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EVIDENCE FOR THE NEGATIVE EFFECTS OF BT (*BACILLUS THURINGIENSIS* VAR. *KURSTAKI*) ON A NON-TARGET BUTTERFLY COMMUNITY IN WESTERN OREGON, USA

Additional key words: *Bt*, species richness, butterfly abundance, diversity.

Bacillus thuringiensis var *kurstaki* (*Bt*) is a gram-positive bacterium commonly occurring in soil (Martin & Travers 1989) and plant leaves (Smith & Couche 1991) with known pathogenic properties for butterfly and moth larvae. Generally, when *Bacillus* bacteria experience starvation or stressful conditions they form a spore, an inactive resting stage (Brock 2000), that is lethal to most Lepidoptera larvae when ingested. Consumption of *Bt* spores by butterfly and moth larvae activates bacterial Cry proteins (toxins) by proteolysis from the larva's gut enzymes (Tojo & Aizawa 1983). Once the bacteria's toxins are activated by proteolysis, the proteins bind to the apical brush border gut microvillae cells (Hofmann et al. 1988) and form an ion channel that disrupts solute concentrations within the cell. Ion pore size increases with increasing gut alkalinity resulting in decreasing solute control with larger pore sizes (Schwartz et al. 1993). The lack of cellular solute control causes water to enter the cell and lyses cells having sufficient numbers of *Bt* ion pores. Presumably, the loss or dysfunction of gut epithelial cells causes mortality of infected Lepidoptera species.

B. thuringiensis var. *kurstaki* is commonly employed as a biological control agent for lepidopteran pests on agricultural plant species, but other *Bt* variants are pathogenic to mosquito and black flies (Hershey et al. 1998), coleopterans, hymenopterans, and orthopterans (Schnepf et al. 1998). *Bt* is suspected of causing abnormally high larval mortality rates in non-target butterfly species like Monarchs, *Danaus plexippus* L. (Danaidae) (Losey et al. 1999), various swallowtail species (*Papilio*) (Johnson et al. 1995, Peacock et al. 1998), and a host of other butterfly and moth species (Wagner et al. 1996, Peacock et al. 1998, Whaley et al. 1998). Miller (1990, 1992) observed a decrease in larval density, diversity, and species richness of lepidopteran guilds feeding on *Quercus garryana* Douglas (Fagaceae) and *Ceanothus velutinus* Douglas (Rhamnaceae) following aerial *Bt* application. Bio-engineering of genes encoding the *Bt* Cry toxins into corn plants and the dispersal of transgenic pollen into hedgerows bordering cornfields is linked to increased larval mortality of native butterfly species when pollen coated leaves are consumed by larvae (Losey et al. 1999, Hansen Jesse & Obrycki 2000).

Elevated larval death rates due to *Bt* not only impacts butterfly and moth populations, but is also capable of altering higher trophic level interactions with insectivorous species. *Sorex cinereus* (masked shrew) males migrated out of a *Bt* treatment area and all shrews altered their prey selection from Lepidoptera larvae to less preferred hemipterans and hymenopterans after a *Bt* spray reduced larval abundance (Bellocq et al. 1992). Parent birds fed their nestlings fewer caterpillars in *Bt* treated areas (Gaddis & Corkran 1986, Rodenhouse & Holmes 1992, Nagy & Smith 1997) or increased their foraging times compared to birds nesting in untreated areas (Holmes 1998). Although a decrease in Lepidoptera larval abundance did not appear to effect the average nestling weight, survivorship through fledging, or egg size in birds from a *Bt* treated area (Gaddis & Corkran 1986, Rodenhouse & Holmes 1992, Nagy & Smith 1997), lower fat reserves were detected from birds in a Dimilin (a lepidopteran larvicide with impacts similar to *Bt*) treated forest (Whitmore et al. 1993).

Lepidoptera larvae, particularly early instars, experience the acute effects of *Bt* toxins and therefore have received the bulk of the attention from researchers. Conversely, adult non-target Lepidoptera populations in *Bt* treated sites have received very little attention, possibly due to methodological problems accompanying the sampling of adult abundance over potentially large spray areas. However, Whaley et al. (1995) noted substantial reductions in adult population sizes for *Incisalia fotis* Strecker (Lycaenidae) and *Neominois ridingsii* Edwards (Satyridae) in Utah following a *Bt* spray.

