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Non-target impacts of forest defoliator management options: Decision for no spraying may have worse impacts on non-target Lepidoptera than *Bacillus thuringiensis* insecticides

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Abstract

Management programs for major forest defoliators such as gypsy moths or forest tent caterpillars, and crop pests such as the European corn borer have shifted from broad-spectrum insecticides to more environmentally benign microbial pesticides such as *Bacillus thuringiensis* (foliage sprays and transgenic toxin expression in plant tissues). Phytochemically resistant host plants and natural enemies have been used as alternative pest management strategies (including generalist tachinid flies such as *Compsilura*, viruses, microsporidians, and fungi), but all of these have some non-target impacts, as described from literature review. A sequence of lab and field studies were conducted to determine non-target impacts on native Lepidoptera in North America. The conclusions reached are that a decision not to spray *Bt* pesticides (i.e. to allow defoliation and natural pest outbreaks to run their course) could be as bad or worse for non-target Lepidoptera as the microbial insecticides would be. The important concept that must be maintained is that all pest management programs have some risk of negative non-target impacts, but it is the magnitude and relative importance that will remain the most critical issue for environmental impacts and pest management.

Controlling phytophagous insect outbreak defoliations

Management of catastrophic forest defoliators and agricultural/ornamental plants with broad-spectrum insecticides has had short-term successes, but also resulted in serious and extensive non-target impacts. Alternatives to such insecticides have been sought with more specific and environmentally benign microbially derived insecticides such as *Bacillus thuringiensis*, Laird et al. 1990 *Bt*; (Faust and Bulla 1982; Beegle and Yamamoto 1992; Leong et al. 1992; Lambert and Peferoen 1992; Reardon et al. 1994). However, drift of *Bt* insecticides with aerial application has been reported as far as 3 km downwind, depending on method of application (Barry et al. 1993; Swadener 1994; Whaley et al. 1998). On foliage, viable spores have also been recovered weeks (or years) after application (Reardon and Haissig 1984; Huang et al. 1990). Such undesirable environmental distribution of *Bt* sprays have led to even safer genetically engineered plants being developed with tissue-specific expression of *Bt* endotoxins (or other toxins and growth inhibitors such as plant lectins, or protease- and amylase-inhibitors; Babu

et al. 2003). The hopes were to achieve acceptable pest control via 'host plant resistance' (Maxwell and Jennings 1980; Fritz and Simms 1992) using modern transgenic techniques and minimizing the dangers to non-target organisms of all types (Reardon 1994; Robison and Raffa 1994; Wang et al. 1996; Babu et al. 2003; Nap et al. 2003; Conner et al. 2003; Hancock 2003). Nonetheless, undesirable non-target impacts of *Bt* insecticides (whether externally on leaf surfaces or tissue-specific toxin expression) will occur, to varying degrees, depending on many environmental (abiotic) and biotic variables (some known, but most unknown: Smith and Couche 1991; Altmann 1992; Addison 1993; Martin 1994; Moldenke et al. 1994; Ferber et al. 1999; van Emden 1999; Hails 2000; Scriber 2001; Shelton et al. 2002).

Sprays of *Btk* (variety *kurstaki* used against Lepidoptera) employed against European and Asian gypsy moths (*Lymantria* spp), forest tent caterpillars (*Malacosoma* spp), spruce budworms (*Choristoneura* spp.) had negative impacts on many beneficial (biological controls) and non-target invertebrates (Muck et al. 1981; Flexner et al. 1986; Dreistadt and Dahlsten 1989; Melin and Cozzi 1990; Miller 1990; Miller 1992; Chapman and Hoy 1991; James et al. 1993; Addison 1993; Swadener 1994; Johnson et al. 1995; Papp-Herms 1996; Sample et al. 1996; Chenot and Raffa 1998; Peacock et al. 1993 and 1998; Scriber 1998; Whaley et al. 1998; Boulton 1999; Glare and O'Callaghan 2000; Rastall et al. 2003). Based upon phenological overlap with the gypsy moth, host-sharing, and larval habitat (capopy/understory), it was determined that 92–98% of the locally recorded 223 species of Lepidoptera (from 22 families) had measurable vulnerability to Bt sprays in the National Parks of Washington, DC (Venables 1990). In a different study of 42 species of Lepidoptera, all four species of butterflies were extremely sensitive to *Btk*, in contrast to only 10 of the 38 moth species (geometrids, lymantriids, and noctuids were least sensitive; Peacock et al. 1998). It seems that butterfly larvae are generally more susceptible than moth larvae, and therefore may be useful as an 'early warning' signal for environmental danger in different habitats or ecosystems (see also Scriber and Gage 1995; Brown 1997; New 1997).

While the direct measurable impacts of *Bt* on non-target invertebrates have been noted above, there are other ways that *Bt* might indirectly impact non-targets. Although not adequately measured, some of these include the sub-lethal effects of *Bt* on food consumption, growth rates, and increased vulnerability of herbivorous insects to parasites or predators (Johnson and Gould 1992; Johnson 1997). The behavioral preference or avoidance of *Bt* on leaf surfaces has not been thoroughly evaluated for phytophagous insects, but such adaptations to plant protectants may evolve (Gould 1991). Similarly, the sub-lethal impacts of *Bt* (with regard to pathogen susceptibility) remains largely unknown. The relative competitive abilities of *Bt*-impacted phytophagous larvae have not been thoroughly assessed in field or even lab situations.

The fate of *Bt* crystals, spores, and the ecologcal interactions with related microbes remains basically unknown (Doane 1970; Feitelson et al. 1992; Addison 1993; Watanabe et al. 1998). The persistence of toxic and chronic effects of *Bt* applied on leaf surfaces (or genetically engineered into crop plants and trees) remains variable, with little understanding why (Smith and Couche 1991; Martin 1994; Bauer 1997; Scriber 2001). Sprays are differentially effective (Krieg and Langenbruch 1981; Wagner et al. 1996) because of the varied Lepidopteran guilds with different feeding behavior/ecology. Some species are protected/shielded (e.g. stem borers, bud feeders, leaf-miners, leaf-rollers, leaf-tiers, etc) versus exposed/unprotected external feeders (Slansky and Scriber 1985; Scoble 1992; Stamp and Casey 1993; Sheehan 1994) and the bulk of Lepidoptera are living on leaf surfaces (as with the Papilionidae and Saturniidae; Scriber et al. 1995b; Tuskes et al. 1996). Early instars tend to be more susceptible to *Bt* than later instars, but this is not always the case (Navon et al. 1990; Wagner et al. 1996; Peacock et al. 1998). The environmental persistence, genetic exchanges via hybridization, and ecological interactions of *Bacillus* used in control programs with other microbes surprisingly remain largely unknown (Hails 2000).

