Sublethal Effects of *Bacillus thuringiensis* Berliner subsp. *kurstaki* on *Lymantria dispar* (Lepidoptera: Lymantriidae) and the Tachinid Parasitoid *Compsilura concinnata* (Diptera: Tachinidae)

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ABSTRACT Parasitoid-pathogen interactions were examined using gypsy moth, *Lymantria dispar* (L.), *Bacillus thuringiensis* Berliner, and *Compsilura concinnata* (Meigen). The objectives of this study were to quantify effects of sublethal doses of *Bacillus thuringiensis* (*Bt*) force-fed to gypsy moths and to determine if sublethal doses of *Bt* affected host acceptance and suitability of gypsy moth for *C. concinnata.* Gypsy moths were minimally affected by sublethal doses of *Bt;* development of fourth instar was delayed, and male pupal mass reduced. *Compsilura concinnata* preferentially attacked and had higher superparasitism on noninfected hosts than on *Bt*-treated larvae. Exposure of gypsy moth to both sublethal doses of *Bt* and parasitoids reduced percentage parasitism and host larval survivorship. Effects on *C. concinnata* development varied with host superparasitism status. Parasitoids in *Bt*-treated, superparasitized gypsy moths had shorter larval development times and smaller pupal masses than parasitoids in untreated larvae, while parasitoids in singly parasitized larvae had larger pupal masses than those in superparasitized larvae. Timing of *Bt* infection relative to parasitism is a factor in gypsy moth mortality, but not in parasitoid potential fecundity.

KEY WORDS *Lymantria dispar*, *Bacillus thuringiensis*, *Compsilura*, sublethal effects

GYPSY MOTH, *Lymantria dispar* (L.), is considered one of the most damaging forest insect pests in the northeastern United States (Cameron 1986) affecting >300 host plant species (Doane and McManus 1981). Despite quarantine and eradication efforts, it has continued to spread throughout susceptible forest types, and is now widely established in the eastern United States and Canada (Doane and McManus 1981). It has been the subject of classical biological control efforts with parasitoids, as well as intense spray programs using *Bacillus thuringiensis* Berliner (*Bt*).

Despite widespread use of *Bt* for gypsy moth suppression, the extent of target insect mortality that can be achieved is limited by environmental and technical constraints. In aerial spray programs, larvae often consume a sublethal rather than a lethal dose (Pedersen et al. 1997). Sublethal effects of *Bt* on the pest are often not well understood because most studies involving *Bt* against forest pests have focused on larval mortality (van Frankenhuyzen 1995). Furthermore, sublethal effects on other trophic levels, such as parasitoids of target insects, are poorly understood (Elzen 1989). Ingestion of a sublethal dose may change host attributes that influence parasitoid foraging or oviposition. In addition, parasitoids attacking *Bt*-infected hosts may be directly or indirectly affected by the pathogen.

Interactions between parasitoids and pathogens such as *Bt* are becoming increasingly important as integrated pest management regimes are being used more frequently in forest and agro-ecosystems. These interactions can range from synergistic to competitive, depending on environmental conditions and timing of interactions (Chilcutt and Tabashnik 1997). In some cases, *Bt*-treated larvae develop slower or are smaller than noninfected insects, which renders them more susceptible to attack by parasitoids and results in higher rates of parasitism and better pest control (Weseloh et al. 1983, Mascarenhas and Luttrell 1997). Larvae parasitized before contact with *Bt* often lack the feeding stimulus required to ingest a lethal dose, whereas unparasitized larvae feed normally and are more likely to ingest lethal doses (Hamel 1977, Nealis et al. 1992). Results of several studies indicate that larvae parasitized after *Bt* infection are more likely to perish, along with their parasitoids (Nealis and van Frankenhuyzen 1990, Ulpah and Kok 1996, Blumberg et al. 1997).

Results of studies examining the effects of *Bt* on parasitoids range from no apparent negative effects on various developmental stages or parasitoid emergence (Weseloh and Andreadis 1982, Ulpah and Kok 1996, Atwood et al. 1998) to lower parasitoid survival rates due to premature host death (Nealis and van Frankenhuyzen 1990, Blumberg et al. 1997), lower parasitoid emergence rates (Atwood et al. 1997), increased (Ahmad et al. 1978) or decreased larval development

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times (Weseloh 1984b) and altered parasitoid sex ratios (Wallner et al. 1983).

