### ECOLOGICAL INTERACTIONS BETWEEN THE CABBAGE LOOPER, A PARASITOID, HOST PLANTS AND BACILLUS THURINGIENSIS

by

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### Abstract

The cabbage looper, Trichoplusia ni, is the only lepidopteran causing economic damage to tomato, sweet pepper and cucumber production in greenhouses in British Columbia. Until recently, T. ni was successfully controlled with Bacillus thuringiensis (Bt), but resistance has developed in some T. ni greenhouse populations. Development of resistance has been associated in other studies with fitness costs such as reduced overwintering ability. Here I assess the overwintering survival of Bt resistant and susceptible T. ni pupae. Resistant and susceptible T. ni had similar survival but resistant populations had reduced fecundity and smaller progeny after cold exposure. This fecundity cost is unlikely to be sufficient to select out the resistant phenotype in the presence of Bt treatment and in the absence of immigration of susceptible moths thus resistance to Bt is unlikely to disappear under current greenhouse management practices. As a potential alternative control, a fly parasitoid, Compsilura concinnata was assessed. In the laboratory C. concinnata readily parasitizes and superparasitizes T. ni, with a preference for later instars. Competition in the host when superparasitism occurred reduced host hemolymph protein, and parasitoids emerged at a younger host stage. Host plant affected T. ni and C. concinnata fitness, as well as the ability of the parasitoid to locate its host. In more complex environments such as in the field and on full grown caged cucumber plants, C. concinnata rarely parasitized T. ni. The effectiveness of the parasitoid depends on T. ni life stage and population, the host plant involved and the complexity of the environment. Although C. concinnata is easy to rear in the laboratory and thus has potential for development as an inundative biological control agent, the complexity of this host/pest/parasitoid system suggests that it may be difficult to effectively use this parasitoid in greenhouses.

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### **Chapter 1: General introduction**

In Canada, the greenhouse industry is growing quickly and gaining economic importance. In British Columbia, a large amount of vegetable production is in greenhouses. The three main vegetable crops are tomato (*Lycopersicon esculentum* Mill.), sweet pepper (*Capsicum annuum* L.) and cucumber (*Cucumis sativus* L.). Since these crops have different requirements, greenhouses tend to specialize on one crop only. For all three crops, soil-less growing methods are used. On average, greenhouses produce crops at least 10 months of the year. Greenhouse conditions are very different from field conditions. By being warm, humid, crowded with plants and depleted of natural enemies, they make an excellent environment for many pests, and facilitate the transmission of many plant diseases. For this reason, pest management is extremely important, especially in British Columbia where greenhouse growers are using Integrated Pest Management (IPM) including considerable amounts of biological control (Ministry of Agriculture, Food and Fisheries of BC 1994).

### Study organism

In British Columbia, *Trichoplusia ni* Hübner (cabbage looper) (Lepidoptera: Noctuidae) is the only lepidopteran to cause economic damage to the vegetable production in greenhouses. The species readily infests the three main greenhouse crops and causes substantial economic damage when at high density due to leaf defoliation that reduces yield, and to direct fruit damage. In 1995, growers reported costs for the control of the cabbage looper to be as high as CAD\$5000 per hectare.

*Trichoplusia ni* is a highly polyphagous species native to North America. It readily feeds on 160 plant species in 36 families. Some of these plants are economically important crops, which makes *T. ni* a significant pest (Sutherland and Greene 1984). Adult moths are brownish or grayish with a white dot on the forewings. They are about 25 mm in length with a wingspan of 38 mm. Males can be recognized by the brown hair

tuft on their abdomen (Mitchell and Chalfant 1984). Adults become active at sunset, when they will mate, feed and oviposit. Eggs are laid singly under leaves or on vertical parts of the plant. Larvae are light green in color and go through 5 to 7 instars (Shorey et al. 1962). At the last instar, larvae reach 30 mm in length (Ignoffo 1963). Pupation takes place in a tightly spun cocoon (Shorey et al. 1962). The development rate of *T. ni* depends on temperature and is faster under warm conditions (Elsey and Rabb 1970; Toba et al. 1973).

According to the literature, *T. ni* does not overwinter on the west coast of North America further north than southern California (Oatman 1966) and Arizona. Adults are thought to migrate northward each summer (Mitchell and Chalfant 1984). According to these sources, the entire population of cabbage loopers in British Columbia will have originated each year from these southern regions. Mitchell and Chalfant (1984) therefore suggest that the distribution of *T. ni* is mainly determined by temperature. Some debate exists over *T. ni* migration into greenhouses. Growers tend to believe that moths originate from outdoor populations. However, *T. ni* pupae were found to survive the clean-up procedures occurring between seasons and therefore survivors may maintain an endemic population inside greenhouses (Cervantes, unpublished).

### The problem

In greenhouses, *T. ni* is mainly controlled by *Bacillus thuringiensis* subsp. *kurstaki*, a soil bacterium that produces a crystal containing toxins specific to certain insects. *Bt* is widely used worldwide since it is highly efficient, has very low impact on the environment and natural enemies, and is very safe to humans (reviewed by Tabashnik 1994). Following complaints by growers about reduced efficiency of *Bt* against *T. ni* in greenhouses, Janmaat and Myers (2003) surveyed populations and found high resistance to *Bt* with the highest levels being in greenhouses spraying the most. Due to the development of resistance to *Bt*, the management of *T. ni* has become more problematic and novel ways of control required investigation.

Parasitoids are useful tools in greenhouses for management of some pests. The most famous example may be *Encarsia famosa* used to control the whitefly (Hoddle et al. 1998). Under suitable conditions, they can efficiently control pest species below damaging levels. A suitable parasitoid could potentially be developed as an alternative means of control for *T. ni*. Due to environmental and quarantine issues, it is wise to first assess what parasitoids are already present under field conditions. Population ecology of *T. ni* and its parasitoids has been studied in some areas such as by Clancy (1969) and Oatman et al. (1968) in California, Elsey and Rabb (1970) in North Carolina, Harding (1976), Andaloro et al. (1982) and Sutherland (1966) in New York. All these studies showed that for the most part, the natural enemies were different from one area to another, making a study of the natural parasitoids of *T. ni* in British Columbia relevant.

### **My objectives**

For my thesis research, I worked on two aspects of the cabbage looper system to improve the current management of the pest.

- 1. I assessed the overwintering capabilities of resistant *T. ni* which could potentially influence the evolution of resistance in greenhouses
- I assessed the potential of a parasitoid, *Compsilura concinnata* (Diptera: Tachinidae) as a biological control agent in greenhouses. I focused on two areas:
  - Host-parasitoid interactions
  - o Tritrophic interactions

The evolution and stability of resistance to insecticides is thought to be directly related to trade-offs occurring in the resistant insects. This explains why, when selection from the insecticide is discontinued, resistance is generally reduced in the insect population (McKenzie 1996; Cotter et al. 2004). Fitness costs associated with the development of resistance to *Bt* are various and have been shown in fecundity (Groeters

et al. 1994; Sayyed and Wright 2001; Janmaat and Myers 2003), development time (Liu et al. 1999; Oppert et al. 2000; Akhurst et al. 2003; Higginson et al. 2005), survival (Groeters et al. 1994; Tabashnik et al. 1994; Oppert et al. 2000; Carrière et al. 2001a), mating success (Groeters et al. 1993), and overwintering capabilities (Carrière et al. 2001b). Since *T. ni* were found to be resistant to *Bt* in some greenhouses (Janmaat and Myers 2003) and pupae were shown to survive the period when no crop is grown and no heat provided to greenhouses (Cervantes, unpublished), the survival of the resistant phenotype during that overwintering period is critical to the evolution of resistance in the greenhouse populations. This has important implications for the management of *T. ni*. For this reason, in Chapter 2, I investigated the overwintering capabilities of resistant *T. ni* pupae as a fitness cost associated with the development of resistance to *Bt* under laboratory conditions.

The efficiency of parasitoids is directly influenced by their ecology, interactions with their host and their environment (Godfray 1994). For this reason, it is important to assess all these aspects carefully when looking at the potential of a parasitoid as a biological control agent. I investigated interactions between *T. ni* and a parasitoid isolated from the field, *Compsilura concinnata* Meigen (family: Tachinidae). Host-parasitoid interactions between these species are assessed in Chapters 3 and 4; and the plant-parasitoid interaction in Chapter 5. In a greenhouse environment, it is important to know what host life stages can be parasitized in order to time the release of the parasitoid, and to maximise the number of parasitoids with the highest fitness. Therefore, the stages of *T. ni* parasitized by *C. concinnata* and the impact of *T. ni* and *C. concinnata* were further studied in Chapter 4 by looking at the immune system and blood nutrients levels in *T. ni* following parasitization by *C. concinnata*.

Plants are also known to have important impacts on parasitoids, both directly by impeding or facilitating the location of the host, and indirectly by affecting host quality (Price et al. 1980; Godfray 1994; Bottrell et al. 1998). A parasitoid efficient on one plant can have very low efficiency on another one. In this system, there are three crops

involved; efficiency of *C. concinnata* could vary with the crop. In Chapter 5, I evaluated tritrophic interactions between *C. concinnata*, *T. ni* and the three crops grown. I looked at the capabilities of *C. concinnata* to locate *T. ni* on full grown plants as well as the effect of the crop on *C. concinnata* fitness.

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# Chapter 2: Effects of resistance to *Bacillus thuringiensis* on the overwintering potential of *Trichoplusia ni*

### **Introduction**

The evolution and stability of resistance to insecticides or parasites is strongly influenced by fitness costs (McKenzie 1996; Cotter et al. 2004). Trade-offs between the costs and advantages of a trait may explain why so few insects develop resistance to the widely used microbial insecticide *Bacillus thuringiensis* (*Bt*) in the field, and why most systems show a decrease in resistance when insecticide use is discontinued (Ferré and van Rie 2002). *Bt*, a soil bacterium that produces crystals that are highly toxic to some insects, is a highly effective biopesticide used worldwide. *Bt* has minimal impact on the environment and human health, and it does not disrupt natural enemies (reviewed by Tabashnik 1994). Frequency of use has dramatically increased with the development of genetically modified crops expressing the gene responsible for *Bt* toxin production (Gould 1998).

*Bt* has been extensively used for over 30 years, and for some time it was thought that resistance would never develop in the targeted pests (Tabashnik 1994). Despite the slow development of resistance, the first published case was found in *Plodia interpunctella* in stored grain by McGaughey (1985). Since then, resistance to *Bt* has been selected for under laboratory conditions in several species of Lepidoptera, Diptera and Coleoptera (Tabashnik 1994; Ferré and van Rie 2002). In the field, resistance is rare and so far, even with widespread use of *Bt*, only two insects have been demonstrated to develop resistance under field conditions; *Plutella xylostella* (Tabashnik 1994) and *Trichoplusia ni* (Janmaat and Myers 2003). Fitness costs associated with the development of resistance to *Bt* vary among different species and have received considerable attention in recent years. Costs include; lower survival (Groeters et al. 1994; Tabashnik et al. 1994; Oppert et al. 2000; Carrière et al. 2001a), lower pupal weight or fecundity (Groeters et al. 1994; Sayyed and Wright 2001; Janmaat and Myers 2003), lower mating success

(Groeters et al. 1993) and slower development time (Liu et al. 1999; Oppert et al. 2000; Akhurst et al. 2003; Higginson et al. 2005). However, in other systems, no fitness costs could be detected (Gould and Anderson 1991; Ramachandran et al. 1998).

Costs associated with development of resistance are not always apparent, and some are manifested only in environments where intra-specific competition is strong. For example, lower survival resulting from resistance to organophosphate insecticide in Culex pipiens is especially apparent under conditions of crowding (Bourguet et al. 2004). In some cases, reduced mating success of resistant males can only be detected in competitive environments (Higginson et al. 2005). There is also evidence of differing costs depending on diet. Recently, Bt resistance was shown to have larger fitness costs in Lepidoptera feeding on lower quality plants (Janmaat and Myers 2005). Adverse abiotic conditions can also increase the costs of fitness. Winter represents a stressful period for many insects and the overwintering period can affect the frequency of resistant alleles in a population (McKenzie 1996). Despite the relevance of overwintering to the evolution of resistance, few studies have been conducted on the impact of winter conditions on fitness costs. McKenzie (1994) showed that mortality is selective for the diazinon resistant phenotype of the fly Lucilia cuprina during overwintering. Similarly, Foster et al. (2000) showed that peach-potato aphids (Myzus persicae) resistant to ester-based insecticides have lower winter survival. Gazave et al. (2001) studied the relative level of resistance to insecticides at two loci in Culex pipiens. Frequency of resistant traits decreased during overwintering, indicating reduced survival for resistant individuals. In a study with Bt and Pectinophora gosypiella (pink bollworm), significant fitness costs reflected in reduced overwintering survival were associated with resistance (Carrière et al. 2001b).