Bacillus thuringiensis

Bacillus thuringiensis and related crystalliferous bacteria have been produced in many countries

with widely varying methods and variable results. Before 1996, there were at least 137 insect species (Lepidoptera, Coleoptera, Diptera, and Hymenoptera) known to be susceptible (Heimpel 1967). Since then, the production has been more standardized and contamination of Btk with other microbes or different *Bt* toxins such as *Bti* has been reduced (Martin and Trivers 1989; Reardon et al. 1994; Swadener 1994). Within the species *B. thuringiensis*, at least 16,000 (and perhaps as many as 40,000) strains had been isolated (Lambert and Peferoen 1992; Addison 1993) with at least 34 different subspecies or serotypes (de Barjac and Frachon 1990). Smith and Couche (1991) isolated *Bt* from unsprayed leaf surfaces of hardwood and conifer species. These bacteria were naturally present throughout the year and may have symbiotically functioned to protect leaves from phytophagous insects. It has been shown that *Bt* readily germinates, produces vegetative cells, and can grow and reproduce successfully in soils (Addison 1993). Ultraviolet light kills most of the phylloplane spores of *Bt*, but whether germination, growth, and reproduction occur is still undetermined, but suspected, especially in shaded areas and on the undersides of leaves (Addison 1993; Scriber 1998). The lack of understanding of the natural ecology of *B. thuringiensis* has been clearly recognized but not addressed (Martin and Travers 1989; Lambert and Peferoen 1992; Addison 1993). We do not know the functional role *B. thuringiensis* in the soil (or on leaf phylloplanes; Andrews and Hirano 1991), whether it has any 'natural' hosts, what feeds on it, or how it interacts with other microorganisms (including the closely related natural *B. anthrax* and *B. cereus*, which are capable of exchanging plasmids; Helgason et al. 2000; Kanda et al. 2000).

When *Bt* cell replication is complete, an endospore and a protein crystal are formed within the vegetative cell, called a sporangium. When the cell wall breaks down the spore and crystals are released. The crystal is a large molecule that is not toxic to insects until solubilized by digestive processes in the gut, which releases smaller delta-endotoxin crystals. The spore coat can also contain proteins that act as toxins (Reardon et al. 1994). The crystal types of *Bt* have been classified by Hofte and Whitely (1989) into Cry I and Cry II (both with subgroups) primarily toxic to Lepidoptera, Cry III toxic to Coleoptera and Cry IV toxic to Diptera.

Persistence of *Bt*

The toxic activity of *Btk* toward target Lepidoptera has been reported to disappear within a few days after application to agricultural crops (Ignoffo et al. 1974; Leong et al. 1980; Beegle et al. 1981). *Bt* disappears within a week or two in the soil (West and Burgess 1985; or longer, Petras and Cassida 1985). However, in forest ecosystems, the results have been quite variable, ranging from 1 day for gypsy moths on oak trees (Sundaram and Sundarum 1992) or 3–4 days in conifers for western spruce budworm (Beckwith and Stelzer 1987). In other studies activity against gypsy moths persisted up to 60 days (Miller and West 1987). Toxicity of Btk 30–40 days post-spray was shown for *Papilio* (Johnson et al.1995). Nonetheless the long-held belief (misconception) persists that 'within hours, or more conservatively, within a few days, Bt toxin levels are expected to be ineffective in reducing most lepidopteran larval populations' (Rastall et al. 2003).

Variability in persistence of *Btk* toxicity at a given dose/density (Bryant and Yendol 1988; Radcliffe and Yendol 1993) may be due to the type of foliage (and its natural phytochemical composition; Felton and Dahlman 1984; Meade and Hare 1993, 1994; Krischik et al. 1988; Reichelderfer 1991; Arteel and Lindroth 1992; Appel 1994; Huang et al. 1994), environmental conditions (rain, sun/shade, temperatures etc.; Reardon et al. 1994), and the relative susceptibility of the insect species/genotypes being targeted (Ignoffo 1992; Gould et al. 1992; Lambert and Peferoen 1992; Bauer 1995; Scriber and Haas 1995; Gould 1998; Scriber 1998; Gahan et al. 2001; Ferre and van Rie 2002). The variable susceptibility of different Lepidopteran species may be related to the presence of spores in the *Bt* spray (Pinnock et al. 1971; Burgess et al. 1976), or caused by the different toxicity of various mixtures of crystal proteins (van Frankenhuyzen et al. 1991), or due to synergized toxic effects of *Bt* in the presence of Bt spores or even other bacteria on host foliage (Dubois and Dean 1995).

Might a decision not to spray forests for gypsy moths may be as bad or worse for non-target Lepidoptera (and other organisms)?

Management of defoliating insects such as gypsy moths via insecticidal sprays such as Btk may result in serious non-target effects as described above. However, a management decision not to spray, allowing the defoliator outbreak to run its course, may have equally undesirable non-target impacts. The desiccation of the forest understory impacts many members of delicately balanced communities some with threatened/endangered wildflowers, the high levels of nitrogenous run-off into streams and lakes can pollute the water, and the repeated defoliation of forest trees can create stress-vulnerability and tree mortality (especially in drought years). Perhaps the most significantly negative for phytophagous Lepidoptera, the large and rapid increases of natural enemies of all types associated with outbreak defoliator insect populations can have profound negative effects that may be worse than the *Bt* sprays. An example from North America illustrates some of the issues that may face non-target Lepidoptera elsewhere.

The gypsy moth, *Lymantria dispar (L.)*, has caused serious broadleaf forest defoliations in eastern North America and continues to spread southward in the Appalachian mountains and westward through the Great Lakes region (Gage et al. 1990; Gage and Pijanowski 1993) . The highly accelerated spread through Michigan's northern aspen/birch forests in 1990–1993 (Sharov et al. 1999) was especially alarming for foresters because of the widespread defoliation damage, mixed with two late defoliating spring freezes in 1992 (Scriber and Gage 1995; Gage 1996).

Cyclic periods (8–10 year intervals) of outbreaks follow the initial wave of gypsy moth outbreak and defoliation (Elkinton and Liebold, 1990; Liebhold et al. 1997; Dwyer et al. 2004). These population crashes may occur only after 3 to 4 years depending on many factors, including parasitoids (e.g. *Compsilura concinnata*, Scriber 1998), nuclear polyhedrosis viruses (Kukan and Myers 1997), the fungus *Entomophaga maimaiga* (Hajek et al. 1995; Hajek et al. 1996), how much *Bacillus thuringiensis* is applied in suppression management efforts (Johnson et al. 1995; Scriber 1998), as well as time-delayed maternal effects (Rossiter 1994). Also, in 1994 and 1996, extremely cold periods and winter mortality of gypsy moth egg masses may have contributed directly to population crashes throughout northern Michigan (Smitley et al. 1998).

Severe defoliations by one herbivore species such as the gypsy moth were known to affect the quality of the host for other species (Faeth 1987; Hunter 1992; Denno et al. 1995; Stewart 1996; Karban and Baldwin 1997). Trees may respond to herbivore damage by increasing the allelochemical defenses or decreasing nutrient concentrations (Wallner and Walton 1979; Schultz 1988; Karban and Myers 1989; Bryant et al. 1987; Neuvonen et al. 1987; Tallamy and Raupp 1991; Koricheva et al. 1998). Rapid induced resistance (RIR) can occur within hours or days, whereas delayed induced resistance (DIR) occurs in the year following defoliation and can last for several years (Haukioja et al. 1985; Baldwin 1994; Kaitaniemi et al. 1999; Parry et al. 2003; Nykanen and Koricheva 2004). Induced defenses in plants illustrate adaptive plasticity because the induced phenotype can exhibit higher fitness in environments with strong (defoliating) herbivory, whereas the uninduced phenotypes can show higher fitness in environments not having intense herbivory Karban et al. 1999; Heil et al. 2004). Recent studies have shown that tiger swallowtail butterflies are significantly slowed in growth rate due to rapid induced resistance from forest tent caterpillar defoliation of quaking aspen, *Populus tremuloides* (Dankert et al. 1997). The growth reduction was proportional to the severity of the defoliation. Additional long-term-ecological-research (LTER) sites were established to experimentally manipulate the (genetically identical clonal) hybrid poplar ecosystem by severe defoliation using nearly 10-million (virus-treated) gypsy moth egg masses (see below).