In this study we examined the tritrophic interactions among gypsy moth, *Bacillus thuringiensis,* and *Compsilura concinnata* (Meigen). *Compsilura concinnata* is a polyphagous endoparasitoid, attacking insects in 34 families, the majority of which are lepidopterous larvae (Arnaud 1978). It was introduced into New England and eastern Canada in the early 1900s to control gypsy moth and brown-tail moth (Culver 1919, Sabrosky and Reardon 1976). Adult females are larviparous, attacking mid-to-late instar hosts by piercing the skin and placing a larvae directly into the host gut or hemocoel (Bourchier 1990). Parasitoid larvae develop in the host midgut between the peritrophic membrane and the midgut wall (Bourchier 1991). *Compsilura concinnata* is multivoltine (Webber and Schaffner 1926) and overwinters as mature third instars in prepupae or pupae of alternate hosts (Clausen 1956). *Compsilura concinnata* was chosen for this study because it develops in the host midgut, the site of *Bt* activity, and readily attacks a range of gypsy moth instars (Weseloh 1984a). Furthermore, studies have shown that its development is influenced by host developmental time, diet and stress levels (Weseloh 1984a, Bourchier 1991), host attributes which may be affected by sublethal doses of *Bt*. The specific objectives of our study were to quantify sublethal effects of *Bt* on gypsy moth development, assess host acceptance of *Bt*-infected and non-infected larvae by *C. concinnata*, and to assess host suitability of *Bt*-infected larvae for development of the parasitoid.

Materials and Methods

Insect Rearing. *Gypsy Moth.* Gypsy moth eggs or early instars were obtained from a laboratory stock maintained by the Canadian Forest Service in Sault Ste. Marie, Ontario. For the bioassay quantifying sublethal effects of *Bt* on gypsy moth (experiment 1) and for the *C. concinnata* host acceptance experiment (experiment 2), larvae were placed in groups of ≈200 in sterilized 4.2-liter plastic Rubbermaid (Wooster, OH) containers containing 1-liter of artificial diet (Bioserv, Frenchtown, NJ). The lid of each rearing container had two 9-cm holes covered with Whatman (Hillsboro, OR) filter paper to prevent condensation. The density was adjusted to ≈100 per container as larvae developed. For the experiment examining *C. concinnata* development in *Bt*-infected hosts (experiment 3), larvae were reared in groups of 10–12 in 250-ml cups (Sweetheart, Owing Mills, MD) containing ≈75 ml diet (Bell et al. 1981). All rearing was conducted at 22 ± 1°C, 60% RH, and a photoperiod of 16:8 (L:D) h.

Compsilura concinnata. C. concinnata pupae were collected from fall webworm (*Hyphantria cunea* Drury) in Blind River, Ontario (46° 10′ N, 82° 58′ W) and Prince Township, Ontario (46° 35′ N, 84° 32′ W), in the fall of 1996, and from sentinel gypsy moth larvae released in Olden township, Ontario (44° 44′ N, 76° 51′ W) in the summer of 1998. Specimens were confirmed as *C. concinnata* by J. E. O'Hara (ECORC-AAFC, Ottawa, Canada). Voucher specimens were deposited in the insect collection at the Great Lakes Forest Research Center in Sault Ste. Marie, Ontario, Canada. Colonies originating from both collections were assumed to be homogeneous, because *C. concinnata* has been shown to be genetically similar throughout eastern North America (Sánchez and Cardé 1998). Parasitoids were maintained on gypsy moth larvae in the laboratory before experimentation. For colony propagation, fly pupae and adults were reared at 19 ± 2°C, 50–60% RH, and a photoperiod of 16:8 (L:D) h. Female flies were at least 8 d old before they were provided with fourth-instar gypsy moth (Culver 1919; Fusco et al. 1978). Gypsy moth larvae were exposed for up to 24 h using a female parasitoid:host ratio of between 1:5 and 1:10 in cages measuring 38 by 38 by 45 cm. Larvae were then removed and placed in 250-ml rearing cups (Sweetheart) with ≈75 ml diet (Bell et al. 1981) and reared in groups of eight until parasitoid pupation. Fly pupae were removed from rearing cups and held in small groups (10–12) in petri dishes lined with moist filter paper. Petri dishes were checked daily after adult emergence started, and adults were placed in the parasitoid cages with up to 30 females and 30 males per cage. Adults were supplied with a 20% honey solution applied to sterilized dental wicks, and were misted twice daily (morning, late afternoon) with tap water. A shallow plastic container (11 by 3 cm) filled with wet sphagnum was placed on the cage floor to provide continuous moisture.

Bacillus thuringiensis Preparation. Foray 48B (Novo Nordisk Bioindustrials, Copenhagen, Denmark, now owned by Sumitomo, Tokyo, Japan) was used for all experiments because it is used operationally for control of gypsy moth. The formulation is based on the HD-1 strain of subspecies *kurstaki* and contains a mixture of spores and crystals for a labeled potency of 12.7 billion international units (IU) per liter. A stock solution with a final concentration of 1,270 IU/ml was prepared by diluting 1 ml of Foray 48B with 9 ml phosphate buffered saline (PBS), which contains 0.01% Triton X-100 as a detergent to maintain the homogeneity of solutions (Pedersen et al. 1997). The stock solution was sonicated before preparing serial dilutions in PBS for each experiment (as per van Frankenhuyzen et al. 1997).

