuming the contaminated disc the individuals were kept on artificial diet for the rest of the experiment (Forschler et al. 1992; Manson. 2003; Cook et al. 2003).

**Infection Technique**

Viral dilutions of AcMNPV, prepared following the protocol explained in Appendix 6, were removed from the freezer several hours before dosing and thawed at room temperature inside a 455 ml paper cup with lid to avoid the incidence of light and therefore degradation of the viral dilutions.

Leaves of the three crops were collected from plants cultivated at the UBC Horticulture greenhouse placed onto a tray covered with a damp paper towel on the bottom and a transparent plastic lid to prevent desiccation.

Six trays per crop were labeled to individualize each treatment (Tomato Control, Tomato D1, Tomato D2, ..., Tomato D5, Pepper Control, ..., Pepper D5, ..., etc.). The same
procedure explained in page 38 (Chapter 3 - Cross resistance) was used to set up the bioassays. Mortality, date of death and pupation date were recorded. The total experiment consisted of 2 repetitions for GLEN (tomato and pepper) and GIP on all the host plants, and one repetition for TOMF3 (cucumber) and GLEN (diet and cucumber).

Artificial diet discs

- Two drops of 1 ml of artificial diet each were spread on a microscope slide and a cover slip was placed on top of the drops and pressed down until the drops touched each other and the diet became a thin layer of about 0.5 mm in thickness. The artificial diet was prepared following the procedure explained in Appendix 2.
- Ten microscope slides were placed inside a 9 mm diameter Petri dish and the Petri dish was kept refrigerated to facilitate handling after solidifying. Once the diet was solid, discs were obtained using a cork borer #2. Each slide yielded approximately 23 discs.
- Discs were removed with a spatula from the slide and placed on top of each filter paper piece.

4.3 Statistical Analysis

The analysis of LD₅₀ was done using GENSTAT (1998) by fitting the results to the Probit Analysis outlined by Finney (1971). Time to death was analyzed with a survival analysis performed with JMPin 4.04 where Log Rank and Wilcoxon tests are used to look for differences between treatments. The significance level (α value) for all statistical tests was set at 0.05.
4.4 Results

a. Analysis of Mortality

The analysis of mortality yielded no significant differences among the LD$_{50}$s for larvae from GLEN and GIP colonies infected with AcMNPV on leaf discs from different host plants. The confidence intervals obtained with Genstat 5 after combining replicates overlapped (Tables 4.1 and 4.2), indicating no significant differences among food plants with corrections for natural mortality in the control groups being automatically performed by the program. Furthermore, an analysis of deviance was performed with Genstat 5, with mortality corrections calculated using Abbott’s formula for those treatments with more than 5% of mortality in control groups. No differences were detected for the four food sources used for viral inoculation with this method (dev ratio $GLEN = 2.29$, $p = 0.08$; dev ratio $GIP = 0.53$, $p = 0.66$).

LD$_{50}$ estimates for GIP had the lowest variation and this indicates that the adjustment of doses, which was done prior to the onset of the experiment with this colony, yielded more consistent results. In spite of that, no differences were detected among food disc types.

b. Time to Death

Significant differences in speed of kill were found among doses, with the time to death decreasing with an increase in the dose for all food sources and colonies (Table 4.3). Therefore the time to death was analyzed for dose 5, which caused the highest mortality (more than 90%) for all the colonies and plant types. The reason for choosing this dose is based on potential commercial application (targeting a high level of mortality).
were non-parametric and therefore analyzed with a survival curve performed with JMPin 4.0.4. The analysis of time to death between replicates for GIP colony for dose 5 on each crop yielded significant differences on tomato ($\chi^2_{\text{Log-Rank}} = 15.05; \text{df}= 1; \text{p}= 0.0001$) and on pepper ($\chi^2_{\text{Log-Rank}} = 7.28; \text{df}= 1; \text{p}= 0.007$). Therefore the analysis for the GIP colony data was done with a separate survival curve for each replicate. No differences among plant types were found for GIP replicate #1 (Table 4.4), however, there were some differences for GIP replicate #2 (Table 4.5a). Multiple comparisons were carried out with the Šidák procedure (Hardin et al. 1996) to determine on which crops time to death was statistically different (Table 4.5b).

No differences were observed for GLEN colony between replicates for dose 5 in either pepper ($\chi^2_{\text{Log-Rank}} = 2.65; \text{df}= 1; \text{p}= 0.1037$) or tomato leaf discs ($\chi^2_{\text{Log-Rank}} = 2.23; \text{df}= 1; \text{p}= 0.1353$), so time to death data were combined for both replicates. A survival curve, and Šidák test for multiple comparisons were performed (Table 4.6a and 4.6b respectively).

The mean number of days to death for dose 5, varied from 4.5 to 6.1 days among plant type, colony and replicate. Although differences in speed of kill were detected among food sources, colonies varied in their response. However, the maximum variation among crops was 0.5 and 0.6 of day for GLEN and GIP # 2 respectively.

4.5 Discussion

The host plant can play an important role in mediating the susceptibility of lepidopteran larvae to baculoviruses (Fuxa, 1982; Fuxa and Geaghan, 1983; Keating and Yendol,
1987; Richter et al. 1987; Duffey et al. 1995; Hoover et al. 1998a,b; Ali et al. 1998). However, according to my results, the susceptibility of *T. ni* larvae to AcMNPV appeared to be unaffected by the type of food ingested at the time of infection. These findings concur with a study done by Forschler et al. (1992) on the susceptibility of *Helicoverpa zea* to its nucleopolyhedrovirus (HzNPV) when larvae were fed the virus on cotton, tomato and artificial diet and remained on artificial diet pre and post infection. However, these results contradict many other studies in which the susceptibility of larval hosts to nucleopolyhedroviruses changed with the food plant species (Richter and Abdel-Fattah 1987; Keating et al. 1988; Santiago-Alvarez and Ortiz-Garcia 1992; Ali et al. 1998; Hoover et al. 1998a; Farrar and Ridgway 2000).

One possible explanation for my findings is that as *T. ni* is highly susceptible to AcMNPV, any variation with food plant might be hard to detect. Other lepidopteran hosts, *Spodoptera exigua*, *Heliothis virescens* and *Helicoverpa zea*, have been found to vary in their susceptibility to AcMNPV on different host plants (Hoover et al. 1998; Hoover et al. 1998a; Hoover et al. 1998b; Bianchi et al. 2000; Hoover et al. 2000). According to Washburn et al. (1995) *T. ni* is highly susceptible to AcMNPV while *H. virescens* is very susceptible and *H. zea* is highly resistant. In many other studies, other hosts and NPVs were used and the LD$_{50}$s or LC$_{50}$s were always much higher than those found here (Richter and Abdel-Fattah 1987; Keating et al. 1988; Forschler et al. 1992; Santiago-Alvarez and Ortiz-Garcia 1992; Hunter and Schultz 1993; Ali et al. 1998; Farrar and Ridgway 2000). Richter and Abdel-Fattah (1987) and Ali et al. (1998) found the susceptibility of *S. frugiperda* and heliothine Lepidoptera to vary among different crop plants, however no differences were detected for highly virulent viruses similar to my
Another factor that could have reduced my ability to detect differences in susceptibility among food sources is the stage at which the larvae were infected. Most studies in the literature were conducted on larvae in their 4th instar. At this stage, larvae are likely to be less susceptible to the virus, and therefore variation in the susceptibility with host foliage is more likely to be revealed.

The size of the leaf disc or diet disc must not be overlooked. The leaf discs I used were small (4 mm Ø) compared to the ones most authors used (5 –9 mm Ø). Richter et al. (1987) stated that it is likely that the differences in susceptibility were related to the pest being stressed by consumption of less suitable host plants, although the possibility of an antiviral agent in one or more of the plant species cannot be ruled out. It could be that a threshold amount of antagonistic or synergistic compounds must be present in the leaves to detect differences and in such a small piece of leaf the proportion is not adequate. So the question that arises then is: would results have been different if larvae were maintained on the different plant foliage for the whole experiment or at least for a longer period of time before and after infection? Forschler et al. (1992) found that host plants did not affect the activity of HzSNPV against corn earworm larvae unless the foliage was fed to larvae for the 24 h before administration of the virus. They found significant differences on cotton when larvae were fed continously and were dosed on crops, however, no differences were detected between tomato and artificial diet in any of their experiments. Richter et al. (1987) conditioned larvae to the plants by rearing them individually from the first instar of one generation through the second instar of the next
generation before testing. However, Schultz et al. (1992) stated that neither food quality before nor after infection influence mortality, and only the quality of foliage entering the gut in concert with the virus influences infection.

In many studies larvae were starved for 18-24 h prior to bioassays to void their guts from previous food (Keating et al. 1988; Hunter and Schultz 1993; Hoover et al. 1998; Hoover et al. 1998a). I only starved the larvae for 3 h prior to infection to assure that they would be hungry enough to finish eating the whole leaf/diet disc in 24 h. Even though the voiding of the guts could possibly help to detect differences among host plants, that situation would be very unlikely to occur outside laboratory conditions.

Other factors could possibly regulate the acquisition of virus in real situations and could therefore modify the susceptibility of the host to AcMNPV in commercial crops. Those include: the amount of leaf consumed on each crop that will in turn determine the actual amount of virus ingested, the foraging behaviour that can help the insect escape from the virus, and the persistence of the virus on the different crops that is also determined by plant architecture and leaf morphology.

Regarding the speed of kill, even when differences were detected for some of the repetitions; the highest difference among crops (cucumber, tomato and pepper) was never more than 0.6 of a day, a difference that would not be relevant in an agricultural situation. Post infection food source however is very likely to regulate the speed of kill. Hoover et al. (1998) stated that the faster the larvae grow on a host plant the quicker they die, due to an increase of viral replication. This indicates that in order to realistically evaluate speed of kill, it would have been better to test the larvae on the same food source for the whole
experiment given that great differences in growth rates exist among the food sources tested (see Appendix 7). Moreover, other factors such as consumption and foraging behaviour may also play very important roles in affecting the speed of kill.

4.6 Conclusions

No differences in susceptibility of T. ni to AcMNPV associated with host plant were detected in laboratory bioassays. In addition, no biologically meaningful differences in time to death occurred in these bioassays. However, even though bioassays are a good tool for basic research, the realistic scenario should not be overlooked.

Bioassays must be complemented with experimental applications in greenhouses to more accurately predict the performance of AcMNPV on the different crops.
Table 4.1. LD$_{50}$ and 95% confidence intervals (95% CI) in PIBs/larva, slope for the relationship between dose and mortality and % mortality of controls for replicates of *T. ni* colonies inoculated with AcMNPV on different diet discs obtained using PROBIT ANALYSIS (Genstat 5).