What follows is a summary of selected studies undertaken in our Lepidoptera research program to address *Bt* non-target impact issues as well as impacts of decisions not to spray *Bt* (and impact of transgenic *Bt* in crops/forests). The experiments below represent a research overview to illustrate some major remaining gaps in our knowledge of non-target impacts and intended to highlight important future needs for additional research;

Direct impacts of aerially applied Btk sprays on non-target Lepidoptera (experimental results with giant silkmoths of the Saturniidae and butterflies of the Papilionidae)

Different host plant species

At four different sites in Michigan, we placed first and second instar larvae on Btk-sprayed and unsprayed control tree species at 1-days post-spray, including: 1) the silkmoth, *Callosamia promethea*, on black cherry, BC of the Rosaceae; white ash, WA of the Oleaceae; tulip tree,TT of the Magnoliaceae; and spicebush, SB of the Lauraceae) 2) the Eastern tiger swallowtail butterfly (*P. glaucus*, on BC, WA, TT, and hop-tree or HT of the Rutaceae), and 3) the Canadian swallowtail butterfly (*P. canadensis*, on BC, WA, Amalenchier, AM of the Rosaceae, Quaking aspen, QA, and Balsam poplar, BP, both of the Salicaceae). Sprays of Foray 48B were applied by backpack sprayer to our trees at the rate calibrated to equal to that achieved in aerial sprays of forest canopies (30 BIU/ha with 40 IU/cm^2; BIU = billion international units/IU = infective units; see Johnson et al. 1995). Early instar larvae are most useful and ecologically meaningful in survival bioassays (Zalucki et al. 2002), and at 5-days post-spray (short-term impacts) we found near-total mortality of larvae on leaves sprayed with *Bt*, regardless of the host plant (94% of *P. canadensis*; 93% of *P. glaucus*; and 89% of *C. promethea*; Johnson et al. 1995), and essentially all of the larvae on *Bt* sprayed foliage were dead or gone by day 8.

The impact on larvae placed on BC tree leaves at an 'intermediate' interval of 12 days post-spray (n = 202 larvae on 15 sprayed/15 unsprayed trees for *P. glaucus* and n = 120 larvae on 10 sprayed/10 unsprayed trees for *P. canadensis* after an 8-day exposure) was as great as the 1-day ('short term') post-spray. For *P. glaucus*, 90% of the larvae died or disappeared compared with only 41 % of the control larvae under natural field conditions and predation; and nearly identical results were seen for *P. canadensis* larvae (Johnson et al. 1995).

Two consecutive years of 'long term' experiments on non-target impact assessments (at time intervals of 0-d 10-d 20-d, 30-d, and 40-d post-spray placement of larvae) consistently showed significantly more daily mortality on the *Bt*-sprayed trees than on the control trees. This statistically significant mortality on *Bt*-sprayed trees (compared to paired unsprayed controls) persisted through 30-days post-spray (at 40 days the difference persisted, but was not statistically significant; p = 0.06). While the *Bt*-toxicity was not diminished significantly in the no-canopy sun and rain treatments of these field studies (Johnson et al. 1995), some attenuation of *Bt*-toxicity was evident after 30-days post-spray in the direct sun/rain treatment compared to the below-canopy shaded treatment in both years of this experiment (Figure 1 in Scriber and Gage 1995).

Different insect species

There were no (host × *Bt* spray) interactions in the ANOVA for *C. promethea*, *P. glaucus*, or *P. canadensis* after 5 days of feeding (see above, and Johnson et al. 1995). The concentrations of *Bt* on the foliage were similar to aerial application rates, and there were no significant (detectable) differences as a result of phytochemical differences between tree species.

The reasons for this extended period of toxicity for these non-target *promethea* silk moths and swallowtail butterflies remained unknown. Based on toxicity to the targeted gypsy moth caterpillars, the conventional wisdom and impression given by the USDA Forest Service was that *Bt* sprays were basically safe for Lepidoptera after 1–2 or a few days since they putatively degraded or were washed away by rain. This clearly was incorrect, but the explanation of these differences in impact still eludes researchers.

Different dosage sensitivities as an explanation of long-term toxicity (?)

In order to see if our non-target Lepidoptera species were simply much more dose-sensitive than gypsy moth larvae to *Bt* formulations, we conducted some basic laboratory studies. In experiments with standard Btk formulations (Foray 48B and Dipel), we determined that the LD-50 for *P. canadensis* was 0.008 IU/cm^2; *P. glaucus* was 0.0014–0.0030 IU/cm^2; *P. palamedes* was 0.004 IU/cm^2; and *P. troilus* was 0.007 IU/cm^2.

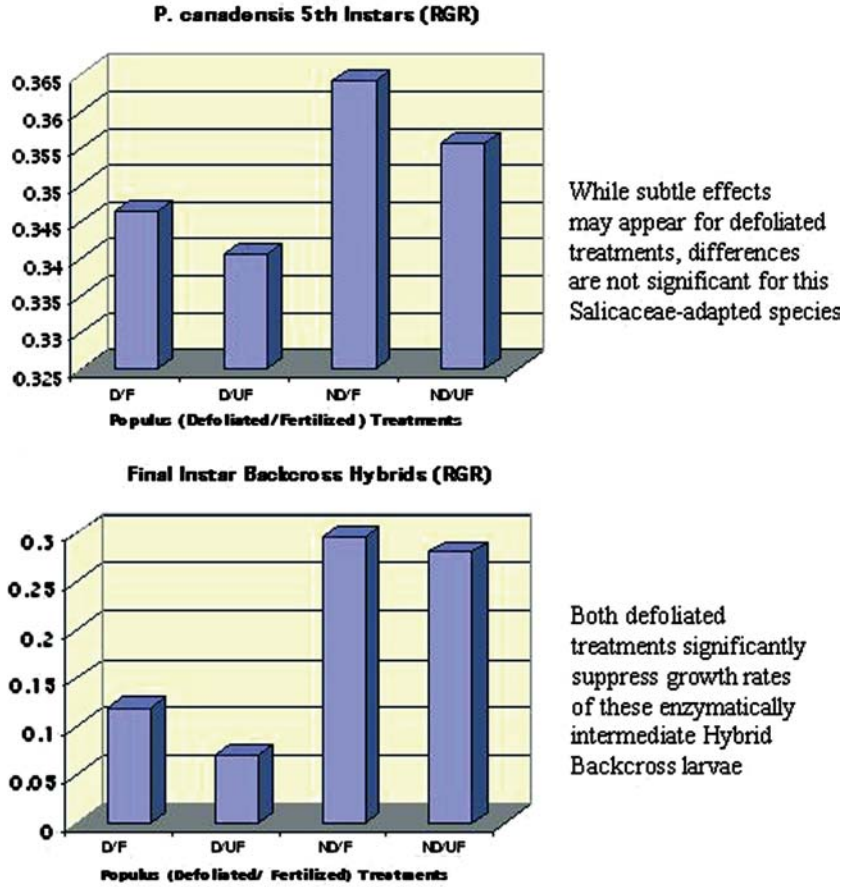

Figure 1. The relative growth rates (mg mg^{-1} d^{-1}) of final instar (n = 9 larvae per treatment/plot, for two field plots) *Papilio canadensis* larvae (TOP); and final instar hybrid backcross larvae (of a hybrid female, *P.g.* x *P.c.* mated to a *P. glaucus* male; BOTTOM) on *Populus* hybrid leaves subjected to four experimental field treatments gypsy moth defoliated (D) and non-defoliated (N), with fertilization (F) and unfertilized (UF). For the hybrid backcross larvae, the two defoliated plots were significantly poorer for larval growth than the two non-defoliated plots (F = 42.6, p = 0.003) but treatments were not significantly different not for the *P. canadensis*. On the Defoliated/Unfertilized leaves, 67% of the hybrid larvae died before pupation, although mortality was observed in any of the three other treatments. These bioassays were conducted in 1997, approximately 2 months after a severe experimental defoliation of *Populus* plots using nearly 10 million gypsy moth caterpillars (Scriber et al. 1999).