Force-Feeding Technique. A force-feeding technique similar to that used by van Frankenhuyzen and Nystrom (1987) for spruce budworm was modified for gypsy moth. This technique ensured quantitative uptake of a known *Bt* dose, thus eliminating variability associated with differential rates of pathogen acquisition (Pedersen et al. 1997). Suspensions of *Bt* were pipetted into a 0.25-ml syringe fitted with a blunted 30-gauge needle. The needle was placed on a Tractor Atlas Microjector syringe drive (model 1003) interfaced with a model 1010 digital Microdoser (Houston Atlas, Houston TX) to deliver 2 μl of Foray 48B suspension directly into the midgut of each gypsy moth larva. Gypsy moth larvae that regurgitated *Bt* after being removed from the needle were discarded.

Experiment 1. Sublethal Effects of *Bacillus thuringiensis* on Gypsy Moth Survival and Development. Effects of sublethal exposure to *Bt* were examined by dosing fourth-instar gypsy moths with a low (0.66 IU per larva, *Bt*L) medium (1.32 IU per larva, *Bt*M) or high (2.64 IU per larva, *Bt*H) dose of Foray 48B. Control larvae were force-fed with PBS. Dose levels were chosen to represent an LD_{25}, LD_{50}, and LD_{95}, respectively, as determined in a preliminary bioassay. A total of 930 larvae were used within 12 h of molting to the fourth instar. The experiment was conducted over 2 d as dictated by availability of newly molted larvae. Force-fed larvae were reared individually in 30-ml creamer cups with 10 ml gypsy moth diet at 20 ± 1°C, 50–60% RH, and a photoperiod of 16:8 (L:D) h. Cups were checked daily for insect mortality or molting. Larvae were weighed with a digital balance (Sartorius, New York) 1 d after molting. Head capsules were collected, placed individually into gel caps and measured using a WILD Digital Length Measuring unit (Heerbrugg, Switzerland) to obtain larval sizes. Larvae were transferred every 5–6 d into new cups with fresh diet. Diet was removed from the cups on the day of pupation to prevent fungal infections and pupae were weighed 24 h after pupation when the pupal case had hardened. Development was monitored until adult emergence, and newly emerged adults were sexed.

Experiment 2. Acceptance of *Bt*-Treated Hosts by *C. concinnata*. Acceptance of *Bt*-treated larvae by female *C. concinnata* was investigated by providing sexually mature females with a choice of dosed and undosed larvae. Fourth-instar gypsy moths were selected within 24 h of molting and randomly assigned to one of the following three treatments: untreated (C, control), treated with PBS (PBS), or treated with 0.85 IU of Foray 48B (*Bt*), which represented approximately an LD_{50} for this cohort of larvae. Each larva (160 per treatment) was marked with a dot of colored correction fluid on the abdomen posterior to the prolegs to indicate which treatment it had received. One hour after the dosing procedure, larvae were placed together in a cage to which 12-d-old female *C. concinnata* were added in a 1:10 parasitoid:host ratio. Parasitoids had not previously been exposed to any hosts. Gypsy moth larvae were exposed for 24 h before being removed from the cage. They were then placed in 25 by 100-mm glass vials (Canadawide Scientific, Ottawa, ON), and immediately covered with boiling water to preserve insects and facilitate dissection. The number of parasitoid larvae as well as their location in the host midgut (anterior, middle, or posterior section) were recorded. The experiment was conducted over 2 d as dictated by availability of newly molted fourth instars.

Experiment 3. *Compsilura concinnata* Survival and Development in *Bt*-Treated Hosts. The fate and performance of *C. concinnata* in *Bt*-treated gypsy moth was examined in relation to the timing of parasitization (before or after dosing with *Bt*) in a no-choice experiment. One thousand fourth instars were selected within 12 h after molting and were randomly divided over the following treatments: *Bt* dosing followed by exposure to *C. concinnata* (*Bt*-P, $n = 365$), exposure to *C. concinnata* followed by *Bt* dosing (P-*Bt*, $n = 386$), and exposure of undosed larvae to *C. concinnata* (control, C, $n = 209$). Larvae in the *Bt*-P and P-*Bt* treatments were dosed with either 2.8 or 4.7 IU per larva, in roughly equal numbers, to bracket the desired response range (LD_{30}–LD_{60}) as determined from a preliminary bioassay with this cohort of larvae. Larvae assigned to the *Bt*-P treatment were force-fed in the morning. In the afternoon, larvae from all treatments were placed in cages with 10-d-old *C. concinnata* females in a 1:10 ratio, using two cages per treatment. After 24 h of exposure to the parasitoids, larvae were removed from the cages and larvae in the P-*Bt* treatment were force-fed *Bt.* All larvae were placed individually in 30-ml creamer cups with 10 ml diet and monitored daily for gypsy moth mortality and the appearance of parasitoid pupae. Gypsy moth deaths were recorded, and dead larvae were dissected to quantify the number of immature parasitoids that did not survive *Bt* treatment. Parasitoid pupae were held under conditions of 22 ± 1°C, 60% RH, and a photoperiod of 16:8 (L:D) h until they emerged. Multiple parasitoids that emerged from one host were recorded and parasitoid pupae were tracked individually. Pupae were weighed on a digital scale (Sartorious) once the pupal case had hardened (≈2 h after pupation). Emergence dates and sex of adult flies were recorded.