*Trichoplusia ni* (cabbage looper) is the only lepidopteran causing economic problems in vegetable greenhouses in British Columbia (see Chapter 1). Greenhouse populations at several geographically separate locations have developed strong resistance to *Bt* subsp. *kurstaki* (*Btk*), making control of the pest problematic (Janmaat and Myers 2003). In British Columbia, greenhouses are in production on average 10 months of the year, with an intense clean-up procedure to prevent the carryover of pests between

production seasons. During this period, the greenhouse is unheated. It has been shown that cabbage looper pupae can survive the cold period during clean-up (Cervantes and Myers, unpublished). Unless there is a fitness cost reducing survival or fecundity of resistant individuals, overwintering resistant individuals are likely to persist in greenhouses. This has consequences for the resistance levels in the next growing season and the long term efficacy of *Bt* as a biological control in the system. This system provides a unique situation to test the relationship between resistance and stress, relating to the evolution of resistance in-situ.

In this study, I compared the overwintering capabilities of susceptible and resistant T. ni to Btk (Dipel, Abbott Laboratories) as well as resistant T. ni whose parents were selected with Btk. I evaluated the survival of T. ni after the overwintering period as well as the resistance levels of their offspring. I hypothesized that resistant T. ni would have lower survival than susceptible insects at cold temperatures, and that this would reduce the overall frequency of resistant alleles in the offspring population. I also hypothesized that selection for resistance (i.e. ingestion of Bt) would negatively affect the overwintering capabilities of resistant T. ni.

### **Material and methods**

### **Colony rearing**

The cabbage loopers used in this study are from three original colonies designated as RC, Glen and Gip. RC is a colony that has been reared under laboratory conditions for more than 15 years and has never been in contact with *Bt*. The other colonies originated from insects collected in 2001 from commercial greenhouses in British Columbia. Glen originated from a greenhouse producing peppers, while Gip was started from cabbage loopers collected from a tomato greenhouse. These initial populations of cabbage loopers showed high levels of resistance to *Bt* (Janmaat and Myers 2003).

Cabbage loopers were reared in groups of 15 larvae in 175 ml Styrofoam cups filled with 20 ml of wheat germ based artificial diet (Ignoffo 1963) at a temperature of 25°C and light:dark photoperiod of 16:8 hours. Pupae were taken out of their cocoons and bleached for 5 min in a 0.6% bleach solution before being placed in 30 cm high cylindrical wire mesh cages with a 20 cm diameter at 20-25°C until hatching. Upon emergence, adults were fed 10% sucrose solution. Paper towels were put on the outside of the cage to provide oviposition sites. Egg sheets were changed every 2 to 3 days and sprayed with a 0.2% bleach solution, allowed to dry and then were stored at 9°C until use (maximum of 10 days). Egg sheets were placed in 4 litre buckets at 25°C until hatching.

### Selection

Neonates were put in groups of 25 in 175 ml Styrofoam cups filled with 20 ml of wheat germ based artificial diet at a temperature of 25°C and photoperiod of 16:8. At the third instar (five days old), larvae were transferred to new Styrofoam cups containing artificial diet mixed with a commercial wettable powder of *Btk* solution (ratio *Bt* solution:diet = 1:10). *Btk* solution was added to cooling diet before solidification. Two days later, the surviving larvae were transferred to normal artificial diet in groups of 20 individuals. Surviving pupae were bleached to reduce viral infections and caged.

For both Glen and Gip, colonies were split in two; one half was selected for resistance to Bt every one to two generations (hereafter called Glen Bt and Gip Bt), the other half (Glen C and Gip C) were never exposed again to Bt. Gip Bt was further divided and selected at different Bt concentrations. After a few generations, four new colonies were formed and named after the concentration of Bt used for selection (Gip Bt20 was selected at 20 000 IU/ml, Gip Bt40 at 40 000 IU/ml, Gip Bt60 at 60 000 IU/ml and Gip Bt160 at 160 000 IU/ml). These colonies showed a range of resistance levels to Bt; each colony represents a treatment in the experiment. In order to counteract the potential direct effect of Bt ingestion, colonies were left for one generation without Bt exposure before the experiments were started, except when stated.

### Assessment of resistance

The lethal concentration required to kill half the population (LC<sub>50</sub>) of each colony was measured for the generation that was used to test overwintering capability. Neonates were put on artificial diet in groups of 25 in 175 ml Styrofoam cups for five days at 25°C. Three hundred individuals were taken from each colony (unless stated otherwise), and divided into six groups of fifty, each of which was subjected to a different dose of *Bt*. Doses were made by serial dilution of the original solution (4 g of Dipel into 40 ml of water giving 160 000 IU/ml) and incorporated into diet. Colonies showing resistance were assigned a range of high *Bt* doses (0, 10 000, 20 000, 40 000, 80 000 and 160 000 IU/ml), while susceptible colonies were given a range of lower doses (0, 625, 1250, 2500, 5000 and 10 000 IU/ml). Larvae were transferred to the *Bt*-diet in groups of five. Mortality was assessed after 3 days by touching the larvae gently with a toothpick. An individual was considered dead if this did not trigger movement.

### Adult survival, deformity and offspring resistance levels following cold treatment

Colonies RC, Glen C, Glen Bt, Gip C, Gip Bt20, Gip Bt40, Gip Bt60 and Gip Bt160 were assessed. An additional assessment was done with Gip Bt40 and Gip Bt160 for which the parents were selected for *Bt* resistance (Gip Bt40 sel and Gip Bt160 sel). Neonates from respective colonies were reared in groups of 15 on artificial diet until pupation as described above. Pupae were put singly in 30 ml plastic cups and randomly assigned to spend 0, 1, 2, 3, 4, 5, or 6 weeks at 10°C. The overwintering temperature of 10°C was chosen as it represents the average temperature in greenhouses in the winter when no light and heat are provided. Pupae in the control were kept at 25°C with a photoperiod of 18:6 until emergence. Pupae in the cold temperature treatments were first kept for 24 hours at 17.5°C before being placed at 10°C (photoperiod 12:12) to reduce the potential thermal shock. After the required time at 10°C, pupae were put at 17.5 °C for 24 hrs, and then at 25°C with a photoperiod of 18:6. Adult emergence was recorded every 2 to 3 days. Adult deformity was assessed visually; moths showing wing abnormality were

recorded as deformed. Adults were caged separately for each colony and treatment combination, and eggs collected every 2 to 3 days. The resistance levels of the offspring were assessed as described above.

### **Statistical analysis**

All  $LC_{50}$ s were determined with Probit analysis using Genstat 5 (Genstat 1998). When mortality in the control was less than 5%, the control was removed from the analysis. A 95% fiducial limit was calculated for all colonies. Resistance ratios were calculated by dividing the  $LC_{50}$  of resistant colonies by the  $LC_{50}$  of their respective susceptible colony.

All further statistical analyses were done in JMP IN 4.0 (2000). The relationship between Bt resistance and the percentage mortality over time at 10°C was assessed using a linear regression between the log transformed LC<sub>50</sub> values and the arcsin square root transformed percentage mortality at each week spent at 10°C. To test the importance of time spent at 10°C on the proportion of mortality and deformed moths, Chi-square analysis followed by Pearson tests were carried out. Nominal logistic analysis and Wald tests were used to test for an interaction between the effects of overwintering treatment and resistance on deformity. To assess the potential effect of selection, percentage mortality and deformity for Gip Bt40 and Gip Bt160 selected and unselected colonies were compared using pairwise comparisons with Chi-square likelihood ratio tests.

### **Results**

### **Resistance levels**

Colonies selected at different doses of Bt had a range of resistance levels. The LC<sub>50</sub> values of the different populations were significantly different (df=7, deviance ratio=69.30, p<0.001) (Fig 1). RC, Gip C and Glen C were highly susceptible to Bt and were significantly different from all the resistant colonies, but not from each other. Gip Bt20 and Glen Bt showed low levels of resistance while Gip Bt 40, 60 and 160 had higher resistance ratios (Table 1). Gip Bt20 and Glen Bt were not significantly different from Gip Bt40 and Gip Bt160 were different from each other, but not from Gip Bt60. The LC<sub>50</sub> of the colonies that had just been selected (Gip Bt40 sel and Gip Bt160 sel) were not different from the LC<sub>50</sub> of their respective colonies which had one generation without Bt exposure (Fig. 2).

Figure 1.  $LC_{50}$  data and 95% fiducial limits of *T. ni* colonies used to assess overwintering capabilities. Letters above bars indicate significant pair-wise differences.  $LC_{50}$ s were considered significantly different when error bars were not overlapping.



Table 1. Resistance ratio (resistant: susceptible) of colonies used to assess overwintering capabilities. Results for Probit analyses of the effect of dose on mortality are shown (all slopes are significant at p<0.001). Resistant ratios were calculated relative to  $LC_{50}$  of the reciprocal susceptible colony.

Population	Resistance ratio	Probit slope (± s.e.)
RC	1	0.944±0.112
Gip C	1	1.275±0.155
Glen C	1	1.061±0.117
Glen Bt	2.45	0.842±0.139
Gip Bt20	2.88	0.206±0.033
Gip Bt40	11.02	0.627±0.091
Gip Bt60	16.78	$1.103 \pm 0.122$
Gip Bt160	23.10	0.949±0.117
Gip Bt40 sel	15.64	0.793±0.099
Gip Bt160 sel	18.92	0.694±0.096

Figure 2. LC<sub>50</sub> data and 95% fiducial limits of *T. ni* colonies Gip Bt40 and Gip Bt160 after selection and after one generation without Bt exposure



### Adult survival, deformity and offspring resistance levels following cold treatment

Cold kills moths; time spent at 10°C as pupae was negatively correlated with the proportion of moths that emerged (Pearson  $\chi^2=376$ , p<0.001). However, mortality after exposure to 10°C at the pupal stage was not related to the resistance level of the colony (control: F<sub>1,7</sub>=0.064, p=0.809; 1 week: F<sub>1,7</sub>=0.864, p=0.389; 2 weeks: F<sub>1,7</sub>=2.286, p=0.181; 3 weeks: F<sub>1,7</sub>=0.008, p=0.933; 4 weeks: F<sub>1,7</sub>=2.660, p=0.154; 5 weeks: F<sub>1,7</sub>=2.350, p=0.176), except for 6 weeks (F<sub>1,7</sub>=22.196, p=0.003). This effect remains significant when subjected to a sequential Bonferroni correction. After having spent 6 weeks at the cold temperature, mortality was higher for susceptible *T. ni* than for more resistant colonies with higher LC<sub>50</sub>s (Fig 3).

Figure 3. Relationship between the proportion of *T. ni* pupae that died after being exposed to  $10^{\circ}$ C for 6 weeks and the colonies' LC<sub>50</sub>s



Moth deformity was strongly influenced by the time pupae spent at 10°C (Pearson  $\chi^2$ =882, p<0.001). The proportion of perfectly formed moths decreased with the number of weeks spent at 10°C, with no moths being normal after 3 weeks. The resistance level had no effect on deformity; perfectly formed and deformed moths were distributed equally among colonies having different LC<sub>50</sub>s (Wald=3.23x10<sup>-9</sup>, p>0.999) (Fig. 4).

Figure 4. Proportion of deformed moths emerging from pupae that were exposed to  $10^{\circ}$ C for 0, 1 and 2 weeks in relation with the LC<sub>50</sub>s of different colonies



### **Offspring resistance level**

No moths laid eggs after having spent three weeks at  $10^{\circ}$ C as pupae. After two weeks, Glen C and Glen Bt had no offspring, and Gip Bt40, Gip Bt60 and Gip Bt160 colonies produced a few live larvae (approximately 15 surviving after five days) compared to over a hundred for Gip C (101 larvae) and RC (133 larvae) and Gip Bt 20 (168 larvae). The LC<sub>50</sub> could only be assayed for the last three colonies.

For most resistant colonies, there was a loss of resistance in subsequent generations, i.e.  $LC_{50}$ s of the offspring are lower than the  $LC_{50}$  of their respective parents (Fig. 5). The overwintering treatment had no significant effect on resistance of offspring, except for offspring of Gip Bt20 exposed to cold for two weeks, which had significantly lower  $LC_{50}$ s than those exposed for one week ( $\chi^2$ =3.74, p=0.024).