(Scriber 1998). These lethal doses (LD-50s) are all considerably less than the 30–43 IU/cm^2 actually deposited on forests in aerial sprays of *Bt* for gypsy moths and the average LD-50 of 2.7 IU/cm^2 (Radcliffe and Yendol 1993).

Although low-dose toxicity prevailed on black cherry (*Prunus serotina*) and tulip tree *(Liriodendron tulipifera)* leaves, we were able to detect different host plant-LD-50 dosage responses for second instar *P. glaucus* larvae. On tulip tree, 87–100% of all larvae died on leaves with all *Bt* doses (including; 0.0004, 0.0017, 0.0023, 0.0030, 0.004, 0.0070, 0.0170, 0.050 IU/cm^2), compared to only 14.6% mortality on the tulip tree leaf controls (96 larvae/dose × 8 doses; larvae from 24 different families; Scriber and Haas 1996). In contrast to this highly enhanced mortality on tulip tree leaves, larvae in the same experiment on black cherry (Foray 48B leaves dipped and maintained in individual petri dishes with a larva at 24 °C; 18:6 photoperiod; for 4-day survival) were much less sensitive (LD-50 was between 0.002 and 0.004 IU/cm^2; with only 19% mortality at 0.0005 and 9% on the control; Scriber and Haas 1996).

Potential synergism from phyllosphere (phylloplane) mibrobes as an explanation of long-term toxicity (?)

Toxicity for *Papilio* caused by such low doses, might partially explain the long-term persistence of *Bt* toxicity in our field studies. We examined the possibility that these low doses were also partially from a synergism resulting from other bacteria that were found naturally present on leaf surfaces used as natural food in our *Papilio* (e.g. *Serratia marcessans*, Leah Bauer, pers. comm.) during both in our field studies and in some lab studies. Previous research (Miyasono et al. 1994; Dubois and Dean 1995) had shown that at least 14 different bacterial strains (including those of *Klebsiella* sp., *Erwinia* sp., *Pseudomonas* sp., *Xanthomonas* sp., *Actinomyces* sp., *Corynebacterium* sp., *Flavobacterium* sp., and *Escherichia coli*) were of effective synergizers of at least one of the Cry IA *Bt* toxins. Unlike *Bt* spores which contain some protein toxins, in the absence of the CryIA toxins, none of these bacterial cell or spore synergists exhibited any toxicity or growth inhibition (Miyasona et al. 1999; Dubois and Dean 1995). Ulceration of the caterpillar midgut resulting from cell lysis and loss of peritrophic membrane integrity from *Bt* toxins are hypothesized to have facilitated this invasion of the hemolymph by bacterial opportunists. This type of interaction may, to varying extents, explain some of the laboratory mortality in insect culturing (whether on leaves or on synthetic diets, especially butterflies; Cohen 2003; se also Dillon and Dillon 2004). In our lab, it appears that hybrids between *P. canadensis* and *P. glaucus* may be better able than the parental species to survive the unavoidable lab-pathogens while we were using late summer tree leaves for larval rearing (Scriber et al. unpublished). Leaf sterilization with 5% bleach solutions gave some improvement in our rearing success, but this technique was also tedious and variable.

In an attempt to determine the possible synergistic effect of leaf surface microbes with different *Btk* application doses, Haas and Scriber (1998) used 4 commercial *Bt* (Foray 48B) doses (0.268, 0.034, 0.008, and 0.004 IU/cm^2) and control leaves of tulip tree and black cherry with tiger swallowtail (*P. glaucus*) butterfly larvae. Each experiment included a pre-treatment leaf dip of 5% Clorox bleach followed by a 10-minute water rinse (with a no-dip control leaf). The *Btk* dosage effects were clearly observed for all four treatments (cherry leaves with and without bleach treatment and tulip tree leaves with and without bleach). However, removal of the microbial community from the leaf surface did not decrease the *Bt* toxicity dose required to kill *Papilio*, as predicted if microbes synergize *Bt* toxicity (Dubois and Dean 1995).

In fact, the growth and survival of larvae were slightly poorer for the bleached leaves, perhaps reflecting inadvertent leaf-wax removal from the bleach treatment and subsequently faster leaf desiccation or an unintentional leached/reduced nutritional value. The actual reasons for poorer performance on our experimentally bleached leaves remains unknown, but we verified that very low doses of *Btk* will kill *Papilio*, even when not synergized by any leaf surface microbes. The LD-50 doses were between 0.0006 and 0.0025 IU/cm^2 for these four treatments (860 larvae from 58 different females evenly distributed across treatments; Haas and Scriber 1998). This extremely low dose corresponds to dosage results obtained in previous lab studies of four *Papilio* species (Scriber and Haas 1996) and are much lower (1000×) than the 2.7 IU dose required for gypsy moth larvae (Radcliffe and Yendol 1993; Reardon et al. 1994).

Natural enemies and slowed growth with sub-lethal exposure (?)

Reduced growth rates of pathogen infected (or *Bt*-sprayed) larvae can result in enhanced parasitism rates (Andreadis et al. 1983; Reardon et al. 1994; Peacock et al. 1998). The interaction of pathogens, parasites, and competitors has not been comprehensively researched for many herbivores (but see Denno et al. 1995). However, the possibility of direct competition from severe defoliators such as gypsy moths (or indirect competition from such defoliators via impacts on 'enemy-free space' or induced resistance in host trees), as a result of outbreak defoliations must be seriously considered for non-target impacts from non-spray management decisions (Scriber 1992; Redman and Scriber 2000). Some of the mortality in field studies above may have been partially related to some kind of different behavior of larvae feeding on *Bt*-sprayed trees that may have rendered them more vulnerable to predators or parasites because of slowed

growth rates (Reardon et al. 1994; Wraight et al. 2000; Zangerl et al. 2001).

Larval performance of Salicaceae-'adapted' (*P. canadensis*; Hwang and Lindroth 1995) and unadapted (*P. glaucus*; Lindroth et al. 1988) swallowtail butterflies is related to levels of phenolic glycosides in *Populus* leaves. One of these species *(Papilio canadensis R & J)* showed little reduction in larval growth rates or survival on any *Populus*, while the other (*P. glaucus* L.) found Salicaceae basically toxic for all larvae; Scriber et al. 1995a). In feeding studies using leaves sampled from these four treatments of defoliated/fertilized hybrid poplars we did see these all-or-none survival and growth patterns for larvae of each of the parental species (i.e. all *P. glaucus* died). However, significant leaf suitability differences were detected among the four treatments (defoliated, fertilized) and reflected in growth rates of hybrid and backcross larvae (Scriber et al. 1999, Figure 1). As with other field studies of defoliated and re-leafed trees (Redman and Scriber 2000), we found no differences in ability of adult females of either species to detect these phytochemically and nutritionally induced leaf treatment differences (Figure 2a, b, below).