Data Analysis. Host and parasitoid developmental variables (mean development times and weights) were analyzed using one-, two- and three-way analyses of variance (ANOVA) (GLM module; Systat, Wilkinson 1997). Percentage data (survival, adult emergence, adult sex ratios, rates of parasitism, location of parasitoids in host midgut) were analyzed in two- and three-way contingency tables using *G*-tests (for two-way: X-TAB module; for three-way: LOGLIN module; Systat, Wilkinson 1997). Multiple comparison procedures for ANOVAs and contingency tables were carried out using Tukey's honestly significant difference test and simultaneous test procedures, respectively (Sokal and Rohlf 1995). Pupal weights for male and female gypsy moth in experiment 1 were analyzed separately using a one-way ANOVA because no transformation could be found to normalize the bimodal distribution caused by sex dimorphism.

Results

Experiment 1. Sublethal Effects of *Bt* on Gypsy Moth Survival and Development. Survival of gypsy moth larvae 6 d after force-feeding was dose-dependent and differed significantly among treatments, with survival rates of 12.6, 37.4, 58.2, and 98.6% in the *Bt*H, *Bt*M, *Bt*L, and control groups, respectively ($G = 134.01$, df = 3, $P < 0.001$). Most of the *Bt*-induced mortality occurred within 6 d of *Bt*-dosing during the fourth larval stadium (Fig. 1). An additional 20% mortality at the end of the fourth stadium occurred in all treatments, for reasons unknown.

Despite significant differences between males and females (data not presented), treatment means were

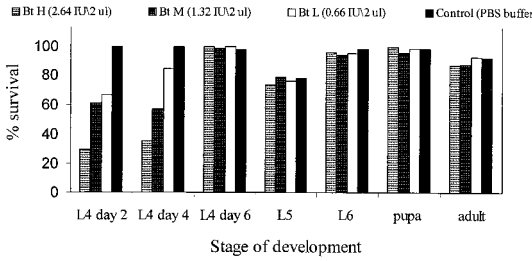

Fig. 1. Percentage survival of gypsy moth to adult after being force-fed *B. thuringiensis.* Survival is expressed for each instar as a percent of insects alive at the end of the previous instar (or number of days after dosing, in the case of fourth instar). Number of larvae force-fed were 145, 220, 214, and 340 for control, *Bt*L, *Bt*M, and *Bt*H treatments, respectively.

pooled for ease of presentation because there was no interaction between sex and *Bt* treatment for any of the variables measured. Total larval development time (from the first day of the fourth stadium to pupation) was significantly affected by treatment ($F = 3.269$; df = 3, 226; $P = 0.022$) with larvae in the *Bt*H treatment developing significantly slower than in the control. The difference in total larval development time between treated and control larvae was due primarily to slower development of treated larvae during the fourth stadium ($F = 6.71$; df = 3, 224; $P < 0.001$), as there was no significant effect of treatment on the duration of the fifth ($F = 1.950$; df = 3, 226; $P = 0.122$) or sixth ($F = 0.73$; df = 3, 106; $P = 0.534$) stadia (females only) (Table 1). There was no effect of *Bt* treatment on the duration of the pupal stage ($F = 163$; df = 3, 222; $P = 0.182$).

Differences in larval development times were not reflected in larval size (head capsule measurements) or larval weight (data not presented). There were no significant effects of treatment on head capsule measurements of fourth, fifth, or sixth instars (fourth: $F = 0.667$; df = 3, 225; $P = 0.573$; fifth: $F = 2.399$; df = 3, 222; $P = 0.069$; sixth: $F = 1.643$; df = 3, 107; $P = 0.184$). Similarly, there was no effect of sex or treatment on mean weight of fifth instars ($F \geq 0.790$; $P \geq 0.179$; for treatment: df = 3, 223, for sex: df = 1, 223), and no effect of treatment on mean weight of female sixth instars ($F = 0.454$; df = 3, 107; $P = 0.715$). The *Bt* treatment, however, caused a significant reduction in weight of male pupae ($F = 3.68$; df = 3, 118; $P = 0.014$), but did not affect female pupal weight ($F = 0.064$; df = 3, 107; $P = 0.979$) (Table 2).