Figure 5. LC<sub>50</sub>s data and 95% fiducial limits of *T. ni* parents and their offspring, related to the time parents spent at 10°C as pupae. Probit analysis between dose and mortality are not shown. All slopes were significant at p<0.001



### Selection effect

The two colonies for which parents were selected for *Bt* resistance, Gip Bt40 and Gip Bt160 showed different patterns of mortality with exposure to cold. The overall mortality of offspring from parents that underwent selection was significantly higher than that of offspring whose parents were not exposed to *Bt* for Gip Bt40 ( $\chi^2$ =10.85, p<0.001), but not for Gip Bt160 ( $\chi^2$ =1.14, p=0.286) (Fig 6). Proportions of individuals with deformities were not significantly different when comparing selected colonies to unselected colonies for Gip Bt40 ( $\chi^2$ =2.464, p=0.116) and Gip Bt160 ( $\chi^2$ =1.167, p=0.280). On the other hand, resistance of selected colonies was significantly affected by the overwintering treatment. The LC<sub>50</sub> of offspring whose parents spent 1 week at 10°C was significantly higher than the LC<sub>50</sub> of offspring whose parents were kept at warm temperatures (Gip Bt40 selected  $\chi^2$ =8.80, p=0.003; Gip Bt160 selected  $\chi^2$ =12.00, p<0.001) (Fig 5).

Figure 6. Proportion of pupae that died after being exposed to  $10^{\circ}$ C for 0 to 6 weeks after selection for *Bt* resistance (selected) and when one generation was unexposed to *Bt* (unselected) prior to the experiment



b) Gip Bt160





### **Discussion**

Adverse conditions may magnify the fitness costs to insects associated with resistance to insecticides. Winter can be a stressful period in an insect's life (McKenzie 1996). For this reason, it was predicted that one cost of resistance to *Btk* could be reduced ability of *T. ni* to survive the overwintering period. I found significant evidence of negative effects of the overwintering period on survival, moth deformity and fecundity of *T. ni*. However, contrary to the original prediction, the survival of resistant individuals was not reduced to a greater degree than controls by exposure to a 10°C stress mimicking the overwintering period in greenhouses. Furthermore, higher pupal emergence was observed in resistant pupae exposed for 6 weeks to 10°C (see Fig. 3). Other studies have found that resistance reduces overwintering success of the resistant phenotype (McKenzie 1994; Foster et al. 2000; Carrière et al. 2001b; Gazave et al. 2001) or that overwintering mortality was not related to resistance (Daly and Fitt 1990). This study is the first to describe an increase in survival associated with resistance, and is only the second account of a potential advantage due to resistance, following Hollingsworth et al. (1997) who

The increased survival of the resistant phenotype at 10°C could be caused by a correlated response; positive or negative effects of one trait resulting from selection for another trait (Hedrick 1999). In this case, it is possible that an allele responsible for resistance is positively influencing one of the components required for longer survival at cold temperatures. More than one gene is involved in resistance of *T. ni* to *Btk*, (Janmaat et al. 2004). It is therefore possible that these different genes each have one or more pleiotropic effects on life-history traits of *T. ni*. In this case, a possible scenario is that one gene has an antagonistic pleiotropic effect (fitness cost) as shown by Janmaat and Myers (2003; 2005) while another has a positive pleiotropic effect, increasing survival during overwintering.

Although there were clear positive effects of resistance on survival, these cannot be directly translated into increased fitness. A fitness advantage inferred by survival at

colder temperatures is only relevant if surviving individuals can reproduce. In this study, increased survivorship was only significant after six weeks at 10°C and at that time none of the populations produced offspring. In fact, when comparing mortality between colonies with different resistance levels during the reproductive phase (i.e. control, 1 and 2 weeks at 10°C) there was no difference in survival. It is likely that levels of deformity influence reproductive fitness as well. Deformity of moths increases rapidly with time spent at 10°C during the pupal stage, and deformed moths are unlikely to be viable in a field setting. Resistant and non-resistant populations were not significantly different in terms of the proportion of moths that were deformed. Therefore it seems likely that resistance does not confer either a direct advantage or disadvantage in terms of survival or mating success after overwintering.

While there is no evidence for costs of resistance in terms of survival or the likelihood of reproducing, there is clear evidence for a fitness cost related to fecundity in this study. Resistant colonies produced relatively few offspring (<20, often none) after having spent 2 weeks as pupae at 10°C, while some of the susceptible colonies produced over 100 offspring. Reduced pupal weight and therefore fecundity of *T. ni* is a characteristic associated with *Bt* resistance (Janmaat and Myers 2003; Caron, unpublished). This cost has the potential to decrease the development of resistance in the population. After the overwintering period, even if both resistant and susceptible phenotypes survive, the susceptible moths, with their higher fecundity, will have more progeny and could therefore dilute the resistant alleles in the population over time.

The reduction of resistance when selection was discontinued also suggests that fitness costs are associated with resistance. The  $LC_{50}$ s declined between parents and offspring independently of different overwintering treatments (Fig. 5). This reduction in resistance was expected since resistance in *T. ni* is partially recessive (Janmaat and Myers 2003; Janmaat et al. 2004; Kain et al. 2004). However, for some of the low to moderately resistant colonies (Gip Bt20, Gip Bt40 and Gip Bt60), the resistance levels of the parents and their offspring varied little. Moderately resistant colonies of *Plutella xylostella* were shown to lose their resistance more slowly than highly resistant ones. Sayyed and Wright

(2001) hypothesized that when resistance is relatively low, fitness costs are less detrimental, and thus, resistance can remain in the population longer without selection. My results provide evidence for associated costs reducing levels of resistance in the absence of selection. However, resistance may be maintained without selection in low to moderately resistant populations.

A parental effect resulted from the ingestion of Bt (i.e. selection) by resistant cabbage loopers. For one of the colonies tested, resistant pupae whose parents had been selected had lower survival after an overwintering period than those whose parents were not exposed to Bt. Selected parents of this colony had offspring with longer total development times, which was not the case for other resistant offspring (Caron, unpublished). Results with the pink bollworm were similar in that when resistant strains were fed Bt-diet, offspring had lower fertility, embryogenesis and ability to enter cotton bolls (Carrière et al. 2001b). Resistance declined in offspring of selected parents that were kept at warm temperatures relative to individuals that spent 1 week at 10°C for resistant colonies. It is possible that parents that spent 1 week at 10°C invested greater resources per offspring, so they are better prepared to grow quickly and deal with the higher stress (i.e. cold temperature), and thus are more tolerant to Bt. Similar results were found in a solitary bee *Megachile apicalis* in which resource allocation per offspring varies with seasons; resources per offspring being greater under stressful conditions (Kim and Thorp 2001).

These results have important consequences for the management of *T. ni* in greenhouses. They suggest that no clear increased cost of resistance occurs in terms of surviving the cold period between crops in greenhouses. *Trichoplusia ni* does not overwinter in field conditions in British Columbia, where populations are re-established by annual migration from southern California (Mitchell and Chalfant 1984). Field populations were shown to have very low levels of resistance (Janmaat and Myers 2003). If resistant populations surviving in greenhouses and non-resistant immigrants mate, the level of *Bt* resistance would decline. Outbreeding with susceptible moths reduces resistance development over time, even if overwintering is not impeded by resistance

(Daly and Fitt 1990). The beginning of crop production in the greenhouse however precedes the arrival of migrant moths in the outside environment. Therefore, if the frequency of the resistant allele is already high at the beginning of the season, and selection continues, resistance will increase. This could explain the rapid evolution of the resistance in greenhouses observed by Janmaat and Myers (2003).

In conclusion, resistance to Bt in T. ni does not reduce the overwintering capabilities of pupae. An increase in survival in resistant pupae was found compared to susceptible pupae when kept longer at cold temperatures. This indicates a potential advantage provided by resistance over susceptible T. ni, with implications for the development of resistance. However, moths surviving after spending a few weeks at cold temperatures were highly deformed and incapable of reproducing. Therefore this increase in survival associated with resistance did not infer an increase in fitness, and is irrelevant in evolutionary terms. However there is the potential that enhanced survival may preadapt T. ni populations for future adaptations that do enhance fitness. This study emphasizes the need to study fitness costs associated with the development to resistance carefully. Unless resistance directly impacts the fitness or size of the next generation produced, the effect is not significant to the evolution of resistance in the population.

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## Chapter 3: Effects of inter and intra-species interactions on fitness in a host parasitoid system

#### **Introduction**

Parasitoids have been widely studied as model organisms to test ecological and evolutionary theory. In addition, parasitoids are valuable for control of insects in agricultural settings and have an important influence on population dynamics of hosts. Much attention has been paid to host-parasitoid interactions that are extremely strong, since for parasitoid offspring, the host represents the only resource available for their development. Host quality therefore determines the overall fitness of the adult parasitoid and is affected by the host species used, host stage and size at parasitization, as well as previous parasitization (Godfray 1994; Brodeur and Boivin 2004). For idiobiont parasitoids, which stop the development of the host at parasitization, host stage and size are important characteristics. Larger or older hosts should yield larger parasitoids, potentially with higher fitness (Fidgen et al. 2000; Bezemer and Mills 2003). For koinobiont parasitoids, which develop with the host, more variable results are expected. Size or age of the host may affect parasitoid fitness as a function of size of parasitoids (MacKauer and Chau 2001), weight or development time (Elzinga et al. 2003; Harvey et al. 2004).

Host quality is also influenced by previous parasitization. Superparasitism results from multiple parasitization events in a host from the same parasitoid species, involving one female (self-superparasitism) or different females (conspecific superparasitism) (van Alphen and Visser 1990; Godfray 1994). Parasitoids can either be solitary, with only one offspring arising from a host even if more than one egg were laid, or gregarious, with multiple offspring developing to maturity in a single host. Interestingly, superparasitism is common, not only for gregarious parasitoids, but also for solitary parasitoids (van Alphen and Visser 1990). In gregarious parasitoids, the immature parasitoids compete for resources, and this potentially reduces the amount of resource available per parasitoid (van Alphen and Visser 1990). The effect of superparasitism on parasitoid fitness can be

variable. In many cases, progeny from superparasitized hosts are smaller and/or weigh less (van Alphen and Visser 1990; Potting at al. 1997; Harvey et al. 1998; Elzinga et al. 2003; Santolamazza-Carbone and Rivera 2003) though there are exceptions (Gu et al. 2003). Superparasitism has also been shown to both shorten (Potting at al. 1997) and lengthen development time (Gu et al 2003).

This study examines the effects of host attributes and superparasitism on the success of a tachinid parasitoid, Compsilura concinnata Meigen. Most studies addressing host-parasitoid interactions have been carried out on hymenopteran parasitoids (Potting et al. 1997; Harvey et al. 1998; Cloutier et al. 2000; Fidgen et al. 2000; Mackauer and Chau 2001; Elzinga et al. 2003; Gu et al 2003; Santolamazza-Carbone and Rivera 2003; Harvey et al. 2004) rather than dipteran parasitoids (reviewed by Feener and Brown 1997). All members of the family Tachinidae are parasitoids and display various parasitization strategies (Belshaw 1994). Polyphagy (parasitizing more than one species) is more frequent in tachinids than hymenopterans (Belshaw 1994, Feener and Brown 1997; Stireman and Singer 2003). This is thought to be due to strategies that have evolved to avoid host immune systems such as making a respiratory funnel to avoid the lack of oxygen following encapsulation or remaining in specific tissues where the immune system has limited access, such as glands, nerve ganglia or muscles (Feener and Brown 1997). Furthermore, host discrimination seems less highly evolved in tachinids than in hymenopteran parasitoids. For example, while there is strong evidence that hymenopteran parasitoids can discriminate hosts that have been already parasitized; no proof has been found that the same behavior has evolved in tachinids (Belshaw 1994).

Included amongst the species affected by *C. concinnata* is *Trichoplusia ni*, commonly known as the cabbage looper, a native of North America. The cabbage looper is a significant agricultural pest (Sutherland and Greene 1984) and is the only lepidopteran causing economic problems in vegetable greenhouses in British Columbia (see Chapter 1). Its status is aggravated by the development of resistance to *Bacillus thuringiensis* subsp. *kurstaki*, making its management problematic (Janmaat and Myers 2003). A preliminary field survey showed that *C. concinnata* parasitized over 6% of *T. ni* 

in a broccoli field in Delta, British Columbia. While a number of studies have been done on the biology of *C. concinnata* (Culver 1919; Fusco et al. 1978, Weseloh 1980 and 1984a, Ishiki and Shima 2003), none have studied *T. ni* as a host. The aim of this study was therefore to assess the interactions between *C. concinnata* and *T. ni*. as part of an evaluation of its potential as a biological control for *T. ni* in vegetable greenhouses. I was interested in addressing three main questions:

- 1. What is the occurrence and frequency of *C. concinnata* in *T. ni* populations in the field?
- 2. What is the role of host stage in determining parasitoid host choice and parasitoid fitness in laboratory populations? I hypothesized that since C. concinnata is a koinobiont parasitoid, the host stage at parasitization would have no effect on host choice or parasitoid offspring fitness
- 3. What is the frequency and effect of superparasitism on parasitoid fitness? I hypothesized that superparasitism would reduce fly fitness, due to fly larvae intra host competition.