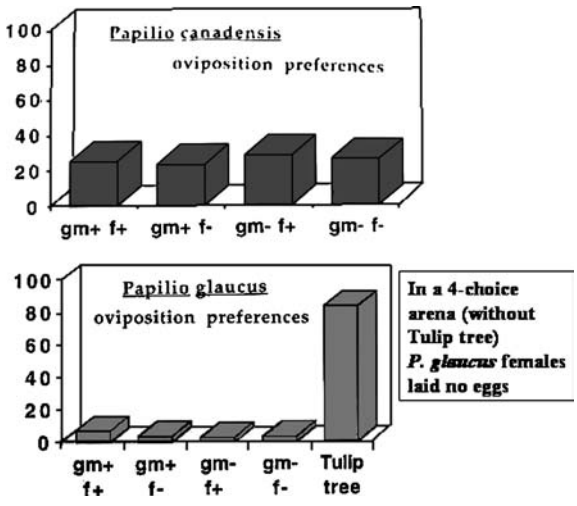

Figure 2. The percentage of *Papilio canadensis* eggs (TOP) laid on leaves of four different hybrid poplar treatment leaves (gypsy moth defoliated/ fertilized, GM+ F+; and GM+ F−, GM− F+, GM− F−, respectively) in early September 1997. No significant differences were observed for *P. canadensis* female choices(Chi-square $p > 0.90$; 16 females). There were no significant differences in the very small percentages of *P. glaucus* eggs (BOTTOM) on leaves from 4 *Populus* treatments with a normal tulip tree host in 5-choice arena (no eggs were laid by any female in arenas with only the 4 *Populus* leaves; $n = 28$ females in 1997, 44 females in 1998).

Other possibilities remain unknown (vegetative growth and resporulation on leaves?)

The extent to which *B. thuringiensis* reproduces and increases on leaf surfaces remains basically unknown, although it is known to persist on leaves for long periods of time (Leong et al. 1980; Smith and Couche 1991) and may have impacts even if it doesn't increase vegetatively with resporulation and increased concentrations of toxins. The undersides of leaves may protect some spores from sun light and rain. The pollen, dust, insect frass, and aphid honeydew exudates tend to accumulate on tree leaves later in the summer, and might provide abundant nutrients for leaf surface microbes such as *Bacillus* and others.

Indirect impacts causing non-target effects (Decision for no *Btk* spray)

Biological control of gypsy moths with natural and introduced parasites, predators and pathogens (fungi, bacteria, viruses, and microsporidians) have been shown to have variable non-target impacts on Lepidopteran species. When gypsy moth populations increase to outbreak levels, associated natural enemies abound that can attack native non-target Lepidoptera (Elkinton and Liebold 1990; Elkinton et al. 1990; Gould et al. 1990; Ferguson et al. 1994; Redman and Scriber 2000). This is especially true of generalist parasitoids such as the tachinid fly *Compsilura concinnata* (With at least three generations/year) that kills more than 200 species of butterflies and moths (Culver 1919; Webber and Schaffner 1926; Williams et al. 1992; Scriber 1998). It has been estimated that 68%–81% of two large silkmoth species (*Callosamia promethea* and *Hyalophora cecropia*; Boettner et al. 2000) and up to 62% of *Actias luna* moth larvae (Kellogg et al. 2003) are killed by *Compsilura*. However, while these species have persisted even after 100 years of parasitism by the introduced *Compsilura*, other Lepidoptera have apparently been extirpated in New England (Schweitzer 1988;

Louda et al. 2003). In addition to these direct attacks by introduced and natural parasites, the vulnerability to introduced and natural pathogens (fungi, microsporidians, and viruses) is an issue for non-target Lepidopterans in the same community as defoliating herbivores (Elkinton et al. 1991; Hajec et al. 1995; Wesoloh and Andreadis 1997; Redman and Scriber 2000). Direct competition may also occur between phytophagous insects and these targeted defoliator pests (Scriber 1992, 1998; Stewart 1996). The costs of evaluating non-target impacts of potential biological control introductions (which can evolve and change in time; Louda et al. 1977) would be huge and unrealistic (Hawkins and Marino 1997), yet seem important enough to deserve serious study (Stiling and Simberloff 2000). An excellent way to start might include sentinel species that are large, well-known to the public, and common (Stamp 1990; Bossart and Carlton 2002) much as described by Boettner et al. (2000) and (Kellogg et al. 2003)

Direct competition of forest defoliators with native non-target species of Lepidoptera

Northern Michigan suffered severe and extensive defoliations from gypsy moths and forest tent caterpillars during the 1980's and early 1990's (Gage et al. 1990; Gage 1996; Sharov et al. 1998 and 1999). Few tree leaves (other than Maples, *Acer*, or ashes, *Fraxinus*) could be found in the heavily defoliated outbreak areas. In addition to these outbreak caterpillars eating leaves with *Papilio canadensis* eggs (as a direct mortality factor; Scriber 1996), there was a serious lack of host leaves of *Papilio* hosts. Trees such as paper birch and other birch/alders (*Betula* spp./*Alnus* spp.), quaking aspen, balsam poplars, big-toothed aspens, and willows (*Populus* spp./*Salix* spp.), shadbush (*Amelanchier* spp.), pin cherry, choke cherry, black cherry (*Prunus* spp.), basswood (*Tilia americana*), and others were extensively, if not completely, defoliated (Scriber 1992). Such severe and widespread defoliation (millions of hectares) certainly posed potential competitive threats for *Papilio* populations and other species of Lepidoptera in these forests (Scriber and Gage 1995). However, determining the precise role of direct competition for phytophagous insect herbivores is difficult for generalist species such as these *P. glaucus* and *P. canadensis* (local specialists on a gyspy moth devoured tree species may have been more seriously devastated).

Indirect competition with native non-targets (enemy-free space)

Increased densities of gypsy moths attract natural enemies of all sorts, which impact other Lepidoptera locally in an 'indirect competition' (Elkinton and Liebold 1990; Elkinton et al. 1990; Gould et al. 1990; Ferguson et al. 1994). Similar patterns of natural enemy increases have been observed in the wake of forest tent caterpillar outbreaks as well (Parry et al. 1997). It was determined that swallowtail butterflies in northern Michigan were negatively affected by gypsy moths in several ways, including loss of usable host foliage (see above; Arteel and Lindroth 1992), depressed growth rates and survival on regrowth leaves (see below), and increased rates of parasitism when placed near gypsy moth infestations (Williams et al. 1992; Redman and Scriber 2000), or even when artificially high numbers were created (Boettner et al. 2000). Some evidence was observed that suggests that gypsy moth body fluids with NPV virus naturally or experimentally painted on plant leaves could also reduce growth and survival of *Papilio canadensis* larvae (Redman and Scriber 2000).