<table>
<thead>
<tr>
<th>Plant type</th>
<th>Colony</th>
<th>Rep #</th>
<th>LD$_{50}$ (95% CI)*</th>
<th>Slope-s.e</th>
<th>% Control mortality</th>
<th>$\chi^2$ Fit of line</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOMATO</td>
<td>GLEN</td>
<td>1</td>
<td>10 (8-17)</td>
<td>0.91 (0.16)</td>
<td>2.8</td>
<td>78.62; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>11 (7-22)</td>
<td>0.66 (0.12)</td>
<td>3.3</td>
<td>62.28; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>GIP</td>
<td>1</td>
<td>18 (10-23)</td>
<td>0.66 (0.15)</td>
<td>10.7</td>
<td>24.15; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>12 (9-17)</td>
<td>0.90 (0.16)</td>
<td>3.5</td>
<td>45.53; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>GIP</td>
<td>1</td>
<td>11 (7-17)</td>
<td>0.81 (0.16)</td>
<td>8.3</td>
<td>34.52; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>14 (11-19)</td>
<td>1.01 (0.16)</td>
<td>0</td>
<td>56.91; p&lt;0.001</td>
</tr>
<tr>
<td>PEPPER</td>
<td>GLEN</td>
<td>1</td>
<td>17 (10-30)</td>
<td>0.58 (0.10)</td>
<td>10.2</td>
<td>56.61; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>19 (11-32)</td>
<td>0.67 (0.11)</td>
<td>9.3</td>
<td>70.79; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>GIP</td>
<td>1</td>
<td>11 (7-17)</td>
<td>0.81 (0.16)</td>
<td>8.3</td>
<td>34.52; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>14 (11-19)</td>
<td>1.01 (0.16)</td>
<td>0</td>
<td>56.91; p&lt;0.001</td>
</tr>
<tr>
<td>CUCUMBER</td>
<td>TOM-F3</td>
<td>1</td>
<td>10 (6-18)</td>
<td>0.57 (0.11)</td>
<td>9.7</td>
<td>35.49; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>GLEN</td>
<td>1</td>
<td>12 (8-19)</td>
<td>0.69 (0.10)</td>
<td>0</td>
<td>73.57; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>GIP</td>
<td>1</td>
<td>11 (8-19)</td>
<td>0.64 (0.14)</td>
<td>7.9</td>
<td>23.03; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>13 (10-17)</td>
<td>0.90 (0.16)</td>
<td>6.3</td>
<td>51.88; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>GLEN</td>
<td>1</td>
<td>20 (12-32)</td>
<td>0.77 (0.12)</td>
<td>0</td>
<td>76.03; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>GIP</td>
<td>1</td>
<td>11 (7-22)</td>
<td>0.52 (0.16)</td>
<td>9.6</td>
<td>14.55; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>12 (9-18)</td>
<td>0.82 (0.14)</td>
<td>8.0</td>
<td>44.86; p&lt;0.001</td>
</tr>
</tbody>
</table>

* LD$_{50}$ (95% CI) automatically adjusted for control mortality by Genstat 5.
Table 4.2. Mean LD$_{50}$, and confidence intervals in occlusion bodies of AcMNPV per larva and slopes for each colony obtained with PROBIT ANALYSIS (Genstat 5), combined data used for GIP and GLEN colonies.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Confidence Interval</th>
<th>Cuke</th>
<th>Diet</th>
<th>Pepper</th>
<th>Tomato</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOMF3</td>
<td>LD$_{50}$</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low95%</td>
<td>6</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Up95%</td>
<td>18</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td></td>
<td></td>
<td>0.57±0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dev ratio:0.84</td>
<td></td>
<td></td>
<td>p=0.473</td>
<td></td>
</tr>
<tr>
<td>GLEN</td>
<td>LD$_{50}$</td>
<td>12</td>
<td>20</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Low95%</td>
<td>8</td>
<td>12</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Up95%</td>
<td>19</td>
<td>32</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td></td>
<td></td>
<td>0.69±0.10</td>
<td>0.77±0.12</td>
</tr>
<tr>
<td></td>
<td>dev ratio:0.84</td>
<td></td>
<td></td>
<td>p=0.473</td>
<td>0.62±0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.76±0.10</td>
</tr>
<tr>
<td>GIP</td>
<td>LD$_{50}$</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Low95%</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Up95%</td>
<td>15</td>
<td>14</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td></td>
<td></td>
<td>0.78±0.10</td>
<td>0.68±0.10</td>
</tr>
<tr>
<td></td>
<td>dev ratio:1.19</td>
<td></td>
<td></td>
<td>p=0.313</td>
<td>0.92±0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.81±0.11</td>
</tr>
</tbody>
</table>
Table 4.3. Analysis of time to death among doses by colony, repetition and crop performed using survival curves and Log Rank Test in JMPin 4.0.4

<table>
<thead>
<tr>
<th>GIP #1</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuke</td>
<td>$\chi^2 = 32.7; df = 4; p &lt; 0.0001; range D1 - D5 = 9.3 - 5.0 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>$\chi^2 = 17.0; df = 4; p = 0.002; range D1 - D5 = 8.3 - 4.7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepper</td>
<td>$\chi^2 = 26.3; df = 4; p &lt; 0.0001; range D1 - D5 = 9.2 - 4.8 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>$\chi^2 = 22.7; df = 4; p = 0.0001; range D1 - D5 = 8.7 - 4.5 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GIP #2</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuke</td>
<td>$\chi^2 = 22.7; df = 4; p = 0.0001; range D1 - D5 = 8.9 - 5.0 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>$\chi^2 = 15.3; df = 4; p = 0.0041; range D1 - D5 = 9.0 - 4.9 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepper</td>
<td>$\chi^2 = 12.9; df = 4; p &lt; 0.0110; range D1 - D5 = 8.8 - 5.6 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>$\chi^2 = 15.9; df = 4; p = 0.0030; range D1 - D5 = 8.7 - 5.4 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GLEN #1</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuke</td>
<td>$\chi^2 = 9.95; df = 3; p &lt; 0.0413; range D1 - D5 = 8.5 - 5.8 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>$\chi^2 = 9.77; df = 3; p = 0.03; range D1 - D5 = 9.5 - 6.1 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepper</td>
<td>$\chi^2 = 9.68; df = 3; p &lt; 0.021; range D1 - D5 = 8.6 - 5.7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>$\chi^2 = 32.4; df = 3; p &lt; 0.0001; range D1 - D5 = 9 - 5.2 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GLEN #2</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepper</td>
<td>$\chi^2 = 20.5; df = 3; p = 0.0001; range D1 - D5 = 9.6 - 5.3 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>$\chi^2 = 19.8; df = 3; p = 0.0006; range D1 - D5 = 9.5 - 5.4 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.4. Mean time to death in days (± SEM) of *Trichoplusia ni* that fed AcMNPV (dose 5) on discs of different food sources for the first replicate of GIP colony.

$\chi^2_{\text{Log Rank}} = 2.7; \ df = 3; \ p = 0.4394$. Same bold letters indicate no significant statistical differences.

<table>
<thead>
<tr>
<th>GIP #1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuke</td>
<td>5.0 ± 0.21 a</td>
</tr>
<tr>
<td>Diet</td>
<td>4.7 ± 0.20 a</td>
</tr>
<tr>
<td>Pepper</td>
<td>4.8 ± 0.22 a</td>
</tr>
<tr>
<td>Tomato</td>
<td>4.5 ± 0.25 a</td>
</tr>
</tbody>
</table>
Table 4.5a. Mean time to death in days (± SEM) of *Trichoplusia ni* that fed AcMNPV (dose 5) on discs of different food sources for the second replicate of GIP colony.

$\chi^2_{\text{Log Rank}} = 13.97; \text{df} = 3; p = 0.0029$. Same bold letters indicate no statistical differences after multiple comparisons (see Table 4.5b.)

<table>
<thead>
<tr>
<th>GIP #2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuke</td>
<td>$5.0 \pm 0.14 \ a$</td>
</tr>
<tr>
<td>Diet</td>
<td>$4.9 \pm 0.16 \ a$</td>
</tr>
<tr>
<td>Pepper</td>
<td>$5.6 \pm 0.13 \ b$</td>
</tr>
<tr>
<td>Tomato</td>
<td>$5.4 \pm 0.18 \ b$</td>
</tr>
</tbody>
</table>
Table 4.5b. Multiple comparisons for non-parametric data of time to death among leaf-disc types for GIP colony replicate #2 using the Šidák procedure, with a $\gamma$-level or comparison wise-error rate of 0.0042 (Hardin et al. 1996).

<table>
<thead>
<tr>
<th>Group</th>
<th>$\chi^2$*</th>
<th>p</th>
<th>Significance **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuke vs Diet</td>
<td>0.06</td>
<td>0.4263</td>
<td>NS</td>
</tr>
<tr>
<td>Cuke vs Pepper</td>
<td>10.16</td>
<td>0.0014</td>
<td>S</td>
</tr>
<tr>
<td>Cuke vs Tomato</td>
<td>11.03</td>
<td>0.0009</td>
<td>S</td>
</tr>
<tr>
<td>Diet vs Tomato</td>
<td>13.02</td>
<td>0.0003</td>
<td>S</td>
</tr>
<tr>
<td>Diet vs Pepper</td>
<td>11.40</td>
<td>0.0007</td>
<td>S</td>
</tr>
<tr>
<td>Pepper vs Tomato</td>
<td>0.75</td>
<td>0.3867</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Chi-squared values with their associated probabilities (p) obtained after performing a Wilcoxon/Kruskal Wallis test in JMPin for each pair.

** Significance (S) based on the $\gamma$-level value = 0.0042.
Table 4.6a. Mean time to death in days (± SEM) of *Trichoplusia ni* that fed AcMNPV (dose 5) on discs of different food sources for the second replicate of GLEN colony.

$\chi^2_{\text{Log Rank}} = 17.4; \text{df} = 3; p = 0.0006$. Same bold letters indicate no statistical differences after multiple comparisons (see Table 4.6b)

<table>
<thead>
<tr>
<th>GLEN</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuke</td>
<td>5.8 ± 0.09 bc</td>
</tr>
<tr>
<td>Diet</td>
<td>6.1 ± 0.16 c</td>
</tr>
<tr>
<td>Pepper</td>
<td>5.5 ± 0.10 ab</td>
</tr>
<tr>
<td>Tomato</td>
<td>5.3 ± 0.10 a</td>
</tr>
</tbody>
</table>
Table 4.6b. Multiple comparisons for non-parametric data of time to death among leaf disc types for GLEN colony using the Šidák procedure, with a \( \gamma \)-level or comparison wise-error rate of 0.0042 (Hardin et al. 1996).

<table>
<thead>
<tr>
<th>Group</th>
<th>( \chi^2 ) *</th>
<th>( p )</th>
<th>Significance **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuke vs Diet</td>
<td>2.66</td>
<td>0.1028</td>
<td>NS</td>
</tr>
<tr>
<td>Cuke vs Pepper</td>
<td>7.9</td>
<td>0.005</td>
<td>NS</td>
</tr>
<tr>
<td>Cuke vs Tomato</td>
<td>10.4</td>
<td>0.0012</td>
<td>S</td>
</tr>
<tr>
<td>Diet vs Tomato</td>
<td>15.6</td>
<td>&lt;0.0001</td>
<td>S</td>
</tr>
<tr>
<td>Diet vs Pepper</td>
<td>11.80</td>
<td>0.0006</td>
<td>S</td>
</tr>
<tr>
<td>Pepper vs Tomato</td>
<td>0.61</td>
<td>0.4359</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Chi-squared values with their associated probabilities (p) obtained after performing a Wilcoxon/Kruskal Wallis test in JMPin for each pair.

** Significance (S) based on the \( \gamma \)-level value = 0.0042.
4.7 References


GENSTAT. 1998. Release 4.1. in Rothamsted Experimental Station, Lawes, Agricultural Trust: Harpenden, UK.


(Lepidoptera: Noctuidae) Larvae. Environ. Entomol. 27:1264-1272.


Chapter 5

The effect of host plants on leaf consumption and movement of cabbage loopers as potential influences on infection by AcMNPV

5.1 Introduction

The cabbage looper, *Trichoplusia ni*, is a generalist insect that feeds on over 160 species of plants in 36 families (Sutherland and Greene 1984). It is the major lepidopteran pest in cucumber, pepper and tomato greenhouses in British Columbia, however its growth and behaviour differ with the host crop.

Despite the fact that nucleopolyhedroviruses (NPVs) have been shown to be effective for the control of insect pests (Granados and Federici 1986, Moscardi 1999), extrapolation of results from laboratory experiments on interactions between viruses and hosts do not necessarily apply to the field situation. Efficacy of NPVs for control of lepidopteran pests not only has to do with the ability of the virus to kill the pest in the laboratory, but also with the probability that the pest will ingest a lethal dose of viral occlusion bodies capable of causing infection and death. Thus foraging behaviour and development time are important factors to consider when attempting extrapolate laboratory experiments to greenhouse situations. For example, in a study of *Spodoptera exigua* in chrysanthemum greenhouses, Bianchi et al. (2001) found that larval development time on chrysanthemum plants was 36% greater than that on artificial diet. Moreover, development can differ from one crop to another associated with differences in food quality and the presence of
antiherbivore compounds. Sutherland (1966) found that larval development of cabbage loopers on potted pepper plants took 11-15 days longer than those on potted cabbage plants.