#### Material and methods

#### Study organism

*Compsilura concinnata* Meigen (Diptera: Tachinidae) was introduced to North-America from Europe between 1906 and 1986 to control 13 different lepidopteran host species, especially the gypsy moth (*Lymantria dispar*) (reviewed by Boettner et al. 2000). The fly is widely distributed in eastern North America, but also occurs on the west coast, where *L. dispar* is rarely present and subject to strict control. *Compsilura concinnata* is a highly polyphagous parasitoid that has over 160 different hosts in North America (Arnaud 1978; Strazanac et al. 2001), mostly Lepidoptera. Despite its high degree of polyphagy, *C. concinnata* has a strong preference for *L. dispar* (Weseloh 1980, 1984b), and was shown to respond to changes in *L. dispar* density (Gould et al. 1990). Since *C. concinnata* has multiple generations during the summer, host species changes throughout the season (Boettner et al. 2000). *Compsilura concinnata* larviposits directly in the larval stage of the host by using a sickle-shape ovipositor formed by the seventh segment of its abdomen. The larva remains inside the host, attached to the tracheoles between the midgut wall and the peritrophic membrane. The fly larva exits during prepupation and pupation of the host and forms a puparium (Culver 1919, Ishiki and Shima 2003).

#### Field survey

#### Field sites

Seven organic broccoli fields were sampled in Delta, British Columbia. No more than four fields were productive at the same time. Fields were visited until harvest. If high densities of diamondback moth (*Plutella xylostella*), imported cabbageworm (*Pieris rapae*) or cabbage looper (*T. ni*) occurred, the fields were sprayed with *Bacillus thuringiensis* subsp. *kurstaki* by the growers.

#### Sampling

Every week from May to October 2004, each field was sampled for the different stages of *T. ni* and adults were monitored using pheromone traps (Phero Tech Inc.) placed around the field. Sampling was achieved by walking at least four rows per field and looking at an entire plant every 30 steps. In addition, on intervening plants, leaves showing damage were inspected for larvae and pupae. Eggs, larvae and pupae were collected into 473 ml paper coffee cups with pieces of leaves. In the laboratory, these were transferred singly into 30 ml plastic cups containing 2 ml of wheat germ based artificial diet (Ignoffo 1963). The larvae were maintained at 25°C until moth or parasitoid emergence. Parasitoids were preserved and identified.

#### **Insect rearing**

#### Trichoplusia ni

Laboratory populations were established to investigate further aspects of the interaction between *T. ni* and *C. concinnata. Trichoplusia ni* were reared according to the rearing procedures described in Chapter 2. Briefly, *T. ni* were reared in groups of 15 larvae on wheat germ based artificial diet (Ignoffo 1963) at a temperature of 25°C and light:dark photoperiod of 16:8 hours. Pupae were placed in mesh wire cages and emerged adults were fed 10% sucrose solution. Paper towels were put on the outside of the cage to provide oviposition sites. Egg sheets were changed every 2 to 3 days.

#### Compsilura concinnata

A laboratory colony of *C. concinnata* was established based on seven individuals originating from the field collection described above. Adult *C. concinnata* were kept in a one cubic metre cage at room temperature (20-25°C) and provided with water and 10% sugar solution. To maintain colony numbers, cups of third or fourth instar *T. ni* were taken from the laboratory populations and put without a lid in the fly rearing cage. After four hours, cups were retrieved and put at 25°C until the emergence of the parasitoid. New individuals were then placed in the rearing cage.

#### Impact of host instar on parasitization rate

To assess the larval stage of the host that can be parasitized, flies were exposed separately to second, third and fourth instar *T. ni* from the laboratory populations. Groups of fifteen larvae were reared in 175 ml Styrofoam cups which were placed one cup at a time in the fly cage at the required instar. Each larva represents one experimental unit (i.e. larvae in cups were at the same stage, size and are unlikely to interact with each other). Only one instar was presented during a 24 hour period, with the instar chosen randomly. *Trichoplusia ni* larvae were kept in the cup until prepupation when they were moved singly to 20 ml plastic cups. Cups were checked every day for parasitoid

emergence. Fly puparia were put singly in Petri dishes until fly emergence. The proportion of hosts parasitized of the total number of hosts was calculated for each host instar.

#### Impact of host instar on fly fitness

To assess the effect of host instar on the fitness of parasitoid offspring, I repeated the experiment outlined above, with a slight modification. For this experiment, the second instar was not tested since only one host was found to be parasitized at that instar in the first experiment. Instead, I chose third instar larvae that were close to moulting which I called instar 3.5. As for the previous experiment, *T. ni* of three different ages (third, 3.5 and fourth instars) were presented separately to flies, kept in groups of 15 until host prepupation, and then moved to single cups until parasite emergence. The date when a fly puparium was found, puparium weight, fly emergence and gender were recorded.

#### Assessment of the effects of superparasitism

For both of the experiments above, whether more than one fly offspring resulted from a single host was recorded. When this was the case data were kept separately. As for the second experiment, larval and pupal development time, number of puparia per host, puparium weight, fly emergence and gender were recorded.

#### Statistical analyses

Parasitization levels were calculated as the percentage of caterpillars from the field that yielded viable *C. concinnata* individuals. To assess the level of parasitization and superparasitism of different host instars, I used Chi-Squared analysis (JMP IN 4.0 2000). To assess the effect of the host instar at parasitization on parasitoid fitness, host instar and parasitoid sex were used as factors in a two-way ANOVA comparing parasitoid larval and pupal development times and parasitoid puparium weight separately.

The correlation between puparium weight and parasitoid mortality was assessed separately using a categorical logistic analysis followed by a Wald test. When superparasitism was higher than four flies per host, the data were excluded due to small sample size. Superparasitism relations between sex of offspring and host stage at emergence were assessed. To assess the correlation between superparasitism and larval development time, the number of puparia per host, the host instar at parasitization and sex were used as factors in a three-way ANOVA. Since host instar at parasitization did not have any effect on parasitoid puparium weight and puparium development time, host instar was not included in the two-way ANOVA used to assess the effect of superparasitism.

#### **Results**

#### Field survey

*Trichoplusia ni* populations in the field reached high numbers, peaking first at the beginning of the season (June) and later, increasing steadily to reach a maximum of 60 loopers collected per hour in early September until the end of the growing season (Fig. 1). *Compsilura concinnata* were found parasitizing *T. ni* throughout the field season, but at a very low rate, with a maximum parasitization rate of 3.3%. Only twelve of 2973 *T. ni* collected were parasitized with *C. concinnata*. Other parasitoids were found parasitizing *T. ni* in the field; five Hymenoptera and one other tachinid. None of these parasitoids were found throughout the field season.





#### Impact of host instar on parasitization rate

Trichoplusia ni host stage had a significant effect on parasitization by C. concinnata (df=2,  $\chi^2$ = 46.65, p< 0.001). Compsilura concinnata parasitized all three instars tested and other observations from the laboratory suggested that they may also parasitize the last instar, although host aggression may reduce the success rate of these encounters. It was observed that female parasitoids can suffer substantial damage from fifth instar T. ni which fiercely bite the attacker (Caron, pers. obs). For the three instar levels tested, later instars were strongly favoured (Fig. 2). Only one individual was parasitized at the second instar, whereas up to 50% of fourth instar individuals were parasitized. Figure 2. Effect of host instar on parasitization level of second, third and fourth instar of *T. ni*. The percentage of exposed hosts parasitized and superparasitized are shown with sample sizes above each bar.



#### Impact of host instar on fly fitness

*Compsilura concinnata* larval development time was strongly affected by the host instar at parasitization ( $F_{2, 414}$ =26.571 p<0.001). The mean larval development time within hosts parasitized at instar 3.5 and instar 4 were significantly shorter than those parasitized at the third instar, suggesting that longer exposure to the parasitoid results in an accumulating cost in terms of development time (Table 1, Fig. 4). Larval development time was also influenced by sex ( $F_{1, 414}$ =6.305, p=0.012), males developing faster than females.

Development time for *C. concinnata* puparia was not related to host instar at parasitization ( $F_{2, 414}$ =0.697, p=0.499), but was directly affected by fly gender ( $F_{1, 414}$ =4.719, p=0.030). Similar to the larval development time, puparium development time

was significantly longer for females than for males. Puparium weight was also influenced by fly gender ( $F_{1, 414}$ =161.333, p<0.001) with females being heavier than males. *Trichoplusia ni* instar at parasitization did not affect puparium weight ( $F_{2, 414}$ =0.513, p=0.599) (Table 1, Fig. 5).

Table 1: Larval and pupal development time and puparium weight as a function of *T. ni* instar at parasitization

	Instar 3	Instar 3.5	Instar 4
Larval development time (days)	$11.80 \pm 0.13$	$10.34 \pm 0.31$	$10.37\pm0.16$
Pupal development time (days)	$9.41\pm0.10$	$9.07\pm0.24$	$9.42\pm0.13$
Puparium weight (g)	$0.0452 \pm 0.0005$	$0.0427 \pm 0.0011$	$0.0450 \pm 0.0006$

Mortality at the pupal stage was higher for *C. concinnata* with lower puparium weight (N=460, df=1, Wald  $\chi^2$ =6.501, p=0.011). Also, pupal mortality was marginally higher if the host was at instar 3.5 (10.4%, N=48) than at instar 3 (7.5%, N=254) or instar 4 (2.5%, N=159) (df=2, Pearson  $\chi^2$ =5.996, p=0.050).

#### Assessment of the effects of superparasitism

Superparasitism by *C. concinnata* reached high levels in *T. ni*; the maximum number of flies found per host was eight. The number of hosts superparasitized varied significantly with host stage, with the fourth instar being more readily superparasitized (df=2,  $\chi^2$ =17.34, p< 0.001) (Fig. 2). *T. ni* stage at parasitoid emergence was significantly influenced by superparasitism (df=3, Pearson  $\chi^2$ =93.955, p<0.001), with a higher proportion of superparasitized hosts being at the prepupal stage when the fly larvae emerged (Fig. 3).

Figure 3. *Trichoplusia ni* development stage at emergence of parasite larvae as a function of superparasitism



Fly larval development time was influenced by superparasitism, development was faster when more parasitoid larvae were present in one host ( $F_{3,758}=25.882$ , p<0.001). However, when four flies were found per host, larval development time was not significantly different from three flies found per host (Fig. 4). Host instar at parasitization ( $F_{2,758}=7.057$ , p<0.001) and sex ( $F_{1,758}=1.178$ , p<0.001) also had an effect on larval development time, which is consistent with my previous results. *Compsilura concinnata* sex ratios were influenced by superparasitism with more females emerging from singly parasitized hosts and more males from superparasitized hosts (N=809, df=1,  $\chi^2$ =14.895, p=0.011).



Figure 4. Larval development time of *C. concinnata* as a function of *T. ni* instar at parasitization and the number of flies found per host

*Compsilura concinnata* puparium development time was related to superparasitism ( $F_{3, 771}$ =4.363, p=0.005) and to fly gender ( $F_{1, 771}$ =30.452, p<0.001). Flies emerged faster from hosts that were parasitized four times compared to lower levels of parasitization. Superparasitism reduced puparium weight ( $F_{3, 771}$ = 208.474, p<0.001) (Fig. 5), and affected sex ratios ( $F_{1, 771}$ = 101.082, p<0.001). The interaction between superparasitism and fly gender was also significant ( $F_{3, 771}$ = 13.198, p<0.001), with females losing more weight than males when superparasitized. Superparasitism did not influence the emergence rate of adult flies (N=841, df=3, Pearson  $\chi^2$ =1.831, p=0.608).

Figure 5. Puparium weight of *C. concinnata* as a function of gender and the number of flies found per host



#### **Discussion**

Although *T. ni* reached high densities in field conditions, *C. concinnata* densities were always low and did not appear to be related to the population dynamics of *T. ni*. *Compsilura concinnata* is highly polyphagous and *T. ni* represents only one of its many hosts (Arnaud 1978). It is possible that *C. concinnata* uses *T. ni* as an alternate host and has other preferred hosts in the vicinity. One possible scenario is that *T. ni* is a lifeboat host and only used when other primary hosts are at low population densities. The low rates of parasitization may be also accounted for by habitat separation. Many *C. concinnata* hosts are forest herbivores (Arnaud 1978; Strazanac et al. 2001), and flies may search preferentially and accumulate in these areas. The plant architecture of broccoli and forest vegetation is very different, which may further influence the ability of the parasitoid to detect its hosts or avoid its own predators while detecting hosts. Since all the fields sampled were surrounded by shrubs or trees, it is possible that *C. concinnata* could mostly be segregated from *T. ni* due to habitat preference, and that parasitization of

cabbage loopers represents an overflow of parasitoids from neighboring habitats. It is also possible that T. ni is not as desirable as other hosts for C. concinnata offspring due to nutritional value or post parasitization defenses. However, C. concinnata readily parasitizes T. ni under laboratory conditions, suggesting that the low level of parasitization is not due to non suitability of the host to yield parasitoid progeny. This provides some evidence for a role for the density of other hosts or other habitat types in determining the rate of parasitization of T. ni.