Table 2. Mean pupal masses (g ± SEM) and adult emergence of gypsy moth force-fed PBS buffer or buffer with a high, medium or low dose of *Bacillus thuringiensis* as fourth instars

Treatment[a]	Pupal mass[b] (female/male)	Pupal mass (female/male)	% adult emergence
Control (PBS only)	1.93 ± 0.08a[c] (34)[d]	0.57 ± 0.02a (53)	92.6a (88)
*Bt*L	1.89 ± 0.08a (38)	0.49 ± 0.02b (43)	93.1a (81)
*Bt*M	1.93 ± 0.09a (28)	0.53 ± 0.03ab (16)	90.0a (45)
*Bt*H	1.89 ± 0.15a (11)	0.50 ± 0.04ab (10)	87.5a (21)

[a] Analysis includes insects that developed to adult stage, and did not go through a supernumerary molt.
[b] Pupal mass for males and females was analyzed separately using a one-way ANOVA.
[c] Significantly different means within columns are indicated by different letters ($P \leq 0.05$, Tukey-Kramer HSD for pupal masses, simultaneous test procedures for adult emergence (Sokal and Rohlf 1995).
[d] Sample size.

Adult emergence rates of larvae that survived *Bt* doses varied from 87 to 93% across treatments (Table 2). There was no significant three-way interaction between emergence, sex and *Bt* treatment, ($G = 3.179$, df = 3, $P = 0.365$), so further analyses were conducted in two-way tables. There was no effect of *Bt* treatment ($G = 2.200$, df = 3, $P = 0.532$) or sex on emergence ($P = 0.285$, data not shown). The sex ratio (F:M) was slightly female biased for *Bt*H and *Bt*M treatments (10:9 and 9:5, respectively) and slightly male biased for *Bt*L and control treatments (8:9 and 2:3, respectively), but the effect was not significant ($G = 6.951$, df = 3, $P = 0.075$).

Experiment 2. Acceptance of *Bt*-Treated Hosts by *C. concinnata.* Gypsy moth larvae treated with *Bt* experienced significantly lower rates of parasitism than larvae in control and PBS treatments ($G = 23.84$, df = 2, $P < 0.001$) (Table 3). The force-feeding procedure itself did not affect the rate of parasitism as the proportion of parasitized larvae in the control and PBS treatments were not significantly different. Parasitized larvae in the *Bt* treatment contained significantly fewer parasitoids than larvae in the control or PBS

Table 1. Mean larval and pupal developmental times (days ± SEM) of gypsy moth force-fed PBS buffer or buffer with a high, medium or low dose of *Bacillus thuringiensis* as fourth instars

Treatment[a]	Total larval developmental time	Duration of instar			Pupal duration[b]
		Fourth	Fifth	Sixth[c] (female only)	
Control (PBS only)	22.55 ± 0.26a[d] (88)[e]	6.97 ± 0.18a (86)	9.25 ± 0.11a (88)	12.11 ± 0.14a (35)	12.43 ± 0.08a (86)
*Bt*L	23.14 ± 0.27ab (81)	7.66 ± 0.18b (80)	9.23 ± 0.11a (80)	12.35 ± 0.13a (38)	12.22 ± 0.09a (81)
*Bt*M	23.09 ± 0.37ab (44)	8.02 ± 0.25b (45)	9.23 ± 0.16a (45)	12.35 ± 0.16a (26)	12.48 ± 0.12a (44)
*Bt*H	24.35 ± 0.53b (21)	8.38 ± 0.35b (21)	9.79 ± 0.22a (21)	12.42 ± 0.25a (11)	12.32 ± 0.18a (19)

[a] Analysis includes insects that developed to adult stage, and did not go through a supernumerary molt.
[b] Log-transformed to achieve homogeneity of variances.
[c] Transformed to (development time)^3 to achieve homogeneity of variances.
[d] Significantly different means within columns are indicated by different letters ($P \leq 0.05$, Tukey-Kramer HSD).
[e] Sample size.