Foliage consumption affects the amount of nutrients and viral particles ingested and can regulate larval growth and development time. A characteristic of *S. exigua* on artificial diet is that later instars are more resistant than early instars to virus. Bianchi et al. (2000) found however, that the increased resistance of later instars of *S. exigua* to its NPV did not occur on the chrysanthemum greenhouse crop. They suggested that the increase in foliage consumption of later instars resulted in higher doses of the virus and this compensated for their reduced susceptibility to the viruses. Furthermore, variation in the dose of viral particles ingested can affect both: a) the level of infection achieved and b) the time it takes the virus to kill the host (Ignoffo 1964). Ignoffo (1964) found that for *T. ni* as much as 4.3 days difference in time to death occurred with doses ranging from 25,000 PIBs of *T. ni* singly embedded nucleopolyhedrovirus (TnSNPV) /μl diet to 350 PIBs TnSNPV /μl diet. The highest dose killed *T. ni* in 3.7 days while the lowest required 8 days.

Moreover, as NPVs can be horizontally transmitted, the behaviour of infected larvae if different from healthy ones, can greatly affect the spread of NPVs and the chances for the pest to encounter viral particles. If, as is sometimes the case, infected larvae climb to the upper parts of the plants to die (stated by Fuxa 1987 and Moscardi and Carvalho 1993, shown by Vasconcelos et al. 1996 and Goulson 1997, review in Cory and Myers 2003) new upper leaves that are not sprayed, can still potentially become contaminated by the
PIBs released after the liquefaction of dead larvae (horizontal transmission). This can reduce the number of applications of virus required to keep the pest under control.

Several biological and behavioural characteristics of the insect and their interactions with the plants and virus must be studied to determine the potential of NPVs to effectively control cabbage loopers inside a greenhouse environment. The aim of this chapter is to explore the differences in consumption and behaviour of cabbage loopers fed on the three main crops cultivated in greenhouses: tomato, bell pepper and cucumber. Firstly I will focus on consumption rates and the water content of leaves that could be factors in the differences in growth rates of larvae reared on the three crops (see Appendix 7 and Janmaat 2004). Finally, I will describe the behaviour of larvae on the different crops based on visual monitoring in greenhouses as well as movement experiments with healthy and infected caterpillars carried out in the UBC Horticulture greenhouse.

Objective

To describe consumption and behavioural patterns of cabbage loopers fed on different host crops as factors that might influence the potential of AcMNPV for the control of loopers in vegetable greenhouses.

5.2 Materials, Methods and Results

The materials, methods and results will be described separately for each experiment. The statistical analyses were performed with JMPin 4.04 (Student Version of SAS Institute). The level of significance (α-value) was 0.05 in all tests.
5.2.1 Leaf consumption experiment

The aim of this experiment was to test for differences in leaf consumption by *T. ni* larvae from three different colonies. Each of the colonies was originally collected from commercial greenhouses. The colonies from the pepper and the tomato greenhouses were maintained on artificial diet for more than 2 years and the colony coming from a cucumber greenhouse was maintained on artificial diet for five generations before the experiment.

Leaf discs of 9.6 cm\(^2\) were cut using a plastic cutter of 3.5 cm in diameter. Leaf discs were assigned to plastic trays containing six holes per tray. Four trays per treatment were included in each of the three repetitions. On the bottom of each hole, a filter paper disc of the same area as the hole was provided and was dampened with 5 drops of distilled water. The vials were maintained inside a tray provided with a damp paper towel on the bottom and a plastic lid on top to prevent leaf desiccation. Each hole was individually identified and each leaf disc was weighed and its surface was measured with a leaf area meter (LI-COR, model Li-3000).

Larvae from each colony were 8 days old at the time of the experiment. Larvae were reared at 26 °C ± 1°C on artificial diet in 170 ml Styrofoam cups (15 larvae/cup). A wide range of larval sizes was collected from each cup. Larvae were individually starved for 3 hours prior to the onset of the experiment. Leaf discs were cut and let sit for 4 hours at room temperature before recording the fresh weight and leaf area. Larvae were weighed after starvation and the weight was written on each of the plastic containers. In order to test the same range of weights for all the crops, groups of three individuals per colony
with similar weights were put aside to assign one of the three individuals to each crop for
the experiment. Larvae were then assigned to each of the holes containing a leaf disc and
the larval weight was recorded. Larvae were kept inside a growth chamber at 26°C for
seven hours and then the leaf discs were weighed, scanned for area and the final larval
weight was recorded. The experiment was repeated three times, and the three repetitions
were one week apart. Plants were grown at the UBC Horticulture greenhouse as
previously described for other experiments. Leaves were collected from top, middle and
bottom sections of the plants for pepper, cucumber and tomato respectively to reflect the
position in the plant where larvae are normally encountered feeding in commercial crops.

I calculated the grams of leaf consumed, the amount of surface area consumed, and the
weight gained for each individual. Larval weights were bracketed into three ranges: range
1 from 0.02 grams to 0.05, range 2 from 0.06 to 0.09 grams and range 3 from 0.1 grams
to 0.15 grams and data from the three replicates were combined to have a higher sample
size for each range. Larval weights within ranges and above 0.15 grams were not
included in the analysis. Larval weights within ranges and among colonies and crops
were homogeneous (Range 1: \( \bar{x} = 0.034 \text{ g}, N=62, F_{2,59 \text{ crop}} = 0.22, p_{\text{crop}} = 0.8; F_{2,59 \text{ colony}} = 2.2, p_{\text{colony}} = 0.12 \); Range 2: \( \bar{x} = 0.076 \text{ g}, N=100, F_{2,97 \text{ crop}} = 2.54, p_{\text{crop}} = 0.08; F_{2,97 \text{ colony}} = 1.48, p_{\text{colony}} = 0.23 \); Range 3: \( \bar{x} = 0.12 \text{ g}, N=92, F_{2,89 \text{ crop}} = 0.69, p_{\text{crop}} = 0.50; F_{\text{colony}} = 0.23, p_{\text{colony}} = 0.78 \).

The analysis of grams of leaf consumed and cm² consumed were analyzed with a
Factorial analysis, while food conversion index data (weight gained / g of leaf consumed)
were non parametric and were analyzed with a Wilcoxon test.
Results

The amount of leaf ingested varied significantly within the three ranges of larval weight (Fig 5.1). Heavier larvae ingested more mass of fresh leaf of all the crops ($F_{\text{range}} = 46.7$, $p < 0.0001$, df=18, 236). No differences were detected among colonies ($F_{\text{colony}} = 2.0$, $p = 0.137$, df=18, 236) and no significant interactions occurred between colony and crop ($F_{\text{colony} \ast \text{crop}} = 1.41$, $p = 0.23$, df=18, 236). However, larvae consumed significantly more cucumber than tomato and pepper ($F_{\text{crop}} = 111.86$, $p < 0.0001$, df=18, 236), see Fig. 5.2. On average, cucumber fed larvae consumed 3.5 times more fresh leaf weight after seven hours than larvae reared on tomato and pepper with the level of consumption being similar for the last two crops.

For leaf area consumed (Fig. 5.3 and 5.4), all the colonies were similar ($F_{\text{colony}} = 0.88$, $p = 0.41$, df=18, 236), but showed a significantly higher level of consumption of cucumber leaves, followed by tomato leaves and then by pepper leaves ($F_{\text{crop}} = 279.3$, $p < 0.0001$, df=18, 236). On average, larvae reared on cucumber consumed about 10 times more leaf area than larvae reared on pepper and almost 5 times more than larvae reared on tomato. Although leaf discs were the same size in diameter they did not weigh the same. In addition the size of leaf veins varies among crops and T. ni do not feed on those although they contributed to the leaf disc weight. Cucumber leaf discs were on average lighter than tomato and pepper in that order ($\bar{x}_{\text{cucumber}} = 146 \text{ mg} \pm 0.003 \text{ mg}$, $\bar{x}_{\text{tomato}} = 160 \text{ mg} \pm 0.002 \text{ mg}$ and $\bar{x}_{\text{pepper}} = 184 \text{ mg} \pm 0.003 \text{ mg}$). These differences were statistically significant ($F = 56.51$, $p < 0.0001$, df=2, 251). Differences were also detected among larval weight categories ($F_{\text{range}} = 62.96$, $p < 0.0001$, df=18, 236) with heavier larvae consuming about 3.3
times more surface than larvae in the lowest range (Fig. 5.4).

Regarding the efficiency of food conversion (ECI) as a function of the larval weight, no differences were detected among colonies ($\chi^2 = 0.61$, df=2, $p=0.752$). Differences were detected among weight range categories ($\chi^2 =8.08$, df=2, $p=0.0175$) and crops ($\chi^2=17.64$, df=2, $p=0.0001$). Overall, there was a trend that larvae fed on pepper had higher food conversion efficiencies than larvae fed on tomato and cucumber (ECI<sub>pepper</sub> = 0.63 ± 0.053, ECI<sub>tomato</sub> = 0.50 ± 0.052, ECI<sub>cucumber</sub> = 0.44 ± 0.048). In the case of weight ranges, heavier larvae had higher food conversion indexes compared to smaller larvae with ECI<sub>range3</sub> = 0.61± 0.063, ECI<sub>range2</sub> = 0.53± 0.049 and ECI<sub>range1</sub> = 0.44± 0.051.

5.2.2 Water content experiment

Differential growth rates of larvae on different plants could possibly be related to the water content of leaves. To determine if the water content of leaves at different positions on plants was governing the foraging behaviour of larvae observed in the greenhouses, plants in commercial greenhouses were arbitrarily divided vertically into three thirds and leaves were collected from each of the three positions (top being the first third of the plant, middle the second third and bottom the last third) in four consecutive visits. Leaves were collected from the same commercial greenhouses (one cucumber, one tomato and one pepper greenhouse). Fifty leaves per position were brought to the laboratory each time, in a cooler with ice packs to keep the leaves fresh, and then one leaf disc per leaf was cut with a cork borer #13 (1.53 cm Ø). Leaf discs were weighed, and placed in individually labeled paper envelopes, and dried in an oven at 65 °C for 72 h. Dry weights were then recorded and the water content (mg of water), mg of water per cm$^2$, mg of dry
weight/cm² and % water were calculated.

Results

The fresh weight of leaf discs did not differ among visits for the cucumber crop ($F_{cucumber} = 1.89$, $p = 0.13, df=3,513$). Fresh weight did differ among collections for the pepper crop ($F_{pepper} = 53.28$, $p < 0.0001, df=3,595$) but maintained the same trend among positions in all the replicates. For tomato leaves differences in fresh weight among collections were found ($F_{tomato} = 66.42$, $p < 0.0001, df=3,594$) and in two of the replicates the fresh weight was higher for bottom leaves and for the other two replicates weight was higher for middle leaves. In all the cases differences were not greater than 15% among replicates and therefore, the disc weights of all the replicates were pooled together for further analysis. A summary of all the results obtained in this experiment is shown in Table 5.1.

The fresh weight of leaf discs differed with the location of the leaf on the crops. For cucumbers, discs weighed significantly more in the bottom position followed by the middle and the top. The same significant trend occurred for pepper. However for tomatoes, even though differences were detected, bottom and middle leaves had similar average weights but they were heavier than discs cut from leaves from the top (Table 5.1).

Differences in water content also occurred with leaf position. For cucumbers, differences were detected between bottom leaves and the other two positions, with no significant differences between middle and top. For pepper, water content of bottom and middle leaf discs did not differ, but they were significantly higher than that of leaf discs collected at
the top position. For tomato leaves all positions differed, with the bottom leaf discs having the highest percentage of water, followed by the middle and the top discs. The amount of water (in mg) per mg of dry matter was compared for each crop and all positions. For cucumber, discs from the bottom portion had significantly lower content of water per mg of dry matter with no differences between middle and top positions. For pepper discs, bottom and middle position registered the same ratio while they differed from the top position ratio. For tomatoes, the water content to dry leaf weight ratio varied among all positions with a decrease in the ratio from bottom to top.