For the parasitoid larvae, the host is the only resource available and thus host size is critical. However, C. concinnata is a koinobiont parasitoid and will therefore not kill the host at parasitization; the fly larva will grow with the host. The size of the host at parasitization is therefore not crucial to the fitness of the adult fly. Weight at the pupal stage is a good indication of fitness since it is a function of fecundity (Bourchier 1991) and mortality (this study) in C. concinnata. Host stage at parasitization was shown to have no effect on the parasitoid puparium weight, indicating that a younger or smaller host at parasitization has the same resource potential as an older or bigger host. Bourchier (1991) found the opposite results when C. concinnata was paired with L. dispar, younger hosts at parasitization yielded smaller parasitoids. This different result emphasizes the variability arising between host species used by the same parasitoid, as shown by Harvey (2000) with Cotesia glomerata and two of its host species. Other factors also come into play in the level of parasitization of different instars. Under laboratory conditions, C. concinnata accepts T. ni at the second, third and fourth instar, but only rarely did second instars yield parasitoids. Second instar T. ni larvae are very small (Shorey et el. 1962), and parasitization by C. concinnata larvae may impair the physiological functions of the host.

Despite the lack of an effect of host instar on parasitoid pupal weight, larval development time of the parasitoid was shorter in later host instars. This result is consistent with previously published data: in the case of *L. dispar*, *C. concinnata* was found to parasitize every instar of the pest, but development rate was slower in earlier host instars (Weseloh, 1982, 1984a). Similarly Ichiki and Shima (2003) showed that in

*Bombyx mori*, *C. concinnata* larval development was slower when the host was parasitized earlier during its development. *Compsilura concinnata* larvae grow very slowly at first by remaining between the host peritrophic membrane and midgut until the host reaches prepupation and then they develop rapidly (Culver 1919; Ichiki and Shima 2003; also see Chapter 4). The larval stage of the host represents a lag time in parasitoid development. By parasitizing an older instar, less time is required until the host reaches prepupation, and therefore parasitoid larvae have a shorter development time. Considering this, one would predict that it might be better for a fly to parasitize an older instar since the amount of time that the parasitoid larva is exposed to predation risk via consumption of its host is reduced.

Survival to adult emergence is another measure of parasitoid fitness. Weseloh (1982) found reduced fly emergence when *L. dispar* was parasitized at later larval stages. For *T. ni*, I found that mortality was lower when the host was parasitized at the fourth instar than when the host was parasitized late in the third instar (instar 3.5). This could be caused by the biochemical changes occurring in the host close to molting (Chapman 1974). Only a few days separate the third instar from the fourth instar at 25°C, therefore, a noticeably larger third instar would be close to molting. Some of the enzymes used in molting are also important to the immune system of the host (Gillespie et al. 1997; Wilson et al. 2001). The activation of the enzymes in the molting process could increase the immune response of the host to parasites and therefore impede the first instar parasitoid larva before it penetrates the peritrophic membrane. Host quality is therefore influenced not only by the size of the host stages, but also by the physiological processes taking place.

*Compsilura concinnata* is a gregarious parasitoid, and more than one offspring can reach maturity in a single host. Superparasitism was shown to be frequent in *T. ni*, consistent with other studies on other hosts of *C. concinnata*: Kellogg and her colleagues (2003) found a maximum of seven adult flies emerging from the luna moth (*Actias luna*), while Eichhorn (1996) found a maximum of five adult flies emerging from *L. dispar*. In this study, superparasitism was higher in fourth instar hosts. Theory predicts that the

number of fly larvae per host should be a function of the fitness return per number of flies (van Alphen and Visser 1990). Therefore, if larger hosts provide more resources and can sustain a higher number of parasitoid larvae, this can explain the higher superparasitism found in older hosts. In highly superparasitized *T. ni*, puparium weight was lower, indicating potentially lower parasitoid fecundity. On the other hand, larval development time was faster. In superparasitized hosts it is likely that resource availability is lower per individual, which may result in larvae being resource limited and forced to mature faster. This will reduce the puparium weight and larval development time. This is supported by the host stage at emergence of the parasitoid; a higher proportion of parasitoids emerged from prepupae than pupae under higher superparasitism.

Competition within the host may have knock-on effects for the population dynamics of the parasitoid. Females were shown to develop more slowly than males at the larval stage and were in higher proportion in singly-parasitized host. By developing more slowly, females are probably being outcompeted by the faster growing males. Similar results were found by Santolamazza-Carbone and Rivera (2003), who showed that superparasitism of the snout beetle (*Gonipterus scutellatus*) by a parasitoid wasp *Anaphes nitens* had more impact on females than males: sex ratio was skewed towards females at low rates of parasitization and more males were produced from superparasitized host. Therefore there is the potential for superparasitism to skew sex ratios and influence population dynamics of the next generation of the fly.

Since fitness is reduced under high superparasitism, it should be an advantage for females to detect if the host is already parasitized (Brodeur and Boivin 2004). Superparasitism is very common in dipteran parasitoids and can reach high levels, giving the impression that they cannot discriminate if the host is already parasitized (Feener and Brown 1997). In fact, it was shown by Weseloh (1983) that *C. concinnata* could not discriminate hosts already parasitized by a braconid parasitoid. Superparasitism in gregarious parasitoids is disadvantageous for parasites arriving later due to competition (Godfray 1994; Feener and Brown 1997). In the case of *C. concinnata*, intra-host competition may not provide selection pressure for host discrimination of parasitized

hosts because larval development is delayed until the host reaches the prepupal stage. Therefore, parasitoid larvae being laid later in time are not disadvantaged and have the same potential to consume the available resource as the first laid parasitoid.

If host species are aggressive, it is not advantageous for female parasitoids to spend time assessing host quality (Feener and Brown 1997). This seems to be the case for the host-parasitoid system studied here. *Trichoplusia ni* at later instars were extremely aggressive and it was observed that *T. ni* could injure female *C. concinnata* by biting (Caron, pers. obs.). Parasitization encounters by *C. concinnata* are extremely fast (less than a second (Weseloh 1980)), indicating that no assessment of the host seems to take place. However, parasitization was found to be triggered by movement of the host (Weseloh 1980; Caron pers. obs.). Active hosts are more likely to be healthy, as shown with *Pseudaletia unipuncta* infected with a baculovirus (Hotchkin and Kaya 1983) and *L. dispar* fed sublethal dose of *Bacillus thuringiensis* (Erb et al. 2001). Therefore, using movement of hosts as a cue to fitness could be efficient.

#### Conclusions

This study has shown that the dynamics of the interaction between *T. ni* and *C. concinnata* is influenced by habitat, host population dynamics (host stage present for parasitization) and competition within the host. *Compsilura concinnata* and *T. ni* seemed to have segregated habitats and therefore rarely interact, which may explain in part the low parasitization levels in the field. It is also possible that the parasitization level is influenced by the population dynamics or densities of the other hosts present in the ecosystem. This represents an interesting area for future study. In laboratory conditions, *C. concinnata* parasitized older and larger *T. ni* more readily. This relationship probably evolved since older instars provide a faster development for the offspring, also reducing the time parasitoid larvae risk predation. Superparasitism was shown to be frequent under laboratory conditions and reduced the progeny fitness due to competition within the host. Superparasitism influenced the sex ratio of the fly progeny and females may have been outcompeted due to their slower development. Since *C. concinnata* has not evolved host

discrimination mechanisms, superparasitism could have important implications for the population dynamics of the parasitoid under low host availability.

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### Chapter 4: Host phenoloxidase and hemolymph protein levels of *Trichoplusia ni*, a host of a generalist Tachinid (*Compsilura concinnata* Meigen)

#### **Introduction**

Parasitoids can develop either inside (endoparasitoids) or outside the host (ectoparasitoids) (Godfray 1994). The main challenges for endoparasitoids are to survive the host immune system, and absorb nutrients from the host to complete their development (Vinson 1993). Host immune defenses against pathogens and parasites are varied. The first lines of protection are the cuticle and the midgut. However, if a foreign body succeeds in penetrating the cuticle, different immune functions in the hemolymph will respond. Invertebrates do not have adaptive immune responses (Söderhäll and Cerenius 1998). If the foreign body is small, it can be phagocytized by hemocytes, or trapped and aggregated through the formation of nodules (Gillespie et al. 1997). If the foreign organism is too large for these responses, such as parasitoid eggs or larva, encapsulation can occur. Encapsulation can be due to melanization or to a cellular response whereby hemocytes form multiple layers around the organism; this capsule can also be further melanized. In both cases, it is thought that the parasitoid is asphyxiated, but it may also die due to toxic compounds produced during the immune reaction (Strand and Pech 1995).

Phenoloxidase (PO), an oxidoreductase enzyme, is known to be important to the immune system of insects by triggering melanization and encapsulation of foreign bodies in the hemocoel (Gillespie et al. 1997). Phenoloxidase is part of a cascade of reactions that is initiated by an infection. Prophenoloxidase (proPO) is present in an inactive form in the hemolymph, and is activated when foreign organisms infect. Phenols are oxidized to quinones, which are then transformed into melanin and produce antimicrobial substances as by-products (Söderhäll and Cerenius 1998; Schmid-Hempel 2005). Due to the toxicity of the reaction, the PO cascade has to be regulated by proteinase inhibitors in the hemolymph (reviewed by Söderhall and Aspan 1993). Phenoloxidase levels have

been shown to alter in response to parasitization (Hartzer et al. 2005), and to affect rates of encapsulation of foreign bodies (Cotter and Wilson 2002). It is also involved in wound healing and sclerotization (Chapman 1974; Nation 2002; Mucklow et al. 2004).

Some parasitoids have evolved specific strategies to evade, suppress and avoid host immune system responses (Vinson 1993; Schmidt et al. 2001). Endoparasitoids can evade the immune system by having a similar body surface to the host tissue thereby ensuring the host immune system does not recognize the parasitoid as a foreign body (Vinson 1993). Some hymenopteran parasitoids suppress the host immune system by injecting an endosymbiont virus during the oviposition process that reduces the ability of the immune system to respond to invasion (Whitfield 1994) and the phenoloxidase activity (Shelby and Webb 1999, Bae and Kim 2004). These mechanisms are very specific to the host and are thought to be under constant selection pressure (Godfray 1994).

Dipteran parasitoids do not have viruses to suppress host immune systems, and therefore must rely on other strategies (Feener and Brown 1997). Many avoid the host immune system by remaining in locations where the immune system has limited access (glands, nerve ganglia, muscular tissue), or by forming a respiratory funnel when encapsulated. These strategies are believed to explain the wider host range found in dipteran parasitoids (Feener and Brown 1997). Parasitoids are known to feed on hemolymph nutrients (Baker and Fabrick 2000; Nakamatsu and Tanaka 2004), a process that may be inhibited by hiding in specific tissues, and thus reducing growth. To my knowledge, no study has ever assessed parasitoid larva feeding activity or the response of the host immune system while inside their hiding location.

In this study, the immune response of *Trichoplusia ni* Hübner to a tachinid fly parasitoid, *Compsilura concinnata* Meigen, was assessed. *Compsilura concinnata* was introduced to North America from Europe over an 80 year period to control different pest species (reviewed by Boettner et al. 2000). The parasitoid larviposits directly into the hemolymph of immature stages of its host using a sickle-shape structure formed by the

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seventh segment of its abdomen. First instar parasitoid larvae penetrate the peritrophic membrane of the host and attach to tracheoles with special anal hooks. The larvae remain between the peritrophic membrane and the midgut wall until the host prepupal stage, when they enter the body cavity and grow rapidly. Third instar parasitoid larvae exit the host shortly after prepupation of the host and form a puparium (Culver 1919; Bourchier 1991; Ichiki and Shima 2003). Superparasitism, the presence of more than one parasitoid larva per host, can be frequent (Eichhorn 1996; Kellogg et al. 2003; also see Chapter 3).