It is unknown whether insect hybrids might survive disease better than parental species but this may be the case with our *Papilio*. Much less is known about parasites or diseases of hybrid animals than parasites (e.g. phytophagous insects) and disease of hybrid plants (Moulia 1999; Whitham et al. 1994, 1999). However, increased resistance of animal hybrids have been reported (e.g. hybrid pocket gopher resistance to lice; Heany and Timm 1985; Hafner et al. 1998). In some cases F-1 primary hybrids are more resistant (express a hybrid vigor) than the parental species, but the backcrossed and recombinant genotypes may be more susceptible due to the breakup of coadapted gene complexes of the immune response system (Moulia et al. 1995, 1996). Few studies of hybrid insect resistance to disease or parasites exist, but hybrid honey bees (of European and African subspecies) may have greater resistance to some parasites such as *Varroa* or tracheal mites (Nasr 1997; Erickson 1998; Sammataro et al.

2000). However, no difference in fungal pathogen susceptibility/resistance was found in the hybridizing cicada species (Duke et al. 2002).

Our hybrids between *P. canadensis* and *P. glaucus* show higher survival and growth rates than parental speecies (Donovan 2001; Scriber et al. 2003). We do not rule out the possibility that this was perhaps due to better survival of hybrids on pathogen-laden field-collected leaves in our lab rearing process. Extensive interspecific natural gene introgression and recombination has been extensive across the historical hybrid zone between these *Papilio* since the 1998 regional climate warming in North America Scriber 2002a, 2002b). Differentially sensitive genotypes spawned by introgression might survive in local pockets of high pathogen concentrations. The differential susceptibility/resistance of non-target species and their hybrids has not been investigated for any insect pathogen (bacterial, fungal, microsporidian, nematode, or virus). Surprisingly, the ability of female insects to avoid mating with infected males has been reported for some insects (English 2001) as in mammals (Kavaliers and Colwell 1995). Such differential mating behavior across the *Papilio* hybrid zone (see Deering and Scriber 2002) could indirectly affect the survival of local populations (or particular genotypes) with exposure to pathogens/parasites in defoliation outbreak areas (Dwyer et al. 2004).

Indirect competition with native non-targets (induced phytochemical resistance in previously defoliated plants)

In recent years, we have seen the natural spread of gypsy moths into southwestern Michigan Long Term Ecological Research (LTER) poplar plots. This was an excellent position from which to document effects on tree physiology, allelochemicals, insect population dynamics and nutrient cycling under natural infestations with simultaneous experimental manipulations of the hybrid poplar plantations of the Kellogg Biological Station. In 8 Lepidopteran species of *Populus*-feeders on experimentally defoliated (with and without Nitrogen fertilization) and undefoliated (with and without Nitrogen), it was found that catastrophic (near-total) experimental defoliations did result in altered leaf quality and slowed growth or smaller pupae or reduced fecundity for subsequent phytophagous insects in the same year, and in 2–3 years post-defoliation (Scriber et al. 1999; Parry 2000). The variation we observed between defoliation and fertilization treatments may have related to the structure of the particular tannins (Ayres et al. 1997) or the biochemistry of different herbivore guts (Barbehenn and Martin 1994) as well as the availability of carbon and/or nitrogen to the plants (Herms and Mattson 1992; Arnold and Schultz 2002; Lovett et al. 2002). In *Populus tremuloides*, experimentally defoliated using *Malacasoma disstria*, previous years of defoliations did not affect the parasitoids (Parry et al. 2003) although other herbivores were affected by experimental defoliation of this aspen species (Dankert et al. 1997). Experimental defoliation of birch and sugar maples showed interactions of sunlight/shade and nitrogen fertilizer on leaf quality for other Lepidoptera (Govenor 1998). Meta-analyses of 68 studies of damage-induced changes in woody plants show that herbivore responses to defoliation depend upon the plant species, the type of damage, and the timing of damage (Nykanen and Koricheva 2004).

In order to determine the extent of potential impact of *Populus* defoliations and potential phytochemical induction (Lindroth 1992; Bryant et al. 1993; Hunter and Schultz 1995; Lindroth and Hwang 1996; Dankert et al. 1997; Wait et al. 1998; Peters and Constabel 2002) on genotypes of generalist herbivores, we created interspecific *Papilio* hybrids and backcrosses between adapted and unadapted species using hand-paired lab crosses (Scriber et al. 1999). In attempts to simulate a defoliating insect outbreak in experimental forests of genetically identical hybrid poplar trees (540/plot within larger plots at the LTER Long term 'agroecology' research site at Kellogg Biological Station in Michigan), two million gypsy moth eggs used in 1996 achieved 30–40% defoliation. Essentially total defoliation was achieved in 1997 plots by allowing nearly 10 million hatching gypsy moths to ascend from containers tied to the individual tree boles. All gypsy moth egg masses collected in the winter of 1996–1997 were surface sterilized (10% formalin) to kill virus. The highest feeding activity and defoliation rate coincided with the later instars and occurred in late June. Our feeding bioassay experiments with *Papilio* species,

hybrids, and backcrosses began July 3rd and ran through late August in 1997 and 1998 using the regrowth leaves from the four treatments of LTER treatment plots number 1 and 2. Oviposition studies were conducted on leaves from plots 1 and 2 in 1997 and 1998.

Female *Papilio glaucus* were, in both 1997 and 1998, directly obtained from one population in southeastern United States (Clarke Co. GA), and *P. canadensis* females from Northern Michigan (Emmet, Cheboygan, and Charlevoix counties). Females were individually placed into clear round plastic containers (10 cm high 25 cm diameter) with *Populus* leaves of respective treatments (++, +−, −+, −−) for defoliation and fertilization, respectively. These multi-choice oviposition arenas were stacked on rotating platforms aligned in front of a bank of 100 W incandescent bulbs on a 4 h on/off cycle. The rotation of containers was set so that each of the leaves (supported in a water-filled florists' aquapic) passed in front of the light 10 times per hour. Since the butterflies flutter and bounce along inside of the dishes at the side facing the lights, the multi-choice arena provided a slowly moving sequence of leaves to each female. The treatments were randomized each time they were replaced. Eggs were removed from each container and counted daily (see Scriber 1993 for details of the oviposition arenas). Females were fed daily with a 20% honey water solution.

The leaves bioassayed in this study came from the four treatments of two large plots in the LTER site at the Kellogg Biological Station. Upper crown branch samples were taken from 14 trees per plot with pruning poles above 2.5 m June 28 1997 and August 4 1997. Leaves were immediately flash frozen in liquid nitrogen. Methanol was used to extract phenolic compounds. Total phenolics were quantified using the Folin-Dennis assay on a Rapid-Flow autoanalyzer. Condensed tannins were quantified using a modified sulfuric acid procedure (Nitao et al. unpublished) on the same equipment. Leaf water content was determined for each treatment from subsamples taken at the time of experimental set-up of field-sampled leaves brought to the lab on ice in ziplock bags. Leaf nitrogen (1997) was determined on a dry weight basis with an autoanalyzer. Leaf water was taken at several additional sample dates. Difference between leaf treatment means for leaf water, nitrogen and total phenolics after ANOVA (using GLM procedures) were determined as Least Significant Differences (LSD; SAS 1989; Table 1).