5.2.3 Behaviour of cabbage loopers on different hosts

a. Visual monitoring for larval location inside greenhouses

Once a week, visual monitoring of 20 plants\(^1\) was done inside a tomato and a pepper greenhouse for an entire year with the aim of determining which part of the plants was preferred by cabbage looper larvae in each of the crops. Plants were arbitrarily divided into three portions (top, middle and bottom) and the number of individuals and their developmental stages were recorded. The accumulated number of individuals per month was calculated.

Results

Table 5.2 shows percentage and total number of individuals of all the instars recorded in a tomato and a pepper greenhouse during each month of the year. In the case of the tomato greenhouse, cabbage loopers were mainly found on the bottom portion of the

\(^1\) Except after the clean-up (in January) when 100 plants were checked.
plants until August. However, as the population increased and Bt was sprayed, moths began laying eggs in the upper portion of the plants. Of all the individuals collected inside the greenhouse (N=902), 73% were found at the bottom and middle portion while only 27%, primarily eggs and first instars, were found on top of the plants ($\chi^2 = 8.3$, $p=0.016$). In the case of the pepper greenhouse (N=453) 87% of the individuals were found on the top portion regardless of the instar and the remaining 13% were found in the middle and bottom portions ($\chi^2 = 336$, $p<0.0001$).

b. **Larval movement on plants at the UBC greenhouse**

Objectives:

1) To describe larval foraging behaviour on each crop.

2) To test if healthy caterpillars behave in the same way as infected larvae in order to predict the potential for horizontal transmission on each crop.

Materials and Methods:

A colony of cabbage loopers maintained on cucumber leaves for 3-5 generations was used for the movement experiments in all the crops. Three day-old eggs were allowed to hatch. After two days, neonates were transferred to Styrofoam cups containing artificial diet and remained there for six days. Six day-old larvae were given a viral dose of 2 $\mu$l of a solution containing $1.7 \times 10^5$ PIBs of AcMNPV per ml on cucumber leaf discs that were cut with a cork borer #2 (4 mm $\Omega$). Controls were dosed with 2 $\mu$l of distilled water on leaf discs. Larvae were allowed to feed on discs for 24 hr and were then released in numbers of one per plant on the last leaf at the bottom of the plant. Extra control and
treated larvae were maintained feeding on the crop as back ups for replacements.

Assessment started one day after the release. Whenever the released larva was not found during two consecutive assessments, the larva was replaced in the position where it was found the last time. Replacements were only made during the first 3 days of the experiment. After that time no new releases were made.

The total number of expanded leaves of each plant at the moment of the release was recorded. The assessments were done twice a day (morning and evening) and the total number of expanded leaves of each plant, larval stage and larval position were recorded each time.

Plants for this experiment (grafted tomato “Rapshodie”, sweet bell pepper and English cucumber) were donated by Houweling Nursery Limited, and were brought into the UBC greenhouse and maintained on a flooding bench provided with 120 ppm of 15-5-15 Cal-Mg Scotts Petters Excel® fertilizer for the rest of the experiment. Plants with at least 6 expanded leaves were used for the experiment and were potted in 1 gallon plastic pots. Plants were strung up with cotton threads from the GH structural wires in order to maintain plants upright and to avoid instability of the pots caused by bench flooding. An unfolded paper bag was placed at the bottom of each plant to give falling larvae the opportunity to climb up again to the plant. The last expanded leaf on each plant at the moment of the release was tagged with colored flagging tape. To record the position of larvae, new expanded leaves were indicated relative to the leaf number above the last expanded leaf at the moment of the release, e.g., Lf–1 was the first expanded leaf above the color flagging tape, and Lf–2 indicated the 2nd newly expanded leaf above the
colored flagging tape. Plants were separated 1 m from each other to prevent larvae from moving onto another plant. Fifteen control and treated plants were used for each replicate. Plants hosting virus infected caterpillars and control larvae were arranged alternately on the bench.

Two replicates were conducted for each crop, but each crop and replicate was tested at a different time. A contingency table was used to analyze the data. Each replicate and crop was analyzed separately. The null hypothesis was that the position of the larva on the plant would be independent of treatment (control vs. virus).

Results

For all crops and replicates I fail to reject the null hypothesis, indicating that the larval position on the plant was independent of the virus treatment (Table 5.3). Infected larvae behaved in the same way as the control larvae. However, a greater proportion of the larvae were found on the middle of the plants in cucumber crop ($\chi^2_{cucumber1} = 53.6$, $p<0.0001$; $\chi^2_{cucumber2} = 30.16$, $p<0.0001$), bottom of the plant in tomato crop ($\chi^2_{tomato1} = 56.16$, $p<0.0001$; $\chi^2_{tomato2} = 69.7$, $p<0.0001$) and top of the plants in pepper crop ($\chi^2_{pepper1} = 25.5$, $p<0.0001$; $\chi^2_{pepper2} = 6.13$, $p=0.045$). In the case of the cucumber and pepper crops there was a trend for larvae to start moving up after the release while in the tomato crop larvae mainly remained on the leaf where they had been released. However, in the pepper crop the pattern was more diverse with larvae feeding up to the very top of the plant and most leaves had bites taken from them. Overall, approximately 20% of larvae were replaced in the first 3 days. Mostly all larvae became established when they reached the 4th instar. A 90-100% mortality was achieved in the virus treatment between 5 and 9
days after infection, and when that happened the experiment was terminated.

5.3 Discussion

Larval growth and feeding behaviour of *T. ni* differed considerably on the three crops under study. As tomato and pepper belong to the same family, Solanaceae, these host plants were expected to be more similar than cucumber that is in the family Cucurbitaceae. However, this was not the case. The three plant species differ in their nutritional requirements and growth rates. Hydroponic solutions for each crop contain different amounts of macronutrients such as nitrogen, phosphorous and potassium, as well as calcium, magnesium, sulfate, manganese, boron, and copper\(^2\). In commercial greenhouses, pepper plants are grown at higher densities per square meter (3.3 – 3.5 pl/m\(^2\))\(^2\) followed by tomato (2.2-2.5 pl/m\(^2\))\(^2\) and cucumber (1.2-1.4 pl/m2)\(^2\). The planting density can be used as an indirect measurement of the crop growth rate, the higher the density the slower the growth of the crop. Besides, cucumber greenhouses can have 2-3 crops during the year while tomato and pepper greenhouses have only one. Interestingly, *T. ni* growth follows the crop growth rate. In addition, the three crops have different architectures, leaf morphologies and chemical compositions (Everett 1981, Atherton and Rudich 1986, Bosland and Votava 2000, Choudhury and Copland 2003). Cucumber plants have alternate undivided palmately lobed leaves and branched or branchless tendrils (Everett 1981). Tomato plants have compound leaves arranged alternately. Each leaf has 1 terminal leaflet and up to 8 large lateral leaflets with smaller folioles

interspersed with the large leaflets. Leaflets are irregularly lobed with toothed edges and covered with glandular and non-glandular hairs (Atherton and Rudich 1986). Pepper plants are more compact and more erect than tomato plants and have shiny, glabrous simple leaves (Bosland and Votava 2000). Choudhury and Copland (2003) showed that the lamina of cucumber leaves had 1.5 times and 5 times more hairs than the same surface of tomato and pepper leaves respectively. However all the hairs were non glandular in the case of cucumber, while 100% were glandular in pepper leaves and only 33% were glandular in tomato. The dominant secondary compounds present in cucumber, pepper and tomato plants are cucurbitacins, capsaicinoids and tomatine respectively. Heterogeneity in leaf morphology, leaf size, nutritional chemistry and secondary chemistry can affect plant-herbivore interactions (Denno and Mc Lure 1983, Price 1991, Jones et al. 1993, Suomela et al. 1995, Hartley and Jones 1997).

Among the experiments conducted, the larval weight gained and the development time of *T. ni* were higher and faster on cucumber leaves followed by tomato and pepper (see Appendix 7). These results agree with (Janmaat 2004 and Janmaat and Myers 2005). However, Sutherland (1966) found a great difference in the development time of *T. ni* among crucifers and cucumber and peppers, although he was unable to detect differences between cucumber and tomato. He found that it took *T. ni* approximately 24 days to develop from egg to adult on cucumber and tomato and 32 days on pepper (no temperature mentioned).

Water and nitrogen are fundamental requirements in insect nutrition and are an important component of tissues (Wigglesworth 1984). According to Bernays and Chapman (1994)
caterpillars prefer food with high water content, and given a choice of food of the same species they will tend to select the foliage with the highest water content. These findings are in accordance with the results obtained from the movement experiment and the visual monitoring of larvae in greenhouses together with leaf water content measurements for *T. ni* feeding on tomato and cucumber plants. On those crops, larvae mainly foraged in the position in which leaves had the greater water content. This was not the case however for larvae on pepper plants. Despite the fact that differences in water content of tomato, pepper and cucumber foliage were detected, those differences were not greater than 10% independent of the position. Therefore, water alone was not sufficient to explain the great differences in consumption and behaviour observed in the experiments. However, it partially explained the differences in development time given that larvae ate almost 4 times greater mass of cucumber leaves, and consequently water, than tomato or pepper leaves after seven hours.

Given that water alone cannot fully explain the differences in behaviour and consumption rates of larvae, plant chemistry must be responsible for this differential behaviour. The amount of primary and secondary compounds in plants can be regulated by the addition of supplemental nutrients. Fertilization typically increases foliar nitrogen levels (Minkenburg and Ottenheim 1990, Estiarte et al. 1994, Wilkens et al. 1996) and decreases the leaf phenolic content (Estiarte et al. 1994, Orians et al. 2002). Phenolics are widely considered deterrents, antifeedants or toxins that can change the nutritional quality of plant tissues for herbivores (Lambers 1993). Consequently, differences in foliar nitrogen can have big effects on herbivore behaviour and growth (Minkenburg and Ottenheim 1990).
Capsaicinoids are phytochemicals present in pepper plants. They are the phenols associated with the pungency found in hot peppers and are synthesized by the placenta in the fruits, although they have also been found in the stems and leaves of the plants when fruits are present (Estrada et al. 2002). Estiarte et al. (1994) showed that pepper plants fertilized with higher levels of nitrogen had a lower content of phenolics, a higher quality of leaf and *Helicoverpa armigera* ate more on fertilized plants. Raven and Smith (1976) stated that the allocation of nitrates taken up by the root system is mainly to the shoots. This generates an increased concentration of nitrogen and depletion of carbon in growing points that retards the production of lignin and phenols in shoots and favours it in the roots. These findings might be useful to explain why cabbage loopers mainly forage in younger leaves (top position) of pepper plants that might have greater digestibility and palatability than older leaves.

The most remarkable secondary compounds present in cucumber are the tetracyclic triterpenoids: cucurbitacins. Cucurbitacins are responsible for the bitter taste of leaves, fruits and roots in Cucurbitaceae. The amount of cucurbitacins in fruits is affected by the addition of nitrogen. Kano and Goto (2002) showed that the occurrence of bitterness in cucumber fruits was higher in plants cultivated with twice as much nitrogenous fertilizer than control plants. Cucurbitacins are thought to be potent feeding deterrents for insects not adapted to them (Tallamy et al. 1997), but they are also well known as kairomones for the cucumber beetles *Diabrotica* (Chrysomelidae: Galerucinae: Luperini) in which they promote compulsive feeding (Chambliss and Jones 1966, Sharma and Hall 1973a, Metcalf et al. 1980, Ferguson et al. 1983). Tallamy (1997) found that exogenous addition of cucurbitacins to cucumber leaf discs did not deter the cabbage looper and stated this
might be an adaption from previous exposure of low doses of cucurbitacins in crucifers. It might be that cucurbitacins together with other compounds act as phagostimulants for the cabbage looper. It might also be that the consumption levels on cucumber are a measure of the maximum feeding potential for T. ni, and secondary compounds in pepper and tomato plants are acting as feeding deterrents.