Information is currently limited on the host-parasitoid interaction of *C. concinnata* and *T. ni* inside the host. The present study had two goals. First, I assessed phenoloxidase activity in *T. ni* in response to parasitization by *C. concinnata*, to ascertain whether the parasitoid triggers an immune response and then avoids encapsulation by hiding in specific tissues. I hypothesized that since *C. concinnata* is only in contact with the host immune system for a short period of time (Bourchier 1991; Ichiki and Shima 2003), phenoloxidase levels would not respond to parasitization. Secondly, I assessed protein levels in the host hemolymph as an indication of nutrient consumption by immature parasitoids. It was hypothesized that since *C. concinnata* larvae grow slowly while between the peritrophic membrane and the midgut, the protein levels would be unaffected by parasitization.

#### Materials and methods

#### **Insect rearing**

#### Trichoplusia ni

*T. ni* were reared according to the rearing procedures stated in Chapter 2. Briefly, cabbage loopers were reared in groups of 15 larvae on wheat germ based artificial diet (Ignoffo 1963) Adults were kept in cylindrical cages in groups of 200 and were fed 10% sucrose solution. Paper towels were put on the outside of the cage, providing oviposition sites and harvested regularly.

#### Compsilura concinnata

The colony of *C. concinnata* originated from a *T. ni* larvae collection in fall 2004 (see Chapter 3). Adult *C. concinnata* were kept in cages and provided with water and 10% sugar solution. Groups of 15 fourth instar *T. ni* in cups containing artificial diet were provided daily for larviposition. Retrieved cups were kept at 25°C until parasitoid puparia were found, at which stage they were added to parasitoid cages.

#### Phenoloxidase assay

For this experiment, T. ni that had been in the parasitoid cage and therefore potentially parasitized and T. ni not exposed to the parasitoid (colony RC, see Chapter 2) were assessed. PO activity was measured based on the methods described in Wilson et al. (2001). At the fifth instar, caterpillars were bled by immobilizing them on a piece of parafilm and removing one of the prolegs. Blood pearling on the parafilm was collected with a pipette and 10  $\mu$ l was put into an Eppendorf tube containing 240  $\mu$ l of ice-cold Dulbecco's phosphate buffer saline. Samples were vortexed and put at -20°C for 24 to 48 hours to disrupt hemocyte membranes. To measure the PO activity, three replicates of 50  $\mu$ l of each sample were put in a 96-well microtitre plate with 100  $\mu$ l of 15 mM dopamine. Proteins were measured in three replicates of 5  $\mu$ l for each sample using the Biorad protein assay kit with Bovine Serum Albumin as a protein standard (Bio-Rad laboratories). Absorbance was measured at 492nm for PO and at 595nm for protein with a Spectramax 190 microplate reader (Molecular Devices Corporation). The amount of protein in each sample was calculated from a standard curve created on the same microtitre plate. The specific activity of phenoloxidase per hemolymph sample is expressed as PO units per mg protein, where one PO unit is the amount of enzyme needed to increase absorbance by 0.001.

To quantify parasitization, *T. ni* that had been in contact with the flies were dissected, and the number of parasites found per host were recorded. Data from non-parasitized and superparasitized hosts were kept separately.

#### **Statistical analysis**

PO activity and protein concentration in hemolymph for *T. ni* which were parasitized, superparasitized, not parasitized and not exposed to the parasitoid (RC) were compared using a one-way ANOVA in JMPIN 4.0 (2000). PO activity was natural log transformed and protein concentration was square-root transformed to approximate normal distribution. The four treatments were not all represented in all of the microplates. To test for a possible difference between microplates, each treatment was analyzed separately with microplate as a factor determining PO and protein. For all four treatments the microplate effect was non-significant and was not included in subsequent analyses (PO: parasitized:  $F_{3, 18}=2.282$ , p=0.121; superparasitized:  $F_{3, 10}=0.485$ , p=0.705; not parasitized:  $F_{2, 24}=2.513$ , p=0.104; RC:  $F_{2, 19}=0.352$ , p=0.709; protein: parasitized:  $F_{3, 18}=2.100$ , p=0.143; superparasitized:  $F_{3, 10}=1.458$ , p=0.317; not parasitized:  $F_{2, 24}=0.070$ , p=0.933; RC:  $F_{2, 19}=0.992$ , p=0.391).

#### **Results**

Dissection of *T. ni* that were presented to *C. concinnata* colony showed that parasitization was frequent. Of the 58 larvae that were dissected, 52% were parasitized. Superparasitism was common in *T. ni*; *C. concinnata* parasitized *T. ni* with up to five fly larvae per host (Table 1), 11 hosts were superparasitized. The total number of parasitization events was 59. All fly larvae were found between the peritrophic membrane and the midgut and were first instars, except for one second instar larva in a superparasitized host.

Number of flies per host	Number of hosts	Number of parasitization events
0	28	0
1	19	19
2	1	2
3	4	12
4	4	16
5	2	10
Total	30	59

Table 1. Number of flies found per dissected host and number of parasitization events

Phenoloxidase activity was not influenced by parasitization in *T. ni*. PO activity in the hemolymph was not significantly different when comparing RC, non-parasitized, singly parasitized and superparasitized *T. ni* ( $F_{3,74}$ =0.604, p=0.614), although there was a non-significant trend towards higher PO levels in superparasitized individuals (Fig 1).

Figure 1. Phenoloxidase activity in hemolymph of RC, non-parasitized, singly parasitized and superparasitized fifth instar *T. ni* 



Protein quantity in hemolymph was significantly lower in superparasitized hosts compared to RC and non-parasitized *T. ni*. Singly parasitized hosts were not significantly different from the other categories of *T. ni* ( $F_{3, 77}$ =3.496, p=0.020) (Fig 2).

Figure 2. Protein quantity in hemolymph for RC, non-parasitized, singly parasitized and superparasitized fifth instar *T. ni* 



#### Discussion

Dipteran parasitoids use two major strategies to overcome host immune responses. First they can keep constant contact with air by forming a respiratory funnel and avoid encapsulation by attaching themselves to host tracheoles. The second strategy is to move to specific tissues where the immune system does not have access, such as glands, nerve ganglia or muscles (Feener and Brown 1997). In *T. ni*, *C. concinnata* adopts the second strategy by remaining between the peritrophic membrane and the midgut for most of the host's development, a strategy it also uses in *Lymantria dispar* (Culver 1919, Bourchier 1991), and *Bombyx mori* (Ichiki and Shima 2003). It is likely that the polyphagy found in this species is facilitated by the use of this strategy to avoid the immune system (Feener and Brown 1997). The success of the strategy is shown by failure of phenoloxidase levels to respond to parasitization. By remaining in between the peritrophic membrane and the midgut, *C. concinnata* larvae are not triggering the encapsulation process which would normally take place when a large foreign body enters the hemocoel.

Interestingly, despite the fact that the cuticle was pierced and that PO is used in wound healing (Chapman 1974; Nation 2002; Mucklow et al. 2004), PO levels did not change after parasitization. The PO level was assessed a few days after the parasitization event. It is possible that the phenoloxidase level was high soon after the piercing of the cuticle, but since the oxidization of phenols into quinones produces toxic by-products (Söderhäll and Cerenius 1998; Schmid-Hempel 2005), the PO cascade was regulated and therefore non-detectable at the time of the bioassay. It is also a possibility that *C. concinnata* has a mechanism to suppress PO activity in the host, as shown in some hymenopteran parasitoids (Shelby and Webb 1999; Bae and Kim 2004; Hartzer et al. 2005) and other organisms (Brivio et al. 2002).

By remaining between the peritrophic membrane and the midgut, *C. concinnata* avoids *T. ni*'s immune system, but at the same time is not in direct contact with nutritional resources. In Chapter 3, I hypothesized that parasitoid development does not occur during the host larval stage, which may limit the parasitoid's nutritional needs. In this study, one *C. concinnata* larvae was found at the second instar when the host was dissected, indicating that some nutrient absorption occured. It was also shown that protein quantity in the hemolymph is lower in superparasitized hosts than in non-parasitized hosts. This is in agreement with Nakamatsu and Tanaka (2004) who showed that proteins and other hemolymph nutrients were depleted faster in heavily parasitized hosts. In the case of *C. concinnata* nutrients are probably absorbed when they are going through the midgut wall, before they enter the hemolymph. The concentration of protein in the hemolymph would therefore be reduced due to lower rates of replacement from the midgut.

#### **Concluding remarks**

Parasitoids can be quite specific in their host range. Host range is thought to be determined mostly by host ecology and taxonomy. Parasitoids can be specialised to one species or one group of insects since they share similar physiology and defence mechanisms. Parasitoids can also target hosts having a similar ecology, such as the same food plants (Godfray 1994). Dipteran parasitoids tend to have wider host ranges than hymenopteran parasitoids, and this may be due to strategies used to avoid host immune systems (Feener and Brown 1997). *Compsilura concinnata* is highly polyphagous and can use hosts in different insect orders, mainly Lepidoptera, but also Hymenoptera and Coleoptera (Webber 1926; Arnaud 1978). These orders differ in many ways but share many anatomical similarities. By remaining between the peritrophic membrane and the midgut, *C. concinnata* avoids activation of the phenoloxidase cascade and can access nutrients, therefore does not require strong adaptation to the host.

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# Chapter 5: Biological control of *Trichoplusia ni* on greenhouse vegetable crops using a parasitoid, *Compsilura concinnata:* the role of plant structure and quality

#### **Introduction**

Parasitoids have been widely used as biological control agents and have been shown often to be very efficient at controlling herbivore pests at below damageable levels in agriculture settings. A thorough understanding of the ecological interactions between herbivore hosts and parasitoids is essential to achieve good control of pest species. Plants can also have strong effects on parasitoids, either by influencing host location, or by influencing host quality and therefore parasitoid fitness (Bottrell et al. 1998). These types of tritrophic interactions are essential to the study of biological control in multi-crop systems where parasitoids can have different efficiency on different plants.

Plants can influence the ability of parasitoids to detect their hosts via effects of plant morphology such as plant size, pubescence, epicuticular waxes and presence and shape of plant parts (Price et al. 1980; Bottrell et al. 1998). For the parasitoid, Trichogramma evanescens search ability (Gingras et al. 2002) and parasitization rates (Gingras and Boivin 2002) were decreased with increased complexity of the host plant. Similarly, search efficiency of the parasitoid Trichogramma minutum was reduced on complex foliage (Lukianchuck and Smith 1997). Parasitoids have been shown to detect chemical compounds (semiochemicals) released by the host plant (Godfray 1994; Bottrell et al. 1998). These chemicals are released naturally by healthy plants, but in increased amounts by injured plants. Different semiochemicals are released depending on plant species and type of injury (Paré and Tumlinson 1999; van Poecke et al. 2003). Synomones, semiochemicals released specifically by herbivore feeding, can attract parasitoids targeting the herbivore (Bottrell et al. 1998). In Chapter 3, I showed that C. concinnata can effectively parasitize T. ni in a laboratory environment. In the greenhouse situation, C. concinnata must be able to effectively detect chemical cues and negotiate a complex physical environment to locate its host.

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Plants may also influence parasitoids indirectly, via plant quality. Indirect interactions between plants and parasitoids occur when there is an impact of the plant on the quality of the herbivore host. Host-parasitoid interactions are strong as the host provides the only resource for the parasitoid, and parasitoid fitness is directly linked to host quality (Godfray 1994; Brodeur and Boivin 2004; see Chapter 3). Similarly, plant quality has strong impacts on herbivore fitness (Price et al. 1980; Stadler 1998; Awmack and Leather 2002; Nykanen and Koricheva 2004; Stiling and Moon 2005). Plant chemical defences can reduce the quality of the plant to the herbivore, thereby reducing host quality for the parasitoid (reviewed by Price et al. 1980; Godfray 1994), affecting aspects of parasitoid fitness such as clutch size, larval or nymphal survival (Ode et al. 2004; Leather et al. 2005), development time (Karowe and Schoonhoven 1992; Werren et al. 1992; Leather et al. 2005; Setamou et al. 2005), parasitoid emergence (Werren et al. 1992; English-Loeb et al. 1993) and adult longevity (Karowe and Schoonhoven 1992).

*Trichoplusia ni*, commonly known as the cabbage looper, is the only lepidopteran to cause economic damage in vegetable greenhouses in British Columbia by feeding extensively on the three main crops: cucumber, tomato and sweet pepper (see Chapter 1). Until recently, *Bacillus thuringiensis (Bt)* was the main means of *T. ni* control, however, Janmaat and Myers (2003) showed that some populations of *T. ni* had become resistant to *Bt*. For these, *Bt* was no longer an efficient means of control. Greenhouses provide a good environment for the use of parasitoids as they provide constant environmental conditions and few predators within a contained situation. One of the most successful parasitoids in greenhouses is *Encarsia formosa* which is widely used worldwide to control whiteflies (Hoddle et al. 1998). There is therefore potential for parasitoids to be applied to controlling *T. ni* in greenhouses.