The Salicaceae-adapted species *P. canadensis* did well on all treatment leaves (after defoliation and fertilization treatments) and the unadapted species, *P. glaucus*, did poorly on all leaves. Hybrids and backcrosses with intermediate levels of detoxification carboxylesterase enzymes (Scriber et al. 1989) were intermediate and showed reduced growth on the gypsy moth defoliated

Table 1. Leaf nutritional quality assessments (Mean of two plots)

Plot treatments	Leaf water[A] (%)			% Total nitrogen[B] (dry weight)		Total Foliar phenolics[C] (%)
	July 3	July 18	August 18	mid-June	mid-August	Mid-August
Defoliated/N+	80.5 a ± 0.8	73.0 ab ± 1.3	71.2 b ± 0.4	2.14 b ± 0.09	2.96 a ± 0.34	5.95 a ± 0.35
Defoliated/N−	79.6 a ± 2.8	73.5 ab ± 0.9	71.4 b ± 0.5	2.29 ab ± 0.07	2.40 a ± 0.12	5.65 a ± 0.05
Undefoliated/N+	71.4 b ± 0.1	72.7 b ± 0.5	70.2 b ± 0.4	2.75 a ± 0.03	2.89 a ± 0.10	7.10 b ± 0.20
Undefoliated/N−	70.5 b ± 2.7	72.2 b ± 1.6	70.5 b ± 0.0	2.54 ab ± 0.23	2.75 a ± 0.24	7.05 b ± 0.25

Budbreak in hybrid poplars late May-early April; experimental gypsy moth defoliation mid-June to late June; Releafing late-June to July 5th. Larvae of hybrid, backcross, and parental *Papilio* genotypes were bioassayed mid-July to mid-August. Females (second generation) were bioassayed for oviposition in mid- to late-August.

[A] Leaf water means of 2 plots (24 individual trees) by date 1997.

[B] Nitrogen means of 2 plots ($n = 55$ individual trees were sampled in Plot 1 and $n = 58$ trees from Plot 2) 1997.

[C] Total phenolics six to seven weeks post-defoliation were determined on August 11 1997 samples (see Scriber et al. 1999).

ANOVA (GLM, SAS) conducted on plot means significant differences between means for: overall leaf water, June nitrogen and phenolics.

treatments, presumably due to the induced changes in allelochemicals such as specific phenolic glycosides (Lindroth et al. 1988) and nutrients such as lowered nitrogen concentrations (water content can be a very important nutrient, but was not lower in any of these 4 hybrid poplar treatment leaves; see Table 1). The total phenolics in both of the defoliated treatments were actually lower than the undefoliated treatments 2 months after the 1997 defoliation (Table 1) but rose in the subsequent years where they had minimal effect on herbivores (Parry 2000; Parry et al. 2003). It has been suggested that phenolics may perhaps be involved in other plant protection functions (e.g. from photodamage, not herbivores; Matsuki 1996; Close and McArthur 2002). It has also been found that with *Populus deltoides* Nitrogen fertilization changes leaf chemistry (including phenolics, leaf N, salicin, total soluable protein, etc.) via changes caused in leaf development (Wait et al. 1998).

Our hybrid/backcross *Papilio* genotypes had intermediate levels of esterase detoxification enzymes (Scriber et al. 1989) and were intermediately sensitive (compared to the parental species) to the phytochemically altered treatment differences in gypsy-moth-defoliated hybrid poplar regrowth leaves (*Populus* x *euroamericana* c.v. 'Eugenii'; *P. deltoides* x *P. nigra*), showing more growth rate and survival differences (Figure 1; 1999) than 5 other adapted species, including forest tent caterpillars, tussock moths, poplar tent maker, big poplar sphinx, and Fall webworms, as well as the gypsy moths themselves (Parry 2000). The phytochemically induced differences in *Populus* leaves that affected *Papilio* larvae did not differentially affect the ovipositing females of either *P. canadensis* or *P. glaucus* Figure 2), which was also observed for poor quality aspen leaves in northern Michigan after gypsy moth outbreaks (Redman and Scriber 2000). Such subtle differences in the best and worse hosts were not detected or recognized yet for their physiological/ecological impacts, as is the case for other Lepidopteran 'oviposition mistakes' on toxic plants that may have been recently introduced or phytochemically altered (Scriber 1993; Renwick and Chew 1994; Mercader and Scriber 2004). Plant hybrids can serve as bridges or barriers to insect herbivores of various guilds (Whitham et al. 1999).

Genetically engineered (transgenic) crops and forest trees with Bt endotoxin expression in tissues

Transgenic crops occupy more than 40 million hectares on a global scale, and in the past 15 years, more than 100 species of genetically transformed plant species have been produced (Babu et al. 2003). Recently, publicity regarding transgenic (genetically modified) plants has raised levels of concern regarding non-target and other evolutionary impacts (Ferber 1999; van Emden 1999; Pimentel and Raven 2000; Hails 2000; Kanda et al. 2000; Brower 2001; Scriber 2001). The development of a new generation of *Bt*-crops with much higher toxin expression levels (Kota et al. 1999) may heighten concerns. The expression of *Bt* endotoxins in *Zea mays* (corn) pollen that killed monarch butterfly larvae (Losey et al. 1999; Hansen-Jesse and Obrycki 2000; Sears et al. 2001) led to a major environmental impact debate (Ferber 1999; Brower 2001). Subsequent research showed that other butterflies such as *Papilio* species were affected by Bt toxins in pollen only at very high concentrations, however sub-lethal effects were evident (Wraight et al. 2000; Scriber 2001; Zangerl et al. 2001). The results of a series of research studies presented in a special issue of the Proceedings of the National Academy of Sciences in the USA alleviated much of the concern about widespread movement and severe toxic impacts of transgenic corn pollen (which is heavy and thus primarily of only local concern; Pleasants et al. 2001; Zangerl et al. 2001). In addition to pollen, plant anther parts can disperse and contaminate hosts of non-target Lepidoptera such as monarch butterflies on milkweed plants (Asclepiadaceae; Hanson-Jesse and Obrycki 2000; Hellmich et al. 2001). The non-target impacts of *Bt*-corn pollen on the monarch butterfly in North America were generally low, but locally could be severe (Brower 2001; Scriber 2001).

Since most of the focus and research on non-target impacts of transgenic corn pollen was concentrated on the 'in-field' weed-feeding species of Lepidoptera (monarch butterflies and black swallowtails; Oberhauser et al. 2001; Zangerl et al. 2001), we felt it essential to evaluate the potential impact of transgenic corn pollen on two species of *Papilio* that utilize host plants along hedgerows and field edges throughout the Midwest region of North America (below). Similar non-target trans-

genic plant issues may emerge for trees (Raffa 1989; Bauer 1997), and the negative impacts may extend through herbivores that feed on these plants to include the natural enemies that eat them (Chapman and Hoy 1991; Hilbeck et al. 1998). Another concern is that some plant parts (e.g. fruits) expressing Cry3B toxins aimed at Coleoptera may nonetheless affect Lepidoptera (Arpaia 1997). Unlike *Bt* spores and crystals exposed on leaf surfaces from sprays, *Bt*-toxins when produced in plant cells (or in the midgut of herbivorous insects) are not exposed to sunlight and may remain active to subsequently affect herbivores or their entomophagous natural enemies (De Maagd et al. 2001; Groot and Dicke 2002). Toxins from *Bt*-plants (root exudates or other decaying biomass) also bind to the soil and may persist for weeks or months and remain toxic to insects (but not to many other invertebrates; Tapp and Stotzky 1998; Saxena and Stotzky 2001). Unfortunately, the natural ecological interactions of *B. thuringiensis* remains basically unknown (Addison 1993; Groot and Dicke 2002).