The foliage and fruit of the tomato plant also contain polyphenol oxidases and peroxidases (Felton et al. 1989). These tomato foliar enzymes oxidize an array of endogenous compounds including caffeic acid, chlorogenic acid, rutin, cumaric acid, cinnamic acid and guaiacol (Felton and Duffey 1991). The oxidized forms of these compounds, quinones, react with amino acids that contain nucleophilic centres (-SH, NH2); this reaction (alkylation) reduces the digestibility of dietary protein and the bioavailability of amino acids (Felton et al. 1989). Felton and Duffey (1991) and Felton et al. (1992) reported reduced larval growth of some lepidopteran species when tomato foliar protein was pretreated with peroxidase and chlorogenic acid and incorporated in artificial diet. However, when the amount of alkylatable amino acids is significantly greater than the phenolic concentration, the reduction in larval growth is negligible (Felton et al. 1992). Insects adapted to tomato feeding can also cope with the detrimental effects of oxidative enzymes with the aid of catalase. Catalase activity was detected in the midgut tissues and regurgitate of several lepidopteran pests of the tomato plant and when purified catalase was added to tomato foliage, the peroxidase activity was eliminated and the leaves were superior as larval food compared to untreated foliage (Felton and Duffey 1991). In spite of these proposed mechanisms, I was unable to find differences in larval growth among the colony originally adapted to tomato plants compared to the ones
coming from pepper and cucumber. Furthermore there was a trend for the pepper colony to grow faster when exposed to the tomato crop (Appendix 7).

It is important to note that cabbage loopers seem to feed without problems inside commercial greenhouses and they can cause considerable damage to all of the crops. As previously stated, fertilization can greatly affect the leaf chemistry composition and therefore the ability of the pest to forage on the crop. In commercial greenhouses plants have balanced nutrition throughout the year and macro and micronutrients are supplied together with adequate and constant amounts of water and increased CO₂. Elevated CO₂ tends to increase photosynthetic rates, plant growth, and C:N ratio due to a decrease in nitrogen content and an increase in the availability of carbon (soluble sugars, starch) that induces the accumulation of carbon-based defenses (Cure and Aycock 1986, Bazzaz 1990, Kimball et al. 1994, Williams et al. 1998, Chen et al. 2005). Osbrink et al. (1987) reported that leaf-chewing insect herbivores exhibited compensatory increases in foliar consumption rate or a delay in development when reared on plants grown with elevated CO₂ environments. Orians et al. (2002) demonstrated that nitrogenous fertilization applied to isolated lateral roots in tomato generates heterogeneity in leaf morphology, phenolic chemistry and side-shoot growth. Leaflets in direct connection to the supplemented roots were larger and had lower levels of rutin and chlorogenic acid than did leaflets lacking direct vascular connection. An overall assay of foliar chemical compounds (primary and secondary chemistry) is essential to understanding the complex relationship among specific compounds (Duffey and Isman 1981, Isman and Duffey 1982, Felton et al. 1987).
It is possible that the differences in nutrient supplementation to the plants in the
Horticulture greenhouse at UBC are responsible for the differences in behaviour and
pupal weights observed in commercial greenhouses. The pupal weights of individuals
collected in commercial greenhouses were higher than the ones collected after feeding on
foliage from plants grown at the UBC greenhouses (See Table A7.1). In addition the low
weight of pupae grown on plants in the old UBC greenhouse where plants were not
fertilized indicates a potential impact on leaf quality among those plants (Appendix 7).
The leaf weight per cm$^2$ also varied. Weights of leaves collected inside commercial
tomato, cucumber and pepper greenhouses were heavier compared to the ones obtained in
the Horticulture greenhouse. Pupal weights of cabbage loopers in tomato greenhouses
may reflect the consistency in plant quality throughout the year given that they did not
vary in 11 collections made year round. Nevertheless pupal weights of cabbage loopers
collected from commercial tomato greenhouses were higher than those from commercial
pepper greenhouses. Lower pupal weights are associated with reduced fitness (Janmaat
2004, Janmaat and Myers 2005). This indicates that in spite of the balanced nutrition,
pepper plants are a poorer quality resource for the cabbage looper compared to tomato.

Finally, no differences were detected in the behaviour of healthy and virus infected
caterpillars. These results contradict the statements of Evans (1986), Fuxa (1987), and
Moscardi and Carvalho (1993) who reported that infected caterpillars climb to the upper
parts of the plants to die and the findings of Vasconcelos et al. (1996) in *Mamestra
brassicae*, the cabbage moth, and Wahl 1909 (n.s., cited in Vasconcelos 1996) in
*Lymantria monacha*, the nun moth. Both species are at least gregarious at early stages of
their life cycles. The fact that the cabbage looper has no gregarious behaviour and that
only one larva per plant was used in my experiments may indicate that this mechanism is triggered in the presence of other individuals as an ecological adaptation to avoid the contamination of other individuals of the same species. In their absence there is no reason to invest energy to climb up the plants. Given the different foraging behaviour in the three crops under study, climbing to the top of the plants will definitely not be beneficial on pepper where healthy larvae are normally found foraging at the top of the plants. In addition whether a behavioural response is an adaptation will depend on the strength of selection. The occurrence of viral disease in greenhouse populations is low and will not be a strong selective force. Furthermore, as remarked by Myers and Cory (2003), upward movement might not necessarily enhance virus fitness in all hosts.

5.4 Conclusions

Cucumber, tomato and pepper plants differ in quality as food sources for the generalist herbivore T. ni. T. ni grows faster and consumes more foliage in cucumber, followed by tomato and pepper. Moreover, larvae are mainly found foraging in the middle, bottom and top position of cucumber, tomato and pepper plants respectively. This differential behaviour can greatly influence the performance of AcMNPV in commercial greenhouses. I predict better and faster killing in a cucumber crop given the amount of food ingested and the rapid larval growth rate, a longer persistence on tomato crop where the larvae tend to feed at the bottom of the plants, and the highest challenge on pepper crop where it will be difficult to kill young instars given the amount of leaf consumed and where a more frequent application might be necessary to counteract the tendency of the
pest to avoid contact with the viral particles by foraging at the top of the plants.
Fig. 5.1. Average fresh leaf consumed after 7 hours by *T. ni* larvae in three different ranges of weight. Consumption calculated as an average of three colonies fed on three crops. Larval weight range 1 includes larval weights between 0.02 and 0.05 grams of body weight, range 2 between 0.06 and 0.09 grams of body weight and range 3 between 0.1 and 0.15 grams of body weight. F=46.7, p<0.001, N = 254, df_{model}=18, 236, q = 2.36,

![Graph showing leaf consumption by larval weight ranges](image)

\(\alpha = 0.05\)
Fig. 5.2. Average fresh leaf consumed in milligrams after 7 hours by *T. ni* larvae fed on three different crops: cucumber (C), pepper (P) and tomato (T). Consumption calculated as an average of all the colonies for all larval weights. $F=111.86$, $p<0.001$, $N = 254$, $df_{\text{model}}=18, 236$, $q = 2.35$, $\alpha = 0.05$
Fig. 5.3. Average leaf area consumed in cm$^2$ after 7 hours by *T. ni* larvae fed on three different crops: cucumber (C), pepper (P) and tomato (T). Consumption calculated as an average of all the colonies for all larval weights. $F=279.3$, $p<0.001$, $N = 254$, $df_{model}=18$, $236$, $q= 2.36$, $\alpha=0.05$. 
**Fig. 5.4.** Average leaf area consumed in cm$^2$ after 7 hours by *T. ni* larvae in three different ranges of weight. Consumption calculated as an average of three colonies fed on three crops. Range 1 includes larval weights between 0.02 and 0.05 grams of body weight, range 2 between 0.06 and 0.09 grams of body weight and range 3 between 0.1 and 0.15 grams of body weight. Different letters indicate significant differences after Tukey test. $F=62.96$, $p<0.001$, $N = 254$, $df_{model}=18$, 236, $q = 2.36$, $\alpha =0.05$
Table 5.1. Water content experiment results per crop. Statistical analyses were done with an ANOVA in JMPin 4.04 and confidence intervals were used to determine differences among positions within crops. Values are given as mean ± SEM. Different letters indicate significant differences among positions within crop.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Fresh disc weight (mg)</th>
<th>Water content (%)</th>
<th>Water (mg) / dry matter (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>top</td>
<td>middle</td>
<td>bottom</td>
</tr>
<tr>
<td>Cucumber</td>
<td>32.46 ± 0.47 a</td>
<td>37.13 ± 0.48 b</td>
<td>39.84 ± 0.44 c</td>
</tr>
<tr>
<td></td>
<td>82.5 ± 0.3 a</td>
<td>80.8 ± 0.3 b</td>
<td>79.7 ± 0.4 b</td>
</tr>
<tr>
<td></td>
<td>5.1 ± 0.1 a</td>
<td>4.8 ± 0.1 a</td>
<td>4.2 ± 0.1 b</td>
</tr>
<tr>
<td>statistics</td>
<td>F = 67.7, p &lt; 0.0001, df = 2, 514</td>
<td>F = 25.01, p &lt; 0.0001, df = 2, 514</td>
<td>F = 19.4, p &lt; 0.0001, df = 2, 514</td>
</tr>
<tr>
<td>Tomato</td>
<td>53.49 ± 0.73 a</td>
<td>63.85 ± 0.83 a</td>
<td>64.04 ± 1.01 b</td>
</tr>
<tr>
<td></td>
<td>82.9 ± 0.2 a</td>
<td>85.9 ± 0.2 b</td>
<td>88.0 ± 0.3 c</td>
</tr>
<tr>
<td></td>
<td>5.1 ± 0.1 a</td>
<td>6.4 ± 0.1 b</td>
<td>7.6 ± 0.1 c</td>
</tr>
<tr>
<td>statistics</td>
<td>F = 46.0, p &lt; 0.0001, df = 2, 595</td>
<td>F = 126.1, p &lt; 0.0001, df = 2, 595</td>
<td>F = 181.4 p &lt; 0.0001, df = 2, 595</td>
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<tr>
<td>Pepper</td>
<td>44.1 ± 0.54 a</td>
<td>48.1 ± 0.54 b</td>
<td>52.9 ± 0.53 c</td>
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<td></td>
<td>81.1 ± 0.3 a</td>
<td>83.1 ± 0.2 b</td>
<td>83.4 ± 0.2 b</td>
</tr>
<tr>
<td></td>
<td>4.6 ± 0.1 a</td>
<td>5.1 ± 0.1 b</td>
<td>5.1 ± 0.1 b</td>
</tr>
<tr>
<td>statistics</td>
<td>F = 64.8, p &lt; 0.0001, df = 2, 596</td>
<td>F = 25.94, p &lt; 0.0001, df = 2, 596</td>
<td>F = 15.24, p &lt; 0.0001, df = 2, 596</td>
</tr>
</tbody>
</table>
Table 5.2. Percentage of cabbage looper instars found at top, middle and bottom portion of tomato and pepper plants in commercial greenhouses for each month of the year.

<table>
<thead>
<tr>
<th>Month</th>
<th>Crop</th>
<th>Top %</th>
<th>Middle %</th>
<th>Bottom %</th>
<th># Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tomato</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JUN</td>
<td></td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>JUL</td>
<td></td>
<td>0</td>
<td>29</td>
<td>71</td>
<td>51</td>
</tr>
<tr>
<td>AUG</td>
<td></td>
<td>16</td>
<td>30</td>
<td>54</td>
<td>202</td>
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<td></td>
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<td>22</td>
<td>13</td>
<td>31</td>
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*1: 60% of total individuals observed on top position were eggs and first instars

*2: 86% of total individuals observed on top position were eggs and first instars

*3: 65% of total individuals observed on top position were eggs and first instar.
Table 5.3. Percentage of cabbage looper larvae treated with AcMNPV, “Virus” or untreated “Control”, found on top, middle or bottom position of cucumber, pepper or tomato plants.