Table 3. Parasitism, superparasitism and mean number (±SEM) of *Compsilura concinnata* parasitoids in untreated, PBS or *Bt*-treated fourth instar gypsy moth

Treatment	% Parasitism	Mean no. parasitoids/ parasitized host	% Superparasitism[a]
Control	71.7a[b] (159)[c]	1.92 ± 0.09a (114)	51.6a
PBS	63.8a (160)	1.72 ± 0.10a (102)	52.0a
Bt (≈LD_{50})	45.6b (160)	1.55 ± 0.11b (73)	37.0a

[a] Proportion of parasitized larvae with more than one parasitoid per host.
[b] Significant differences within columns are indicated by different letters ($P \leq 0.02$, Tukey-Kramer HSD for means, simultaneous test procedures for % parasitism and superparasitism).
[c] Sample size.

treatment ($F = 14.5$; df = 2, 476; $P < 0.001$). Rates of superparasitism (proportion of larvae containing more than one parasitoid) also tended to be lower in *Bt*-treated larvae ($G = 4.881$, df = 2, $P = 0.087$).

Location of parasitoid larvae ($n = 506$) in the gypsy moth midgut was not affected by either treatment or the number of parasitoids per host (1, 2, or ≥3) ($G \leq 6.62$, df = 4, $P \geq 0.157$) (data not presented). Subsequent analyses in two-way tables confirmed the significant effect of treatment on parasitism status ($G = 36.425$, df = 4, $P < 0.001$). Regardless of treatment or parasitism status, parasitoid larvae in single- and superparasitized hosts were found more often in either middle (33–42%) or posterior (32–46%) portions of the midgut than in the anterior portion (20–27%).

Experiment 3. *Compsilura concinnata* Survival and Development in *Bt*-Treated Hosts. *Parasitism and Host Survival.* Gypsy moth larvae that were force-fed *Bt* and exposed to parasitoids either before or after treatment with *Bt* (*Bt*-P and P-*Bt*), experienced significantly lower survival rates than larvae that were exposed to parasitoids without receiving a *Bt* treatment (control) ($G = 376.10$, df = 2, $P \leq 0.01$) (Table 4). Host survival was measured 7 d after treatment, which was sufficient time for full expression of the ingested dose (Fig. 1). Among larvae exposed to both *Bt* and parasitoids, host survival was significantly higher when parasitoids were introduced after *Bt* ingestion (*Bt*-P) than before *Bt* ingestion (P-*Bt*). Gypsy moth larvae in P-*Bt* and *Bt*-P treatments also

Table 4. Survival, parasitism and superparasitism of fourth instar gypsy moth force-fed *Bt* before or after parasitization by *Compsilura concinnata* compared to control larvae

Treatment[a]	n[b]	% Survival[c]	% Parasitism	% Superparasitism[d]
Control (parasitoids only)	209	99.5a[e]	82.8a	65.3a
Bt-P	365	55.9b	52.6b	47.1b
P-*Bt*	386	25.4c	55.4b	35.4c

[a] Combined over high and low *Bt* doses.
[b] Sample size.
[c] Survival seven days after *Bt*-treatment.
[d] Percentage of parasitized larvae containing more than one parasitoid.
[e] Significant differences within columns indicated by different letters ($P \leq 0.01$, simultaneous test procedures).

Table 5. Percentage parasitism by *C. concinnata* in relation to lethally and sublethally dosed fourth-instar gypsy moth

Treatment	% Parasitism	
	Sublethally dosed larvae	Lethally dosed larvae
Bt-P	64.7a[a] (204)[b]	37.3a (161)
P-*Bt*	71.4a (98)	50.0b (288)

[a] Significant differences within columns indicated by different letters ($P \leq 0.01$, simultaneous test procedures).
[b] Sample size.

experienced lower rates of parasitism and superparasitism than control larvae ($G \geq 35.11$, df = 2, $P < 0.001$) (Table 4). There was no apparent effect of timing of *Bt* ingestion on overall percentage parasitism but superparasitism rates were higher when larvae were parasitized after *Bt* ingestion (*Bt*-P) rather than before (P-*Bt*). Timing of *Bt* ingestion had no effect on parasitism of larvae that survived the *Bt* treatment ($G = 1.37$, df = 1, $P = 0.242$), but in lethally dosed larvae, parasitism was significantly higher when it occurred before *Bt* ingestion ($G = 6.81$, df = 1, $P = 0.009$) (Table 5).

Parasitoid Fate and Development. Treatment means for male and female parasitoids were pooled for ease of presentation because there was no interaction between parasitoid sex and *Bt* treatment for any of the variables in either parasitized or superparasitized hosts (Table 6). Ingestion of *Bt* by the host did not affect mean parasitoid larval development time (from larviposition date to pupation) in singly parasitized hosts ($F = 1.17$; df = 2, 155; $P = 0.313$), but parasitoid development was significantly longer in *Bt*-treated, superparasitized hosts ($F = 5.81$; df = 2, 534; $P = 0.003$) (Table 6). Timing of *Bt* ingestion relative to parasitization did not affect mean larval development time. Mean pupal development time of *C. concinnata* was not significantly affected by *Bt* treatment in either single or superparasitized hosts ($P \geq 0.603$). In most cases, treatment with *Bt* resulted in lower pupal weights compared with parasitoid pupae from control insects ($P \leq 0.006$).