A recent field survey (see Chapter 3) found seven species of parasitoids attacking *T. ni. Compsilura concinnata* Meigen (family: Tachinidae) was the only parasitoid found throughout the entire field season although at low frequency. *Compsilura concinnata* was introduced to North America from Europe between 1906 and 1986 to control 13 different
species of Lepidoptera, especially the gypsy moth (*Lymantria dispar*) (reviewed by Boettner et al. 2000). It is widely distributed in eastern North America, but is also present on the west coast. *Compsilura concinnata* is a polyphagous parasitoid that has over 160 different hosts in North America (Arnaud 1978; Strazanac et al. 2001). The parasitoid larviposits directly in its immature host, with the larva remaining between the host midgut wall and peritrophic membrane attached to the tracheoles for most of its development. The last instar fly larva exits at the prepupation host stage and forms a puparium (Culver 1919; Ishiki and Shima 2003).

In order to appraise the potential of *C. concinnata* as a control agent on greenhouse crops, I explored two important aspects of the interaction between the parasitoid and *T. ni*.

1. The ability of *C. concinnata* to detect and effectively control *T. ni* in a complex greenhouse environment.

A greenhouse assay was used to ascertain whether *C. concinnata* was capable of detecting *T. ni* in a realistic environment.

2. The effect of plant species in determining the efficacy of *C. concinnata* as a biocontrol agent.

In previous work, *T. ni* was shown to grow faster and produce heavier pupae when fed cucumber, followed by tomato and lastly pepper (Janmaat and Myers 2005). The main objective of this study was to assess the effect of different crop plants on the fitness of the parasitoid. It was hypothesized that cucumber, since it yielded more fit *T. ni*, would have a positive effect on *C. concinnata*.

# Material and methods

# **Insect rearing**

#### Cabbage loopers

The *T. ni* used in this study originated from a colony which has been reared for more than 15 years under laboratory conditions (named RC in Chapter 2 and 3). *T. ni* were reared according to the rearing procedures described in Chapter 2. Briefly, *T. ni* larvae were reared in groups of 15 in 175 ml Styrofoam cups containing a wheat germbased artificial diet (Ignoffo 1963) at a temperature of 25°C and light:dark photoperiod of 16:8 hours. Adults were placed in groups of 200 in mesh cages at 20-25°C and fed 10% sucrose solution. Paper towels were used for oviposition sites and were changed every 2 to 3 days.

#### Compsilura concinnata

The colony of *C. concinnata* originated from flies emerging from *T. ni* larvae collected in fall 2004 from an organic broccoli field in Delta, British Columbia. *C. concinnata* were reared as stated in Chapter 3. Caged adults were kept at temperatures between 20 and 25°C and were provided with water and 10% sugar solution. Cups of third or fourth instar *T. ni* reared on artificial diet were put without a lid in the parasitoid rearing cage. After four hours, cups were retrieved and *T. ni* larvae were kept at 25°C until emergence of the parasitoid larvae. Puparia were collected, bleached and added to the parasitoid cage.

#### Assay under greenhouse conditions

Twenty-eight cucumber plants (*Cucumis sativus*; cultivar: Freda) were obtained from a nursery in Delta, British Columbia. Plants were grown on sawdust bags and watered with fertilized water. Each plant was encased in a two meter high cage (floor area 1 m<sup>2</sup>) made of a textile commercially used in agriculture settings that permits air flow but prevents movement of insects. Plant stems were supported on a wire until the plant reached the top of the cage, and growth tips were removed weekly, except for two which allowed the plant to grow downward. Cucumbers were removed when first formed. Greenhouse temperatures were set at 22°C with a light supplement of 16 hours.

Third instar *T. ni* larvae were randomly selected from the laboratory colony. Twenty individuals were transferred arbitrarily to leaves of each plant. After 24 hours, parasitoids were applied to plants in a randomised design. Zero, one, two or three pairs of mated parasitoid flies were put in the cages (seven replicates for each parasitoid level). Every week, plants were inspected for adult parasitoids. After six weeks, the foliage of the plants was inspected. The total number of leaves (larger than palm size) and the percentage of leaves showing *T. ni* damage were recorded. The number of parasitoids (puparia and adults), the number of *T. ni* empty pupae and larvae were counted. *Trichoplusia ni* larvae were put on artificial diet until parasitoids emerged. Dead larvae were dissected to determine if they were parasitized.

# **Tritrophic interactions**

Tomato (beefsteak grafted on to potato roots, cultivar unknown) and cucumber (cultivar: Naomi) were obtained from a local nursery in Delta. Pepper plants (cultivar: Enza 444) were obtained from the Pacific Agri-food Research Center (Agriculture and Agri-Food Canada in Agassiz). Four week old plants grown on rockwool were transplanted into potting soil and kept on a flood table where they were watered daily with fertilized water. During part of the experiment, cucumber plants showed damage due to thrips and were controlled with predatory mites *Amblyseius cucumeris*.

*T. ni* neonates were placed on the different leaf types in 60 ml plastic cups in groups of five at 25°C. More than three hundred larvae were tested on each leaf type (see Results for exact sample sizes). After four days, mortality was assessed and surviving larvae were transferred singly into 60 ml cups on a fresh piece of leaf. Leaves were

changed and faeces removed when necessary. When larvae had achieved the third or fourth instar, they were weighed. Larvae weighing 20 to 65 mg were put into the parasitoid cage in a 60 ml cup and observed until parasitization occurred (at least one contact with the parasitoid). For 70 of the parasitization events, the time it took for a fly to find and contact the given host was recorded. Parasitoid puparia were collected, weighed and put in a Petri dish until emergence. Date of emergence and sex of the parasitoid were recorded.

# Statistical analyses

All analyses were done in JMP IN 4.0 (2000). For the assay under greenhouse conditions, a linear regression was used to test the relationship between the parasitoid number per cage and the following response variables: the percentage of damaged leaves (arcsine transformed), the number of cabbage looper larvae and the number of empty pupae. The effect of plant type on *T. ni* mortality after four days was assessed using a Chi-Square analysis. To assess the effect of crop and *T. ni* sex on puparium weight and larval development time, a two-way ANOVA was used. The effect of crop type on the time required for *C. concinnata* to contact the host was assessed using a one-way ANOVA. A regression analysis was used to assess the relationship between host larval weight at parasitization and time to contact the host.

#### **Results**

# Assay under greenhouse conditions

Parasitization rates under greenhouse conditions were low, and were not sufficient to control *T. ni* populations or prevent feeding damage in most cages. Only two cages produced parasitoid offspring. One cage had one pair of parasitoids initially and produced six parasitoid offspring. However the *T. ni* population did not persist in that cage, and while parasitoids from the second generation were seen flying in the cage, they could not

reproduce. The other cage had initially three pairs of parasitoids and six puparia were found at the conclusion of the experiment, three of which emerged. Puparia were found mainly on the ground, two were found in the host cocoon. In this cage five *T. ni* larvae were found, but none were parasitized.

There was no relationship between the number of parasitoids put in cages in the greenhouses and the proportion of damaged leaves ( $F_{1, 27}=0.089$ , p=0.769, R<sup>2</sup>=0, y=0.42+0.02x) and the number of *T. ni* larvae ( $F_{1, 27}=3.566$ , p=0.070, R<sup>2</sup>=0.12, y=-0.66+7.27x). The number of empty pupae found was negatively influenced by the number of parasitoids put in cages ( $F_{1, 27}=4.563$ , p=0.042, R<sup>2</sup>=0.15, y=9.91-1.23x) (Table 1), but the data fit the curve poorly.

Table 1. Relationship between number of pairs of parasitoids per cage and proportion of damaged leaves, number of T. ni larvae and empty pupae

Pairs of parasitoids	0	1	2	3
Proportion of leaves damaged	$0.60 \pm 0.20$	$0.16 \pm 0.04$	$0.48 \pm 0.21$	$0.57 \pm 0.15$
Number T. ni larvae	$2.7 \pm 1.6$	$0.1 \pm 0.14$	$16.7 \pm 15.6$	$21.4\pm8.1$
Number T. ni pupae	$9.9 \pm 1.3$	$8.9 \pm 1.7$	$7.3 \pm 1.2$	$6.3 \pm 1.1$

# **Tritrophic interactions**

There was evidence of an effect of plant type on mortality of *T. ni* larvae (N=1425, df=2,  $\chi^2$ = 71.18, p<0.001) (Fig. 1). Mortality after four days was high for *T. ni* larvae reared on pepper, low on cucumber and intermediate for tomato. None of the larvae reared on pepper foliage reached pupation. For this reason, no further data could be gathered for the pepper crop, only cucumber and tomato could be compared with regards to the effects on fitness of the parasitoid.

Figure 1. Percentage mortality of *T. ni* larvae after being reared for four days on cucumber, pepper and tomato foliage. The sample sizes are shown above each bar.



Parasitoid development time differed with crop type (Table 2; Fig. 2). Because sex of the parasitoid also influenced development time and puparium weight, this was also included in the analysis (Table 2). Overall, females took longer to develop at the larval stage than males and had marginally heavier puparia. Larval development time was the shortest (Fig. 2) and puparia were heavier (Fig. 3) when the host had been reared on cucumber.

Table 2. Analysis of variance for larval development time and pupal weight of the parasite with crop and parasite gender (N=59)

	Larval development time			Pupal weight			
Model	SS	F ratio	P value	SS	F ratio	P value	
Crop	49.301	29.981	<0.001	0.00117	12.756	<0.001	
Sex	41.149	25.024	<0.001	0.00176	19.145	<0.001	
Crop*sex	1.366	0.831	0.366	0.00016	1.712	0.196	

Figure 2. Larval development time of *C. concinnata* emerging from *T. ni* reared on cucumber and tomato



Figure 3. Puparium weight of *C. concinnata* emerging from *T. ni* reared on cucumber and tomato



It took significantly longer for *C. concinnata* to contact *T. ni* fed tomato than those fed cucumber ( $F_{1, 69}$ =11.314, p=0.001). On average it took parasitoids 269.8 ± 35.5 seconds to contact tomato fed larvae and 94.2 ± 38.2 seconds to contact cucumber fed larvae. The weight of the host larvae did not influence the time for parasitoids to contact the host (r<sup>2</sup>=0.04, F=2.923, p=0.092).

# **Discussion**

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Efficacy of parasitoids as biological controls in field or greenhouse situations is influenced by both direct effects of plant structure and chemistry, and indirect effects of plants via host quality (Bottrell at al. 1998).

# Direct effects of plant structure and chemistry

Although laboratory trials (Chapter 3) have shown high rates of parasitization of T. ni by C. concinnata, the greenhouse trial found much lower rates. While the successful reproduction of the parasitoid in two replicates, the loss of the T. ni population in one replicate, and the reduced number of T. ni pupae found in cages with higher number of flies provide some evidence of potential for biocontrol, success was generally poor. Of the 21 cages where parasitoids were released, only two cages had a second generation, and this was not influenced by the initial number of parasitoids. Even in the two cages where parasitoids established, there was no evidence of a reduction in leaf damage. Factors which determine the success of the parasitoid in some replicates were not immediately obvious, and future research should aim to determine those factors.

The general lack of establishment by the parasitoid may be due to a number of different factors. Firstly, it is possible that *C. concinnata* could not detect the host in the complex environment of the cucumber plants. Secondly, the parasitoid failed to find the host despite the intensive grazing damage. It is possible that *C. concinnata* does not respond to the semiochemicals produced by the plant under attack and instead based the

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host search on other basis. The main factor triggering parasitization in *C. concinnata* seems to be movement (Weseloh 1980, Chapter 3). On cucumber, *T. ni* appears to have reduced movement (V. Cervantes, personal communication) which may impede the ability of *C. concinnata* to locate its host. Finally, using cages on single plants does not represent an open greenhouse setting. Since *T. ni* were suppressed in one cage where a second generation of the parasitoid was found, there were no hosts available for the new parasitoids. Additionally, the other parasitoids present were likely to be siblings, which may reduce the probability of successful mating and progeny. In an open area, where the parasitoid population would be buffered from local extirpation of its host, I might have found different results. In addition, the cages appeared to result in incidental mortality of both parasitoids and *T. ni* moths.