Transgenic Btk corn pollen does negatively impact North American non-target Lepidoptera (Papilionidae), but so does non-Btk corn pollen!

We determined that transgenic corn pollen that was genetically engineered to express the *Bacillus thuringiensis* var. *kurstaki* toxin against the European corn borer, *Ostrinia nubilalis*, suppressed growth and survival of two North American non-target species (*Papilio glaucus* and *P. troilus*) when dusted on their favorite host tree leaves (Tables 1 and 2). Corn pollen from the same maize genotype, but lacking the *Btk* toxin, also suppressed growth and survival of the two *Papilio* species at basically the same levels as the pollen with the *Btk* toxin (growth rates, RGR's, were approximately half that of larvae fed control leaves with no pollen). While these antibiotic effects in no-choice feeding arenas were clear with pollen dusted on tree leaves at approximately 10% fresh leaf weight equivalents; similar effects were observed at very light dustings (1% fresh leaf equivalents). While significant mortality of both *Papilio* species continued during days 3–7 on fresh pollen-free host leaves, survivors recovered and grew at increased rates that were still slightly less than the control

Table 2. Larval growth performances (2 day and 7 day) of *Papilio glaucus* fed untreated *Liriodendron* leaves and leaves dusted with transgenic (Btk toxins) or non-transgenic (non-Bt) corn pollen. Data are expressed as a mean (±SE)

	Initital larvae (*n*)	48-h survival (%)	Relative[a] growth rate (RGR)	Relative[a] consumpt. rate (RCR)	Approx. digest. (AD)	Efficiency (%) of food conversion		7-day surv. (%)	7-day RGR[a]
						Digested (ECD)	Ingested (ECI)		
Control leaves	(9)	100	0.297a ± 0.056	2.545 ± 0.309	51.4 ± 5.7	23.6ab ± 6.2	11.7a ± 1.7	72.2	0.722a ± 0.036
Btk pollen (10%)	(17)	83.1	0.152b ± 0.027	2.123 ± 0.398	45.8 ± 6.3	43.3a ± 9.5	7.2b ± 1.1	37.5	0.400b ± 0.066
Non-Btk pollen (10%)	(16)	88.2	0.150b ± 0.036	2.985 ± 0.282	54.9 ± 4.5	11.6b ± 3.1	4.9c ± 1.0	23.5	0.420b ± 0.089

[a]RGR and RCR (mg mg^{-1} d^{-1}). Significant differences indicated ($p = 0.05$).

larvae that were not exposed to either pollen type during the 48-h experimental period.

In addition to the recovery of some larvae that consumed leaves with pollen in no-choice studies (Table 3), we observed strong larval feeding deterrence in 2-choice 24-h preference studies in which half of each of the leaves was dusted with pollen (10% fresh weight equivalents Table 4). Larval feeding was basically confined to the non-dusted half of tulip tree leaves *(Liriodendron tulipfera)* for *P. glaucus* . Their growth rates and survival were significantly better than *P. troilus* larvae which apparently failed to discriminate between the treatments (they ate as much of the pollen-covered half of spicebush leaves, *Lindera benzoin*, as the untreated control half).

We conclude that leaves in forest edges or hedgerows adjacent to cornfields may become unsuitable for non-target Lepidoptera species if pollen were to accumulate on the leaf surfaces. However, survival capabilities of larger larvae with strong non-preference in larvae feeding of some species such as *P. glaucus* should allow survival via a change of larval feeding location on the tree. We still do not know what the impact of corn pollen would be for neonate larvae, nor whether ovipositing females would avoid pollen-dusted leaves.

In summary, no pest management tactic is without risk to non-target organisms (Wolfenbarger and Phifer 2000). Generally, the *Bt* pesticide sprays and transgenic plants are of relatively less risk that other insecticides. The natural and altered microbial communities on the leaf phylloplane remain as a black box whose interactions may account for much of the unknown variance in herbivore–plant interactions. Natural interactions among different bacteria synergize the toxicity of *Bacillus thuringiensis* against Lepidoptera 100-fold to 1000-fold (Dubois and Dean 1995), as is the case for other microbial interactions (e.g. virus and microsporidians; Bauer et al. 1998). In defoliator outbreak situations, not using pesticides may in many ways be even be worse for some non-target Lepidoptera due to high levels of community or pest associated pathogens and/or parasitoids. With regard to *Bt*, even generalized butterflies may be more sensitive (than moths) and could serve as non-target impact monitoring or indicator species.

Table 3. Larval growth performances (2 day and 7 day) of *Papilio troilus* fed untreated leaves of spicebush (*Lindera benzoin*) and leaves dusted with corn pollen from transgenic (Btk toxins) and non-transgenic (non-Bt) plants

	Initial larvae (*n*)	48-h survival (%)	Relative[a] growth rate (RGR)	Relative[a] consumpt. rate (RCR)	Approx. digest. (AD)	Efficiency (%) of food conversion		7-day surv. (%)	7-day RGR[a]
						Digested (ECD)	Ingested (ECI)		
Control leaves	(5)	100.0	0.304 a ± 0.070	3.816 ± 0.491	61.4 ± 5.3	16.8 ± 6.7	9.9 ± 4.3	100.0	0.942 a ± 0.006
Btk pollen (10%)	(12)	81.1	0.148 b ± 0.034	4.381 ± 0.983	67.8 ± 4.4	8.7 ± 3.1	5.8 ± 2.1	45.5	0.763ab ± 0.053
Non-Btk pollen (10%)	(11)	83.3	0.136 b ± 0.024	3.869 ± 0.745	54.9 ± 8.8	18.5 ± 9.6	4.0 ± 0.8	58.3	0.588 b ± 0.056

Data are presented as a mean (±SE).
[a]RGR and RCR (mg mg^{-1} d^{-1}). Significant differences are indicated ($p = 0.05$).

Table 4. Larval growth rates (of survivors) at 24 h and 6 days[a] for *P. glaucus* fed tulip tree leaves (and *P. troilus* fed spicebush) half of each leaf was dusted with pollen (Bt or non-Bt) at 10% fresh weight equivalents on one half of the leaf

	No. of initial larvae (*n*)	24 h surv. (%)	24 h RGR	6 day surv. (%)	Visual estimates pollen	Visual estimates control	Calculated leaf area pollen	Calculated leaf area control
Glaucus								
Bt pollen	(8)	87.5	0.179 ± 0.047	62.5	4.4 ± 2.6	23.8 ± 5.9	3.4 ± 2.2	23.5 ± 5.0
Non-Bt pollen	(7)	71.4	0.161 ± 0.050	42.9	4.6 ± 2.9	21.4 ± 8.6	3.8 ± 2.7	20.9 ± 7.9
Troilus								
Bt pollen	(5)	40.0	0.108 ± 0.018	0.0	18.2 ± 16.2	19.0 ± 4.0	21.4 ± 7.9	22.2 ± 4.6
Non-Bt pollen	(5)	40.0	.029 ± 0.009	20.0	9.0 ± 9.6	10.0 ± 4.5	11.8 ± 6.2	13.4 ± 4.7

At right, the relative amounts of leaf consumption for pollen dusted (at 10% leaf fresh weight equivalents) versus undusted control halves of tulip tree leaves by *Papilio glaucus* and spicebush leaves by *P. troilus*.

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