Sex had a significant effect on both parasitoid development time and pupal weights; female parasitoids took significantly longer to develop than males as larvae ($P \leq 0.030$) and pupae ($P < 0.001$) and produced significantly heavier pupae in both single and superparasitized hosts ($P < 0.001$) (data not presented).

Parasitoid adult emergence rates were 91.3, 95.8 and 96.8% in the P-*Bt* ($n = 103$), *Bt*-P ($n = 259$) and control (parasitized only, $n = 370$) groups, respectively, and did not differ significantly between treatments ($G = 4.923$, df = 1, $P = 0.085$). The adult sex ratio was not dependent on treatment or parasitism status of the host; there was no three-way interaction between sex, treatment or parasitism status ($G = 2.135$, df = 2, $P = 0.343$), with adult sex ratios ranging from 0.9:1.0 (F:M) in the *Bt*-P treatment to 1.1:1.0 in the P-*Bt* treatment. Subsequent analyses in two-way tables revealed no interactions between treatment and sex or parasitism status and sex ($P \geq 0.370$) and confirmed the signif-

Table 6. Larval and pupal developmental times (days ± SEM) and pupal mass (mg ± SEM) of *Compsilura concinnata* larvae and pupae in gypsy moth larvae treated with *Bt* either before or after parasitization compared with control larvae

Parasitism status	Treatment[a]	Larval developmental time	Pupal developmental time	Pupal mass[b]
Single	Control	16.67 ± 0.44a[c] (59)[d]	14.23 ± 0.15a (59)	48.48 ± 0.96a (59)
	Bt-P	17.86 ± 0.47a (64)	14.39 ± 0.14a (64)	46.36 ± 0.91ab (64)
	P-*Bt*	16.95 ± 0.61a (38)	14.18 ± 0.18a (39)	43.18 ± 1.17b (39)
Superparasitized	Control	16.35 ± 0.15a (301)	14.01 ± 0.06a (301)	41.06 ± 0.59a (300)
	Bt-P	17.54 ± 0.20b (184)	13.99 ± 0.08a (183)	37.45 ± 0.76b (184)
	P-*Bt*	18.17 ± 0.36b (55)	13.90 ± 0.14a (55)	37.62 ± 1.40b (55)

[a] Only parasitoids that developed to adult were included in the analyses (701/732 pupae).
[b] Pupal mass transformed to 69.47–pupal mass to achieve homogeneity of variances (constant was obtained by adding 1 to the largest pupal mass, as recommended by Tabachnick and Fidell [1996]).
[c] Significant differences within columns and parasitism status indicated by different letters ($P \leq 0.05$, Tukey-Kramer HSD).
[d] Sample size.

icant effect of treatment on rate of superparasitism ($G = 26.479$, df = 2, $P < 0.001$) as seen in Table 4.

Discussion

Sublethal effects of *Bt* on gypsy moth larvae included prolonged development of fourth instars and reduced male pupal mass. The observed effects, however, were minor compared with developmental delays reported for other insects, such as spruce budworm (Pedersen et al. 1997), banded sunflower moth (Barker 1998), tobacco budworm (Abdul-Sattar and Watson 1982) and several other insect pests. Delays in larval development associated with *Bt* have also been observed for gypsy moths, in the laboratory (Weseloh and Andreadis 1982, Wallner et al. 1983) as well as in the field (Weseloh et al. 1983). Although most studies focus on female pupal mass as an indicator of potential fecundity, the negative impact of *Bt* on male pupal mass may also indicate changes in reproductive potential and competitive ability (Savalli and Fox 1998). Similar to our results with gypsy moth, male spruce budworm pupae were also more negatively affected by sublethal doses of *Bt* than females (Pedersen et al. 1997). Adverse effects of *Bt* on male pupal mass does not affect *C. concinnata*, however, because the parasitoids emerge before gypsy moth pupation.

Although sublethal effects on host larvae were small, *C. concinnata* females were able to discriminate between *Bt*-treated and untreated larvae. This was shown by lower rates of parasitism and superparasitism of *Bt*-treated larvae in both choice- (Table 3) and no-choice (Table 4) experiments. How *C. concinnata* discriminates between healthy and *Bt*-dosed larvae is not entirely clear, but may involve host vigor. Weseloh (1980) concluded that host movement and contact with the host integument are important components which elicit examination and attack behaviors in *C. concinnata.* In his experiments, more vigorous hosts were examined and attacked to a greater degree than less active hosts. In previous experiments, we found a significant dose-dependent reduction in the level of activity of *Bt*-dosed gypsy moth larvae over a 24-h period after dosing (measured by comparing larval response to a light stimulus, $P < 0.001$) (data not presented), which may explain why they were less acceptable to *C. concinnata* than untreated larvae. Host discrimination may also involve physiological factors such as color, form, odor, cuticular components, glandular secretions and sound (Tamashiro 1968, Vinson 1976). Color and host texture were found to be important cues in host examination and oviposition for *Exorista japonica*, another generalist tachinid (Tanaka et al. 1999).