<table>
<thead>
<tr>
<th>Cucumber #1</th>
<th>N</th>
<th>%</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>top</td>
<td>15</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>middle</td>
<td>107</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>bottom</td>
<td>49</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>VIRUS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>top</td>
<td>6</td>
<td>5</td>
<td>$X^2 = 3.89, p =0.14$</td>
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<tr>
<td>middle</td>
<td>105</td>
<td>70</td>
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</tr>
<tr>
<td>bottom</td>
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<table>
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<th>Statistical analysis</th>
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</tr>
<tr>
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<td>4</td>
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<td>middle</td>
<td>20</td>
<td>18</td>
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<tr>
<td>bottom</td>
<td>86</td>
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<td></td>
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</tr>
<tr>
<td>top</td>
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<td>3</td>
<td>$X^2 = 0.99 p =0.61$</td>
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<td>bottom</td>
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<table>
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<td>bottom</td>
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<tr>
<td>VIRUS</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>top</td>
<td>119</td>
<td>63</td>
<td>$X^2 = 4.61 p =0.10$</td>
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<td>middle</td>
<td>50</td>
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Continued Table 5.3.

<table>
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<td>6</td>
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<tr>
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<td>middle</td>
<td>65</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>VIRUS</td>
<td>top</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>89</td>
<td>82</td>
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<td>15</td>
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</tbody>
</table>

<table>
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<th>Tomato # 2</th>
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<th>Statistical analysis</th>
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<td>2</td>
</tr>
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<td>20</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td>111</td>
<td>83</td>
</tr>
<tr>
<td>VIRUS</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>middle</td>
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<td>13</td>
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<td></td>
<td>bottom</td>
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<td>87</td>
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</table>

* After grouping top and middle in the same category to avoid suspect $X^2$ (more than 20% of data below 5)

<table>
<thead>
<tr>
<th>Pepper # 2</th>
<th>N</th>
<th>%</th>
<th>Statistical analysis</th>
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</thead>
<tbody>
<tr>
<td>CONTROL</td>
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<td>78</td>
<td>50</td>
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<tr>
<td></td>
<td>middle</td>
<td>42</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td>38</td>
<td>24</td>
</tr>
<tr>
<td>VIRUS</td>
<td>top</td>
<td>65</td>
<td>50</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>bottom</td>
<td>23</td>
<td>19</td>
</tr>
</tbody>
</table>
5.5 References


Duffey, S. S., and M. B. Isman. 1981. Inhibition of insect larval growth by phenolics in


Chapter 6

Conclusions

The fact that the cabbage looper, *Trichoplusia ni* (*T. ni*), can overwinter inside greenhouses and cope with the insecticides applied during the clean-up process poses a great risk for continuing the resistance to *Bacillus thuringiensis (Bt)*.

*Autographa californica* nucleopolyhedrovirus (AcMNPV) has a great potential to be implemented for the management of *Bt* resistance in a rotation application strategy in greenhouses given that no cross-resistance was detected.

Despite the fact that susceptibility of *T. ni* to the virus did not vary with the host plant, the differences in behaviour and foraging capacity shown by the pest on cucumber, tomato and pepper indicate that specific doses and application schedules might be required for each crop to fit with the different pest-crop interactions.

Future work

Further research should be done to explore the differences in efficacy, speed of kill, persistence and horizontal transmission of *Autographa californica* nucleopolyhedrovirus in experimental and commercial greenhouses. Another area of interest is the different chemical composition of leaves of the three crops that might relate to differences in pest behaviour. Finally, studying the ability of resistant cabbage loopers to overcome the clean-up process and understanding the adult immigration dynamics to greenhouses are essential to manage the resistance to *Bt* in protected crops.
Appendix 1

Mass rearing of cabbage loopers

The cabbage looper colonies used for the experiments described in this thesis were reared from egg hatching until adulthood through mass rearing in a controlled temperature room kept at 26°C ±1 °C and 16:8 hr (L:D) on semi-synthetic artificial diet (see Annex 2) unless specified.

Eggs were surface sterilized by spraying with a 0.2% sodium hypochlorite solution. Egg sheets were placed inside 4 L plastic buckets. After neonates hatched, 15 neonates were transferred to 170 ml Styrofoam cups filled with 20 ml of artificial diet. A new container with fresh artificial diet was provided almost every week, depending on the season of the year. Larvae were kept inside these cups until pupation. Pupae were collected, their silky cocoons removed and they were finally soaked in a 0.6% sodium hypochlorite solution for about 6-10 minutes. Pupae were then rinsed in distilled water and let to dry on a paper towel. Pupae, (70-100) for the first two years and (150-200) for the last year, were placed in cylindrical wire mesh cages (Ignoffo 1963) wrapped with paper towel as an oviposition substrate. A 10% sucrose solution was provided inside the cage for adult feeding. Egg sheets were collected every 2-3 days after females started laying eggs. After bleaching the egg sheets, and once they dried at air temperature, egg sheets were kept inside a refrigerator at 9°C until used.
Appendix 2

Artificial Diet Recipe A.2.1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g)</th>
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<tbody>
<tr>
<td>Vanderzant vitamin mix</td>
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<tr>
<td>Wheat oil germ</td>
<td>2 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>500 ml</td>
</tr>
<tr>
<td>Streptomycin sulphate</td>
<td>0.06 g</td>
</tr>
<tr>
<td>Agar</td>
<td>8.25 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>365 ml</td>
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</tbody>
</table>

Preparation of Dry Mix A.2.2

Keep refrigerated until used

<table>
<thead>
<tr>
<th>Ingredient</th>
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</thead>
<tbody>
<tr>
<td>Wheat germ</td>
<td>420</td>
</tr>
<tr>
<td>Cellulose</td>
<td>252</td>
</tr>
<tr>
<td>Wesson salt mix</td>
<td>84</td>
</tr>
<tr>
<td>Casein</td>
<td>294</td>
</tr>
<tr>
<td>Sucrose</td>
<td>154</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>25</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>28</td>
</tr>
<tr>
<td>Sodium alginolate</td>
<td>42</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>10</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td>80</td>
</tr>
</tbody>
</table>

Preparation procedure

1- Bring 365 ml of water to boil in a pot.
2- Add 8.25 g of Agar
3- In a separate bowl mix the rest of the ingredients listed in table A2.1
4- Mix thoroughly with a mixer and add mixture into the pot with the agar. Turn oven to minimum temperature.
5- Mix everything again with the mixer and start filling the cups.
6- Place the cups with a 30 degrees angle of inclination on the border of a tray until diet cools and solidifies.
Appendix 3

Fig. A.3.1 Outside minimum temperatures (in °C) between December 1st and January 10th for the two consecutive winters 2002/2003 and 2003/2004

Winter 02/03: Max Temp: 13.5°C - Min Temp: -2.9°C - Mean Min Temp: 2.7°C - Mean Average Temp: 5.5°C

Winter 03/04: Max Temp: 11.9°C - Min Temp: -12.2°C - Mean Min Temp: 0.4°C - Mean Average Temp: 3.3°C
Fig. A.3.2 Outside average temperatures (in °C) between December 1st and January 10th for the two consecutive winters 2002/2003 and 2003/2004
Appendix 4
Level of Resistance to Bt in GIP colony

The level of resistance to Bt of the two GIP populations (GIP-Resistant and GIP-Susceptible) was determined by a bioassay carried out before running the cross-resistance experiment. This bioassay was conducted by Alida Janmaat and Jessamyn Manson, as part of another experiment. Six day old larvae of both colonies were exposed to increasing doses of Bt. The bioinsecticide was diluted and added to the artificial diet on which larvae were put to feed. Larvae were let to feed on that diet for 48 hr, and after that period, the larval mortality was recorded. The table A.4.1 shows the results of the Probit analysis for both colonies. GIP-Resistant is 16.5 times more resistant to Bt than the susceptible population. Off-spring of the same parents tested in this bioassay were used for the cross resistance experiments detailed in Chapter 3.

Table 4.1

<table>
<thead>
<tr>
<th>Colony</th>
<th>LC50 (IU/ml)</th>
<th>Lower 95% (IU/ml)</th>
<th>Upper 95% (IU/ml)</th>
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<tr>
<td>GIP-S</td>
<td>2180</td>
<td>1574</td>
<td>3018</td>
</tr>
<tr>
<td>GIP-R</td>
<td>35846</td>
<td>25840</td>
<td>49728</td>
</tr>
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</table>
Appendix 5

Viral counting

1. A haemocytometer was used for viral counting.

2. A viral solution diluted 10 times from the original stock (1/10) was used to proceed with counting.

3. The 1/10 solution was mixed with a Vortex machine before each new counting.

4. 10 µl of 1/10 solution was added with a micropipette on to each side of the haemocytometer.

5. 5 squares of 0.2 mm were counted at a time per side and an average was calculated. This procedure was repeated 10 times. A grand average was calculated for the ten time countings.

<table>
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<th></th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
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<td>27</td>
</tr>
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<td>32</td>
<td>25</td>
<td>31</td>
<td>25</td>
<td>22</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>Sq 5</td>
<td>32</td>
<td>34</td>
<td>30</td>
<td>37</td>
<td>36</td>
<td>32</td>
<td>26</td>
<td>20</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>Average (# PIBs)</td>
<td>31.4</td>
<td>27.8</td>
<td>29.2</td>
<td>33.2</td>
<td>31</td>
<td>31.2</td>
<td>21.4</td>
<td>20.8</td>
<td>21</td>
<td>20</td>
</tr>
</tbody>
</table>

**Grand Average = 26.7 PIBs**

6. Cell density (per ml) was calculated as the cell density (# PIBs counted)/grid volume.

7. Grid volume was calculated with the following formula= 0.2 x 0.2 x 0.1 mm grid = 4 x 10⁻⁶ ml.

Cell density (per ml) = 26.7 PIBs/ 4 x 10⁻⁶ ml = 6.7 x 10⁶ PIBs/ml in a 1/10 solution.

Therefore, the density in the stock is: 6.7 x 10⁶ PIBs/ml x 10 = 6.7 x 10⁷ PIBs/ml
Appendix 6

Virus dilution procedure

1. Stock $3.8 \times 10^8$
2. 1/10 200 µl of solution 1 in 1.8 ml of distilled water
3. 1/50 800 µl of solution 2 in 3.2 ml of distilled water
4. 1/100 2000 µl of solution 3 in 2.0 ml of distilled water
5. 1/500 800 µl of solution 4 in 3.2 ml of distilled water
6. 1/1000 2000 µl of solution 5 in 2.0 ml of distilled water
7. 1/2000 1600 µl of solution 6 in 2.4 ml of distilled water
8. 1/5000 800 µl of solution 7 in 3.2 ml of distilled water
9. 1/10000 2000 µl of solution 8 in 2.0 ml of distilled water
10. 1/20000 1600 µl of solution 9 in 2.4 ml of distilled water
11. 1/50000 800 µl of solution 10 in 3.2 ml of distilled water
12. 1/100000 2000 µl of solution 11 in 2.0 ml of distilled water
13. 1/200000 2000 µl of solution 12 in 2.0 ml of distilled water
14. 1/400000 1000 µl of solution 13 in 3.0 ml of distilled water

Initial dilutions  Final dilutions
(after correction by counting)

1. $3.8 \times 10^8$ PIBs/ml  6.7 x $10^7$ PIBs/ml
2. $3.8 \times 10^7$ PIBs/ml  6.7 x $10^6$ PIBs/ml
3. $7.6 \times 10^6$ PIBs/ml  1.3 x $10^6$ PIBs/ml
4. $3.8 \times 10^6$ PIBs/ml  6.7 x $10^5$ PIBs/ml
5. $7.6 \times 10^5$ PIBs/ml  1.3 x $10^5$ PIBs/ml
6. $3.8 \times 10^5$ PIBs/ml  6.7 x $10^4$ PIBs/ml
7. $1.9 \times 10^5$ PIBs/ml  3.3 x $10^4$ PIBs/ml
8. $7.6 \times 10^4$ PIBs/ml  1.3 x $10^4$ PIBs/ml
9. $3.8 \times 10^4$ PIBs/ml  6.7 x $10^3$ PIBs/ml
10. $1.9 \times 10^4$ PIBs/ml  3.3 x $10^3$ PIBs/ml
11. $7.6 \times 10^3$ PIBs/ml  1.3 x $10^3$ PIBs/ml
12. $3.8 \times 10^3$ PIBs/ml  6.7 x $10^2$ PIBs/ml
13. $1.9 \times 10^3$ PIBs/ml  3.3 x $10^2$ PIBs/ml
14. $0.95 \times 10^3$ PIBs/ml  1.7 x $10^2$ PIBs/ml

Doses 5, 7, 11 and 13 were used in susceptibility experiments for TOMF3 and GLEN colonies.
Doses 7, 8, 9, 10 and 11 were used in susceptibility experiments GIP colony as well as in the cross-resistance experiments.
Appendix 7

Larval growth and pupal weights on different host plants

Experiment 1

Is the larval growth the same on the three crops and does the relationship vary with the background experience of the colony?