### **Tritrophic interaction**

I found strong evidence of an effect of crop type on T. ni. Larval T. ni are highly polyphagous and can readily feed on 160 plant species (Sutherland and Greene 1984). Even with such a wide range of potential hosts, generalist herbivores still show preferences (Cates 1980, 1981) and have lower fitness associated with lower quality plant host (Awmack and Leather 2002). Trichoplusia ni raised on peppers displayed high mortality and failure to pupate. Trichoplusia ni had the highest survival on cucumber, a result which is consistent with that shown by Janmaat and Myers (2005), who found that T. ni reared on cucumber leaves were heavier and developed faster. Plants have chemical defenses to fight herbivory (Awmack and Leather 2002) and it is possible that T. ni is less adapted to chemicals produced by pepper, making cucumber the most suitable crop of the three evaluated, followed by tomato. My results may not be true for all T. ni populations - T. ni causes extensive damage to all three crops in greenhouses, including peppers. Cates (1980) showed that polyphagous species can have wide host range over a geographical region, but localized larvae can become specialized. While some populations may be capable of specializing on peppers in greenhouse conditions where no other plants are present, the population in this study shows a clear gradient of fitness

costs associated with host plant, with plant quality ranging from poor (pepper) through intermediate (tomato) to good (cucumber).

Differences in the fitness of T. ni reared on different crops grown in greenhouses provide the basis for the tritrophic interaction with C. concinnata. Development time is an important aspect of parasitoid fitness since the risk associated with predation of the host increases with longer development time. Parasitoid development time was shown to vary with the plant fed to the herbivore, consistent with other studies on similar systems (Karowe and Schoonhoven 1992; Werren et al. 1992; Leather et al. 2005; Setamou et al. 2005). In this study, larval development time was faster for C. concinnata when the host was reared on cucumber rather than tomato. Similarly, C. concinnata puparium weight (which is correlated with fecundity (Bourchier 1991)) was higher when the host was fed on cucumber. Compsilura concinnata emerging from cucumber-fed hosts therefore have higher fitness than those emerging from tomato-fed hosts. These results are in accordance with Bourchier (1991) who showed that Lymantria dispar fed on a diet containing higher levels of tannins, which reduces plant quality to herbivores, produced C. concinnata individuals with slower development time and lower puparium weight. There is the potential therefore that the lower fitness found in C. concinnata emerging from tomatofed T. ni in this study is due to plant chemical defenses. A possible candidate compound for producing chemical defenses in tomatoes is tomatine which has been shown to have tritrophic interactions with parasitoid wasps (Campbell and Duffey 1979).

The ability of the parasitoid to locate the host was also reduced when the host was reared on tomato. Interestingly, when the host was exposed to the parasitoid in the presence of faeces or leaf material, parasitization was found to be faster (Caron, unpublished data), indicating that the parasitoid may use volatiles emanating from faeces or leaves as cues to find the host. Stireman (2002) showed similar results with *Exorista mella* a generalist tachinid, which responded faster to damaged plants and movement, but little to host cues. From these results, we can extrapolate that parasitoids can be subjected to different tritrophic effects on plants due to impact on the host quality reducing the fitness of the parasitoid, but also the reduction of the ability to detect hosts. Studies

assessing efficiency of parasitoids on different plants should consider both direct and indirect plant-parasitoid interactions.

# **Concluding remarks**

This study shows the potential for plants to affect the efficacy of a parasitoid both directly, through structural and chemical means, and indirectly, via tritrophic interactions. *Trichoplusia ni* was shown to have higher fitness when reared on cucumber, followed by tomato and with lowest fitness on pepper. *Compsilura concinnata* was also shown to have higher fitness and locate cucumber raised hosts faster. Therefore, efficiency of *C. concinnata* should be higher on cucumber plants than the other crops tested. However, in the greenhouse trial when full cucumber plants were used, *C. concinnata* did poorly and had no impact on the numbers of *T. ni* found and damage caused to plants. Even though *C. concinnata* has higher fitness on cucumber-fed *T. ni*, poor host location ability, potentially due to plant structure, predominates. Therefore, there are positive indirect (host quality) and direct (host location on leaf) tritrophic interactions, but these are overwhelmed by a negative direct tritrophic interaction (host location on full plant).

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# **CHAPTER 6 - GENERAL CONCLUSIONS**

Control of greenhouse pests represents a significant challenge because of the combined high value of the crop, strict pesticide regulations and favourable conditions for pest outbreaks. *Trichoplusia ni* is the only lepidopteran pest in vegetable greenhouses in British Columbia. Major population outbreaks can occur and cause important economic damage. *Bacillus thuringiensis* (Bt), has traditionally been the main means of control of *T. ni* in greenhouses, but evolution of resistance made it no longer effective in some greenhouses at keeping the pest below damage thresholds (Janmaat and Myers 2003). Control of the pest must therefore be achieved via other means. These could include changes in management, such as reducing greenhouse temperatures between growing seasons to ensure there is no carry-over of Bt resistant phenotypes between seasons. Alternatively, other non-chemical means of control may need to be considered. This may include the use of natural enemies such as parasitoids to reduce pest populations. In order to use this option effectively, there is a need for detailed information on interactions between the pest, the parasitoid and the crops.

# The role of greenhouse management: effects of overwintering temperatures

Evolution of resistance to insecticides such as Bt is strongly influenced by tradeoffs or fitness costs (McKenzie 1996; Cotter et al. 2004). Fitness costs are varied, and can be increased by stressful conditions such as competition (Bourguet et al. 2004; Higginson et al. 2005) or the overwintering period (McKenzie 1994; Foster et al. 2000; Carrière et al. 2001). My expectation was that cooler greenhouse temperatures between growing seasons would reduce viability of *T. ni*, and that this effect would be stronger in *Bt* resistant individuals. Contrary to that expectation, in Chapter 2, I showed that *Bt* resistant *T. ni* pupae had the same low overwintering success as susceptible pupae at 10°C. In fact, *Bt* resistant pupae had higher emergence rates when exposed to 10°C for 6 weeks although these moths produced no eggs. This is the first published account of a resistant phenotype having higher survival than the non-resistant phenotype. Chapter 2 also showed that *Bt* resistant moths emerging from pupae exposed to cold temperatures had smaller viable progeny than susceptible ones. This fitness cost is consistent with that shown by Janmaat and Myers (2003), in a situation where the pupae were not exposed to cold.

While my study and that of Janmaat and Myers (2003) found significant costs associated with resistance, in greenhouse situations where Bt is being applied, very strong selection for resistant individuals is likely. The frequency of the Bt resistant genotype in greenhouses is therefore likely to be a function of two factors:

1/ selection pressure for the genotype in the greenhouse, which is likely to be sufficient to overwhelm fitness costs of resistance and 2/ rates of gene flow from neighbouring populations which are not experiencing selection, and where fitness costs disfavour the resistant genotype. There is likely to be very limited movement between the outside environment where *T. ni* populations show low resistance levels (Janmaat and Myers 2003), and the greenhouse populations. *Trichoplusia ni* does not overwinter in field conditions in British Columbia, but recolonizes from more southerly populations (Mitchell and Chalfant 1984). Chapter 2 shows that resistant *T. ni* can survive the overwintering period, and if selection (i.e. spraying of *Bt*) keeps occurring, resistant individuals will likely pass on their resistance to their offspring early in the season, allowing resistance to build up in the greenhouse. This could explain the rapid appearance of resistance in greenhouses observed by Janmaat and Myers (2003).

Results from Chapter 2 therefore have important implications in the management of *T. ni* in greenhouses, and leave two main choices to the growers.

 Since no *T. ni* were shown to lay eggs after having spent 3 weeks at 10°C, leaving the greenhouse empty and unheated for more than 3 weeks should kill all *T. ni*. One has to keep in mind that temperatures fluctuate during the unheated period and that also might influence *T. ni* survival.

- 2. Early season spraying with *Bt* will produce high levels of resistance if *T. ni* populations persist between growing seasons.
- 3. An alternative to *Bt* should be sought.

# Alternative means of control: evaluation of a parasitoid C. concinnata

As established in Chapter 2 and in previously published work by Janmaat and Myers (2003), under current management, resistance to *Bt* is unlikely to disappear. For that reason, finding an alternative to *Bt* or a means of control which could be integrated with *Bt* is appealing. Parasitoids are already widely used against certain greenhouse pests such as whiteflies (Hoddle et al. 1998). Parasitoids have the advantage that they are compatible with other management practices used in greenhouses to control other pests. They could also form self-sustaining populations in greenhouses, reducing the need for reapplication and therefore minimizing control costs.

I surveyed field populations to find naturally occurring parasitoids of *T. ni*, and established a laboratory population of a dipteran parasitoid, *Compsilura concinnata* Meigen (family: Tachinidae) for appraisal as a means of control for *T. ni*. *Compsilura concinnata* was chosen to be assessed because it was already present in British Columbia, and was the only parasitoid found throughout the field season (see Chapter 3). The potential of parasitoids as biological control agents is directly related to their ecology including interactions with their host and abiotic environment (Godfray 1994). Direct interactions between host and parasitoid are reflected in parasitization rates, host preference behaviors by the parasitoid (Chapter 3) and stress responses by the host (Chapter 4). More complex interactions occur between the host's food plants and the parasitoid via changes in host quality and detectability (Chapter 5).

When *T. ni* was exposed to *C. concinnata* in controlled laboratory conditions in Chapter 3, parasitization rates were consistently high. *Compsilura concinnata* was shown to more readily parasitize later *T. ni* instars. The instar of *T. ni* at parasitization had an

impact on the parasitoid larval development time, with faster development on later stage hosts. This provides a potential advantage to the parasitoid by reducing time spent in the host, during which it is exposed to incidental ingestion by host predators. Superparasitism was frequent in T. ni, as has been shown for other host species (Eichhorn 1996; Kellogg et al. 2003). Superparasitism was more prevalent at the fourth instar, which is the older and largest instar tested, and had an impact on the parasitoid larval development time and puparium weight. This is likely to be due to internal host competition for food, and was reflected in a more rapid reduction of hemolymph protein when superparasitism occurred (Chapter 4). In general, the host exhibited a muted immunological response to parasitization by C. concinnata (Chapter 4). This may be due to the larval parasites lodging themselves between the peritrophic membrane and the midgut, an area which may not to be accessible to the immune system, and thus to avoid triggering the phenoloxidase cascade which is a major part of the encapsulation process (Chapter 4). Compsilura concinnata has a wide host range, affecting over 160 host species in North America (Arnaud 1978; Strazanac et al. 2001). This generalization may be permitted by this immune avoidance strategy.

The effectiveness of a parasitoid may also be influenced by indirect effects of host plants occurring via changes in host quality (Price et al. 1980; Bottrell et al. 1998). *Trichoplusia ni* is highly polyphagous (Sutherland and Greene 1984), but has food crop preferences which may reflect on the quality of the food source, and therefore potentially on host quality for the parasitoid. In Chapter 5, *T. ni* was shown to have highest fitness on cucumber, followed by tomato and then pepper. Following the same pattern, *C. concinnata* had higher fitness when emerging from cucumber-fed *T. ni* and took less time to locate the cucumber-fed hosts under laboratory conditions. This strongly suggests that the effectiveness of the parasitoid in controlling *T. ni* will vary depending on the host plant. The mechanism underlying this tritrophic interaction was not determined in this study, but represents an interesting area for further investigations.

The ability of a parasitoid to detect a host in the natural environment may also be affected by plant structure or chemistry. In greenhouse trials when hosts needed to be

detected on a whole plant, *C. concinnata* did not readily parasitize *T. ni* (Chapter 5). It is possible that some aspect of the greenhouse environment reduces the ability of the parasitoid to detect the host, and therefore control host populations. This is supported by the results of the initial field survey (Chapter 3) which found *C. concinnata* to be rare in the field, and to not track changes in *T. ni* density. The nature of the greenhouse trial in this study was relatively limited, using only one host plant (cucumber) and using caged plants which did not permit free movement of the parasitoid. Future studies may find that the parasitoid is more effective when the parasitoid population can operate on larger spatial scales. Because *C. concinnata* is relatively easy to rear in large numbers, a greenhouse scale inundative release may be worth trying for a definitive evaluation of this parasitoid.

Results from Chapter 3, 4 and 5 suggest that while *C. concinnata* may have some potential as a control agent, a number of difficulties would need to be overcome.

- 1. Tritrophic interactions between host plants, *T. ni* and *C. concinnata* mean that the effectiveness of the parasitoid will vary depending on the crop used.
- 2. A greenhouse release of *C. concinnata* on caged plants was not effective in reducing *T. ni*, or reducing leaf damage.
- 3. Future field trials should be carried out at a larger spatial scale to replicate real host parasitoid dynamics.

# Summary

Resistance to *Bt* in greenhouse *T. ni* populations needs to be addressed as the development of resistance in greenhouse populations is likely to occur under current management practices. A parasitoid could represent a potential alternative. *Compsilura concinnata* offers some useful characteristics in that it is already present in the field, and can parasitize *T. ni* at high rates in some conditions. However, even if the ecology and

behavior of this parasitoid are interesting, the potential of this generalist parasitoid to control effectively *T. ni* in vegetable greenhouse seems low based on this study. A more specific parasitoid with a higher ability to find *T. ni* on the crop plants and which responds to *T. ni* density would be more suitable.

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