Reduced host activity may result in lower rates of parasitism by *C. concinnata* when they encounter *Bt*-treated insects, especially in lethally-dosed larvae (Table 5), where the level of activity is even lower than that of sublethally dosed larvae (see previous paragraph); however, host activity does not explain why rates of parasitism and superparasitism in gypsy moth larvae treated with *Bt* after parasitization were also significantly lower than control insects (Table 4). We expected parasitism rates of larvae in the P-*Bt* treatment to be comparable to control larvae because larvae had similar activity patterns at the time of parasitoid exposure and were exposed under the same conditions. Given this expectation, there was an unexplained loss of parasitoid larvae in the P-*Bt* treatment, either in the 24 h before *Bt* infection, or at some point during the course of infection, with the latter time period being more likely. Because more insects died in the P-*Bt* treatment than in the other treatments, rapidly deteriorating host gut conditions in lethally-dosed larvae may have caused breakdown and death of parasitoid larvae, or parasitoids may have been excreted, rendering them undetectable during dissections. Experiments involving microscopic examination of both *Bt* and *C. concinnata*-infected hosts at regular intervals after infection would enable testing of this hypothesis.

Regardless of the timing or the mechanisms of the interaction between *Bt* and *C. concinnata*, if a parasitoid initially survived, *Bt* had minor effects on parasitoid development. Larval development times were extended and pupal weight was decreased (Table 6). The extended development was more pronounced in superparasitized hosts, suggesting that individual parasitoid development may have been limited by factors such as food availability and/or competition. In

this study, mean larval development times of parasitoids in *Bt*-treated hosts were extended up to a maximum of 2 d (Table 6). In contrast, Weseloh (1984a) reported that *C. concinnata* larval development was accelerated in *Bt*-treated hosts. Longer larval development times in *C. concinnata* may be mitigated by direct or indirect factors. It is possible that parasitoids are directly affected by *Bt* (i.e., bacterial septicemia) or by components of the *Bt* formulation (Flexner et al. 1986). Larvae develop between the peritrophic membrane and the host midgut, the site of *Bt* action, and are thus likely to come into direct contact with crystals and spores. Alternatively, it is possible that *Bt* -induced extended development of the host results in longer parasitoid development; Weseloh (1984a) suggested that *C. concinnata* may be triggered to emerge from hosts when levels of juvenile hormones in the host are low, and ecdysone is high, which occurs just before pupation.

An extended larval development time of 2 d, however, is unlikely to be as critical to the success of *C. concinnata* as a reduction in pupal mass. *C. concinnata* normally experience a slight range of development times and emergence rates, depending on the sex of the fly. The reduction in pupal weight of parasitoids in singly and superparasitized, *Bt*-treated hosts, however, may result in a reduced fitness of females, because pupal weight is positively correlated with potential fecundity in these parasitoids (Bourchier 1991).

Results of our laboratory experiments reveal several factors that may affect the outcome of pest management plans by field application of *Bt*. First, it is possible for gypsy moth larvae to ingest sublethal doses of *Bt* and then resume normal development after a few days. Second, if larval development times in the field are prolonged as they were in the laboratory, *Bt*-dosed gypsy moth may be vulnerable to parasitization by other larval parasitoids. Third, the combination of parasitism and *Bt* treatment will destroy more gypsy moth and in a shorter time period than parasitoids alone. Parasitization by *C. concinnata* followed by *Bt* treatment (P-*Bt*) kills more gypsy moth larvae than either *Bt* treatment followed by parasitism, or parasitoids alone (gypsy moth survival is ≈7, 17, and 20% for P-*Bt*, *Bt*-P, and control, respectively; see Tables 4 and 5). There will, however, be negative impacts on the next generation of parasitoids, because almost 60% of gypsy moth larvae treated with a combination of *Bt* and parasitoids died along with their parasitoids within a few days of *Bt* infection. There are a number of examples where the premature death of the infected hosts resulted in death of parasitoids (Ticehurst et al. 1982 for *C. concinnata* and gypsy moth, Brooks 1993 and examples therein; Blumberg et al. 1997, Chilcutt and Tabashnik 1997, Chenot and Raffa 1998). Thus, if conservation of larval parasitoids such as *C. concinnata* is an important criteria in the pest management program, applications of *Bt* to early instars (1–2) before parasitoids are actively seeking hosts would ensure that more naturally occurring *C. concinnata* survive in the current generation to parasitize larvae that are not killed by *Bt*.

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