Three colonies of cabbage loopers were tested in this experiment: RC (laboratory colony), GIP (originally collected from a Tomato GH) and GLEN (originally collected from a Pepper GH). All of these colonies had been maintained in the laboratory on artificial diet for several generations, however the generation previous to the onset of the experiment they were reared on tomato plants.

Three crops: tomato (*Lycopersicum esculentum*), pepper (*Capsicum annuum*) and cucumber (*Cucumis sativus*) were used to feed the larvae throughout the experiment. Plants were grown from seeds in the UBC greenhouse using the same varieties and growing techniques explained in previous chapters. Leaves were collected from the plants without discriminating any leaf stratum.

Eggs (4 day old) were put to hatch and neonates were collected after 2 days.

Fifteen neonates were placed inside 455 ml paper cups provided with one leaf of tomato, pepper or cucumber. Twenty-five cups per colony and per crop were prepared. Cups were maintained at 26°C ± 1°C inside a controlled temperature room. After 3 days larvae were randomly selected and transferred to Styrofoam cups provided with a crop leaf using the
hanging leaf technique (See Appendix 8 for hanging leaf rearing technique). Ten cups with 3 larvae per treatment were prepared. The larval weights after 5 days were recorded and results are shown in Table A7.1. Data were normally distributed so a factorial analysis was performed with crop and colony as factors. The existence of interactions among those factors was explored. The average larval weight per cup was taken into account for statistical purposes to avoid pseudoreplication. Crop leaves were replaced every other day and larvae were maintained until pupation.

Results

The factorial analysis produced a significant model (F=139.8, p < 0.0001, df= 8, 76). Larval weights varied with food plant (F= 549.33, p < 0.0001, df=2) but colonies did not differ significantly (F=1.79, p=0.1735, df=2) and almost no significant interactions occurred between colonies and food plants (F= 2.49, p= 0.051, df =4). Because colonies did not differ, larval weight data were combined for each of the crops. The results obtained with the ANOVA and Tukey test are shown in Table A.7.1. Overall, after 5 days of feeding on the same crop, cucumber larvae weighed 3.9 times more than tomato larvae and 15.2 times more than pepper larvae. At day 10, larvae in the cups containing cucumber leaves entered prepupation. Only one piece of cucumber leaf was insufficient to maintain 3 large larvae and therefore larvae in the cucumber treatment began pupating before they reached their maximum potential. None of the individuals fed on pepper survived until pupation. On tomato only 3 larvae pupated for GLEN colony and 2 for GIP colony. No pupae were collected from RC colony on tomato.

The factorial analysis for pupal weights was significant (F2.9, p=0.025, df = 5, 36)
Differences between male and female pupal weights of larvae reared on cucumber leaves were detected (F = 9.47, p=0.004, df= 1, 39) and no significant differences were found among colonies (F= 0.34, p= 0.714, df= 2, 38) nor did significant interactions occur between sex and colony (F=0.57, p = 0.5687, df= 2, 38). The mean pupal weight ± SEM of females and males was 170.7 mg ± 5.3 mg and 205.4 mg ± 8.1mg respectively.

**Experiment 2**

*Is artificial diet a better food source than cucumber, tomato and pepper leaves and does previous exposure to the host plant influence this?*

Larvae of GLEN, GIP and RC were released on plants of the three crops and maintained in an old UBC greenhouse facility irrigated manually with water only. After one generation, pupae were collected and caged and offspring were used to set up a new experiment. Pupae of GLEN colony were collected from cucumber and pepper plants, pupae of GIP colony were obtained only from tomato plants and pupae of RC were collected from cucumber and tomato plants.

I tested each colony on artificial diet and also on the crop they had fed on previously. For example: GLEN was tested on cucumber (GLEN-C-F1) and pepper foliage (GLEN-P-F1) while RC was tested on cucumber (RC-C-F1) and tomato (RC-T-F1) and GIP was tested on tomato (GIP-T-F1). For the artificial diet, 80 larvae per colony were maintained individually in 30 ml plastic cups with 2.5 ml of artificial diet. Diet was replaced every 5 days until prepupation. In the case of crops, 90 larvae were maintained individually in 50 ml plastic cups provided with a piece of crop leaf until they reached the second instar.
After this, larvae were transferred to 170 ml Styrofoam cups provided with a leaf of crop with the hanging leaf technique. Leaves were collected from the bottom, middle and top portion of plants in the case of tomato, cucumber and pepper plants respectively according to the foraging behaviour reported by growers in a survey conducted at the beginning of my project. Both types of cups were kept inside a tray supplied with a damp paper towel on the bottom and a lid on top of the tray to prevent leaf desiccation. Leaves were replaced every other day. Larval weight was recorded at day 5. Larvae were checked every other day until pupation. Pupal weight was recorded.

Results

The analysis within colony performed with t-test yielded significant differences between artificial diet and each of the crops (Table A.7.2). Weights of larvae fed on artificial diet were greater than on pepper and tomato except for GIP TOM F1 colony for which the trend was the same but the difference was not significant. The opposite was true for larvae fed cucumber leaves that were significantly heavier than those fed artificial diet.

Differences were found among colonies on artificial diet (F = 14.83, p<0.0001, df = 4, 343) after ANOVA and Tukey test for multiple comparisons (Table A.7.3). Larvae on artificial diet from RC parents fed tomato were significantly heavier as were larvae fed artificial diet of GIP parents that had fed on tomato. No significant differences were detected among the other colonies.

A factorial analysis (F = 71.9, df=7, 440, p<0.0001) yielded significant differences in pupal weights between sexes (F_{sex} = 51.4, p < 0.0001, df=1) with higher weights for males.
than females, also differences among crops ($F_{\text{crop}}=139.3$, $p<0.0001$, df= 3) and no interactions between sex and crop ($F_{\text{sex}\times\text{crop}} = 2.1$, $p = 0.102$, df = 3) meaning that the difference in pupal weight by sex was maintained in all the crops. The mean pupal weights (± SEM) were 236.1 mg ± 1.8 mg and 255.8 mg ± 2.0 mg for females and males fed artificial diet, 201.6 mg ± 4.1 mg and 236.3 mg ± 4.4 mg for females and males fed tomato, 197.6 mg ± 8.0 mg and 224.0 mg ± 6.5 mg for females and males fed pepper and 175.4 mg ± 4.4 mg and 192.3 mg ± 3.9 mg for females and males fed cucumber.

Pupal weights and pupal success show differences in food quality. Higher pupal weights are associated with better fitness. These results show that in spite of the faster growth of larvae on cucumber leaves followed by artificial diet, tomato and pepper, pupal weights were higher on artificial diet. Surprisingly pupal weights were also higher on tomato and pepper than on cucumber. However the pupal success was 78% on artificial diet, 45% on cucumber, 24% on tomato and 17% on pepper. This indicates that even when larvae grow more slowly on artificial diet than cucumber this food source ensures the highest pupal weight and survivorship.

**Experiment 3:**

*Does experience modify the larval growth of caterpillars previously adapted to each food source?*

Three colonies collected from cucumber, tomato and pepper greenhouses were maintained on hanging leaves on their parental crops for six, five and six generations respectively. Plants that were grown at the UBC Horticulture greenhouse as explained in
other sections of this thesis were used for this experiment. Leaves were collected from
different parts of the plant according to previously observed behaviour on each crop. In
the case of cucumber, leaves were collected from the middle portion of the plant, for
tomato from the bottom portion of the plant and for pepper from the top of the plants.
Larvae were kept at 24°C ± 1°C and 16L:8D photoperiod inside a Conviron growth
chamber. Thirty larvae were individually maintained in 170 ml Styrofoam cups with the
hanging leaf technique for the crop comparison and in 30 ml plastic cups with 2.5 ml of
artificial diet for the larvae tested on artificial diet. Leaves were replaced every other day
and diet was replaced every 5 days. Larval weights were measured at day 12. Pupal
success was very poor for tomato and pepper treatments. Only one repetition was done;
the pepper colony was lost due to a virus outbreak. The statistical analysis was performed
first with a factorial analysis with colonies and crops as the main factors and their
interactions. Larval weights were ln transformed before the analysis. This greatly
improved the fit to the model. Secondly, an ANOVA and a Tukey test were used to look
for differences among crops and among colonies.

Results

The factorial analysis model was significant (F = 64.6, df= 11, 170, p<0.0001). Larval
weights were significantly different among crops (F = 207.8, p < 0.0001, df=3) and
interactions between crops and colonies were detected (F = 4.8, p = 0.0001, df=6) but no
differences were detected among colonies (F = 1.8, p = 0.16, df=2). This means that not
all the colonies performed the same way in all the crops. The differences among crops
were significant for each of the colonies. There was a trend for larvae to weigh more on
cucumber, followed by artificial diet, tomato and pepper regardless of the colony (Fig. A.7.1 A, B, C). To look for differences due to parental experience in the crops, an ANOVA and Tukey tests were performed on all the colonies for each of the crops (Fig. A.7.2 A, B, C, D.). No significant differences were detected among colonies for larvae kept on the cucumber, pepper and tomato crop (Fig. A.7.2A, A.7.2B, A.7.2C.). However, there was a trend for higher larval weight for larvae coming from the pepper colony on tomato and pepper crops. On artificial diet (Fig. A.7.2D.), larvae coming from the pepper colony were significantly lighter than larvae coming from cucumber and tomato colonies.

For all the colonies, pupation started at day 14 for larvae maintained on cucumber leaves, 16 days for those on diet, 20 days for those on tomato leaves and 28 days for those on pepper. The pupation success varied with the colony however, on average a 62% of success was recorded on cucumber leaves, 78% on artificial diet, 17% on tomato and 0% on pepper except for the colony coming from pepper for which the pupal success was 10%.

**Pupal Weights**

Pupal weights obtained from individuals pupated in greenhouses as well as in the laboratory is given in Table A.7.4 along with the previous history of each colony, the food used for maintenance and the differences among generations. Data were analyzed in JMPin 4.0.4 with the appropriate test for each case.

**Pupae collected from greenhouses**

During visual monitoring for cabbage loopers in greenhouses, pupae were collected and
kept inside a cooler until returned to the laboratory. Pupae were sexed and weighed. A total of 271 pupae were collected from the tomato greenhouse over 11 visits and 29 pupae from the pepper greenhouse on three different visits. Data were normally distributed so they were analyzed in JMP in 4.0.4 with a factorial analysis with sex and date as main effect factors and their interactions.

Overall, no differences were detected on pupal weights among collections in either greenhouse reflecting consistency in the food quality inside greenhouses year round. The pupal weight of individuals obtained from the tomato greenhouses were heavier than the ones collected from the pepper greenhouse and the sex ratio was approximately 50F:50M in both cases. As pupal weight is also indicative of the food quality, these results show that the tomato crop is a better food source than pepper crop for the cabbage looper.

**Pupae collected from colonies maintained in the laboratory**

Pupal weights were recorded from individuals coming from the laboratory colony and also from colonies collected in commercial greenhouses and maintained in the laboratory on artificial diet and/or crops. For all the crops pupal weights obtained when individuals were reared with the hanging leaf technique inside the laboratory were higher than those reared on plants. The fact that individuals were reared individually (without competition) in the cups against 5-6 larvae/plant and that no fertilization was applied to the plants maintained in the old UBC greenhouse where plants were grown might contribute significantly to those differences. However, pupal weights and survivorship rates varied considerably within crops and among colonies and generations with the hanging leaf technique. This might reflect the inability of loopers to compensate for the lack of some
nutrients or the excess of others given that one leaf is arbitrarily assigned to each cup and
the fact that the crop nutrition was not specifically adapted to their continuously changing
requirements.

Table A.7.1. Mean larval weight and SEM (in mg of larvae per cup) of five day old *T. ni*
larvae reared on cucumber (C), tomato (T) and pepper (P) leaves. Different letters
indicate significant differences detected among crops with a Tukey test.

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Crop</th>
<th>Mean (mg)</th>
<th>Std Error (mg)</th>
<th>Tukey</th>
</tr>
</thead>
<tbody>
<tr>
<td>F= 505.4</td>
<td>C</td>
<td>12.49 a</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>p&lt; 0.0001</td>
<td>T</td>
<td>3.17 b</td>
<td>0.34</td>
<td>q=2.39</td>
</tr>
<tr>
<td>df= 2.82</td>
<td>P</td>
<td>0.82 c</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>
### Table A.7.2

Mean larval weight and SEM of 5 day-old larvae of five different *T. ni* populations reared on parental crop and artificial diet. Each colony was analyzed separately. Different letters indicate significant differences between food source within each colony.

<table>
<thead>
<tr>
<th>Colony</th>
<th>ANOVA</th>
<th>Crop</th>
<th>Mean (mg)</th>
<th>Std Error (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC-TOM-F1</td>
<td>$t= 3.53$, $p=0.0006$, $df=121$</td>
<td>D</td>
<td>6.9 a</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>4.8 b</td>
<td>0.4</td>
</tr>
<tr>
<td>GIP-TOM-F1</td>
<td>$t= 0.56$, $p=0.57$, $df=116$</td>
<td>D</td>
<td>5.0 a</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>4.3 a</td>
<td>0.3</td>
</tr>
<tr>
<td>RC-C-F1</td>
<td>$t= 15.44$, $p&lt;0.0001$, $df=125$</td>
<td>D</td>
<td>3.7 b</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>13.1 a</td>
<td>0.4</td>
</tr>
<tr>
<td>GLEN-P-F1</td>
<td>$t= 7.48$, $p&lt;0.0001$, $df=122$</td>
<td>D</td>
<td>3.7 a</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>1.6 b</td>
<td>0.2</td>
</tr>
<tr>
<td>GLEN-C-F1</td>
<td>$t= 22.9$, $p&lt;0.0001$, $df=129$</td>
<td>D</td>
<td>3.7 a</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>14.8 b</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Table A.7.3. Mean larval weight of 5 day-old larvae of five different T. ni populations reared on artificial diet. Different letters indicate significant differences detected with Tukey test for multiple comparisons.

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Colony</th>
<th>Mean (mg)</th>
<th>Std Error (mg)</th>
<th>Tukey</th>
</tr>
</thead>
<tbody>
<tr>
<td>F = 14.83</td>
<td>RC-TOM-F1</td>
<td>6.9.a 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p&lt; 0.0001</td>
<td>GIP-TOM-F1</td>
<td>5.0 b 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>df=4, 343</td>
<td>RC-C-F1</td>
<td>3.7 c 0.3</td>
<td></td>
<td>q=2.74</td>
</tr>
<tr>
<td></td>
<td>GLEN-P-F1</td>
<td>3.7 c 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLEN-C-F1</td>
<td>3.7 c 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure A.7.1. Mean larval weights of 12 day-old *T. ni* larvae from three different colonies and maintained for the experiment on cucumber (C), tomato (T), pepper (P) leaves or artificial diet (D). Different letters indicate significant differences after Tukey test.

**CUCUMBER colony**

![A](image)

**TOMATO colony**

![B](image)

**PEPPER colony**

![C](image)
Figure A.7.2. Mean larval weights of 12 day-old *T. ni* larvae from three different colonies maintained for the experiment on cucumber (C), tomato (T), pepper (P) leaves or artificial diet (D).

**Pepper**

F = 3.06, p = 0.07
df = 2, 24, N = 27

**Cucumber**

F = 0.75, p = 0.474
df = 2, 53, N = 56
Figure A.7.2. (Cont.) Mean larval weights of 12 day-old *T. ni* larvae from three different colonies maintained for the experiment on cucumber (C), tomato (T), pepper (P) leaves or artificial diet (D). Different letters indicate significant differences after Tukey test.

**Tomato**

<table>
<thead>
<tr>
<th>Colony</th>
<th>Cuke</th>
<th>Pep</th>
<th>Tom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean larval weight (mg)</td>
<td>12</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>F=1.12</td>
<td>p&lt;0.3415</td>
<td>df=2,25</td>
<td>N=27</td>
</tr>
</tbody>
</table>

**Artificial diet**

<table>
<thead>
<tr>
<th>Colony</th>
<th>Cuke</th>
<th>Pep</th>
<th>Tom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean larval weight (mg)</td>
<td>150</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>F=8.37</td>
<td>p&lt;0.0006</td>
<td>df=2,67</td>
<td>q=2.40</td>
</tr>
</tbody>
</table>
Table A.7.4. Mean pupal weights ± SEM of different *T. ni* populations maintained under different rearing conditions.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Collected</th>
<th>Crop of Maintenance</th>
<th>T (°C)</th>
<th>Generation</th>
<th>Pupal Weight (mg)</th>
<th>Sex ratio</th>
<th>% Survival</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RC</td>
<td>Laboratory</td>
<td>Artificial diet</td>
<td>26 +/-1</td>
<td>11</td>
<td>244.8 ± 5.7</td>
<td>262.5 ± 5.2</td>
<td>46F:54M</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Tomato GH</td>
<td>Tomato</td>
<td>11 collections year round</td>
<td>F1,F2</td>
<td>243.2 ± 2.2</td>
<td>270.4 ± 3.4</td>
<td>47F:53M</td>
<td></td>
</tr>
<tr>
<td>GLEN</td>
<td>Artificial diet (Pep GH)</td>
<td>Tomato (Plants)</td>
<td>20-35</td>
<td>F1,F2</td>
<td>195.5 ± 5.9</td>
<td>217.3 ± 6.1</td>
<td>52F:48M</td>
<td>15,10</td>
</tr>
<tr>
<td>GIP</td>
<td>Artificial diet (Tom GH)</td>
<td>Tomato (HL)</td>
<td>24</td>
<td>F1</td>
<td>222.8 ± 7.1</td>
<td>252.7 ± 7.9</td>
<td>52F:48M</td>
<td>15</td>
</tr>
<tr>
<td>GIP</td>
<td>Artificial diet (Tom GH)</td>
<td>Tomato (Plants)</td>
<td>20-35</td>
<td>F1</td>
<td>186.0 ± 8.3</td>
<td>214.4 ± 7.8</td>
<td>55F:45M</td>
<td>20</td>
</tr>
<tr>
<td>Tom</td>
<td>Tomato GH</td>
<td>Tomato (HL)</td>
<td>24</td>
<td>F1,F2</td>
<td>220.0 ± 4.9</td>
<td>249.4 ± 5.4</td>
<td>55F:45M</td>
<td>58,27</td>
</tr>
<tr>
<td></td>
<td>Pepper GH</td>
<td>Pepper</td>
<td>3 collections (Apr, Jun, Jul)</td>
<td>F3,F4</td>
<td>205.2 ± 4.8</td>
<td>222.7 ± 3.7</td>
<td>45F:55M</td>
<td></td>
</tr>
<tr>
<td>Pep</td>
<td>Pepper GH</td>
<td>Pepper (HL)</td>
<td>22</td>
<td>F3,F4</td>
<td>204.7 ± 5.8</td>
<td>224.3 ± 4.7</td>
<td>54F:46M</td>
<td>29,25</td>
</tr>
<tr>
<td>Pep</td>
<td>Pepper GH</td>
<td>Pepper (HL)</td>
<td>26</td>
<td>F5,F6</td>
<td>194.3 ± 4.2</td>
<td>205.5 ± 5.5</td>
<td>61F:29M</td>
<td>50,18</td>
</tr>
<tr>
<td>Pep</td>
<td>Pepper GH</td>
<td>Pepper (HL)</td>
<td>24</td>
<td>F3,F4</td>
<td>220.0 ± 4.9</td>
<td>249.4 ± 5.4</td>
<td>55F:45M</td>
<td>58,27</td>
</tr>
<tr>
<td>GLEN</td>
<td>Artificial diet (Pep GH)</td>
<td>Cucumber (HL)</td>
<td>24</td>
<td>F1,F2</td>
<td>221.1 ± 4.1</td>
<td>236.6 ± 5.0</td>
<td>65F:35M</td>
<td>50,29</td>
</tr>
<tr>
<td>Cucumber</td>
<td>Cucumber GH</td>
<td>Cucumber (HL)</td>
<td>24</td>
<td>F1</td>
<td>195.5 ± 9.8</td>
<td>218.3 ± 2.7</td>
<td>57F:43M</td>
<td>34</td>
</tr>
<tr>
<td>Cucumber</td>
<td>Cucumber GH</td>
<td>Cucumber (Plants)</td>
<td>20-35</td>
<td>F1</td>
<td>178.3 ± 6.2</td>
<td>205.5 ± 5.7</td>
<td>46F:54M</td>
<td>48</td>
</tr>
</tbody>
</table>

HL=Individuals reared with the hanging leaf technique, Plants= Individuals grown on potted plants in old UBC greenhouse.
Appendix 8

Hanging leaf technique

Materials:
- L2 instar larvae
- 170 ml Styrofoam cups and plastic lids
- 60 ml plastic cups
- Paper Clips #3
- Plastic Tray with transparent lid
- Damp paper towel

Method:

1- Remove the bottom of the 170 ml Styrofoam cups with the aid of a cutter.

2- Open the paper clips, as it is indicating in the draw, to imitate a hanger shape.

3- Place the damp paper towel on the bottom of the tray.

4- Cut the crop leaves and keep them in a high humidity environment until their usage to prevent desiccation.

5- Hang the piece of or complete leaf by the extreme numbered as “2”

6- Pass the hanger with the leaf by the extreme numbered as “1” through the small hole in the lid (The orifice comes from the manufacturer).

7- Seal the cup with the lid, the leaf will be hanging in the centre of the Styrofoam container.

8- Introduce one L2 larvae from the opened bottom of the cup.
9- Cover the bottom with a 60 ml cup. Invert the container in order to keep the cup in its regular vertical position.

10- Put the container inside the tray.

This technique will avoid the contact of the larvae with the frass while eating. The leaf will remain free of frass. Feces will be collected in the 60 ml cup at the bottom of the container. The leaves of the three crops can be maintained fresh for 2 days or more depending on the rearing temperature.

This system is adequate to rear larvae from second instar on. Neonates are highly susceptible to low humidity conditions, so they could be reared with another technique up to that stage. Neonates are reared, following Alida Janmaat’s technique, individually in 60 ml plastic cups containing a piece of leaf. Cups are covered with plastic transparent lids and placed inside the same trays used for the other technique (with the damp paper towel on the bottom). The piece of leaf is replaced every two days and the paper towel re-damp when required.