Increased larval mortality from *Bt* use should result in the reduction of adult non-target Lepidoptera populations. The butterfly community should respond to eradication dosages of *Bt* by displaying a reduction in species richness, diversity, and adult abundance in the years following the spray. Furthermore, because *Bt* will likely elevate mortality rates of all butterfly species in the community, reductions in species abundance should be synchronized among species. I monitored a relatively small *Bt* spray, approximately 32 ha, that occurred in the spring of 1997 in western Oregon to assess the effects of *Bt* on the adult non-target butterfly community.

Study site description. Schwarz Park, situated downstream of Dorena Reservoir, Lane Co. Oregon, is a 32.4 ha (80 acres) *Bt* treatment area for a local gypsy moth infestation. The Schwarz Park *Bt* spray site contains three different habitats that presumably received equal dosage treatments, a "groomed," "prairie," and "spillway" habitat. The groomed habitat consists of 14.2 ha (35 acres) of lawn and *Pseudotsuga menziesii* (Mirbel) Franco (Pinaceae), Douglas-fir, forest maintained for camping and recreational use. The spillway habitat is 8.1 ha (20 acres) of young riparian forest interspersed with native upland prairie and cliff face. An area of 10.1 ha (25 acres) of degraded upland prairie with a narrow swath (0.8 ha) of riparian habitat and young Douglas-fir forest compose the remaining prairie habitat. The *Bt* treatment area is bounded by reservoir water to the east, and sporadically clearcut Douglas-fir forests to the north, west, and south. Excluding the nearby residential developments and an U.S. Forest Service tree genetics research site, Schwarz Park is the only area within 5 km that is suitable upland prairie butterfly habitat. On 30 April, 8 May, and 20 May of 1997, three aerial applications of Foray 48B (*Bt*) at 24 B.I.U.s were administered by helicopter at least 15 m (50 feet) above the tree canopy over the Schwarz Park spray area (Bai & Johnson 1997).

Adult surveys and analysis. The 32.4 ha spray area was subdivided into three habitats (spillway, prairie, and groomed) that were systematically surveyed for adult butterflies once every three to four weeks beginning in late April and ending in early June from 1997 through 2000. Target flight periods for sampling were late April, mid May, and late May/early June that correspond with the spring butterfly community flight period. Temporal separation of adult sampling dates by approximately two weeks ensured that most adult butterflies would not be counted twice because the butterflies from the first survey period would likely be dead by the second survey period. In most instances, adult butterflies were accounted for and identified without capturing or disturbance. By avoiding injury and death of the adult population through surveyor disruption, butterfly reproductive capability was not diminished through capturing or disturbance while ovipositing. Survey days were restricted to climate conditions that favored the flight of adult butterflies while maintaining the seasonal timing of the initial survey periods from the original spray year. Adult surveys were performed from 1030 h–1630 h and occurred on days when temperatures were near or above 21°C (70°F) under sunny conditions. Butterfly binomial nomenclature was synonymous with the locally accepted names published by Hinchliff (1994).

TABLE 1. Butterfly diversity for the Schwarz Park spray area. * = significant difference in diversity compared to the spray year, 1997

Year	Shannon Diversity (H′)	Year Comparison (modified *t*-test) of H′
1997	0.9840	
		*1997 × 1998 $t = 2.77$ $P < 0.01$
1998	0.7408	
		*1997 × 1999 $t = 4.92$ $P < 0.0001$
1999	0.5497	
		1997 × 2000 $t = 1.59$ $P > 0.05$
2000	0.8607	

Adult surveys were conducted systematically by walking transects approximately 5 m apart progressing from one end of a subarea, defined by physical barriers, to the other end. Subareas, nested inside the three habitats, were 0.8–2.0 ha and delineated by physical boundaries so survey replication within and between sampling years was consistent. If large numbers of butterflies occupied a subarea (only occurred once in 1997), all butterflies within the site were captured in an aerial insect net and held until the butterflies in the subarea were accounted for, identified to species, and then released. Once all transects in a subarea were surveyed, butterfly data gathering was finished for that particular subarea and then began anew in the next subarea.

The spray year, 1997, was used as baseline data for yearly pairwise comparisons because it was assumed that the majority of the adult spring butterfly species would have overwintered as a pupae or late instar larvae, and would thus be unaffected by the timing of the *Bt* spray. Butterfly abundance was estimated by dividing the total number of butterflies counted per survey by the spray area (32.4 ha). Butterfly diversity was calculated for each year using the Shannon Diversity Index (Magurran 1988). Yearly diversity measurements were compared for significant pairwise differences between the spray year and the three postspray years using a modified *t*-test described in Zar (1996).

Community response. Diversity was significantly lower in the two postspray years, 1998 ($P < 0.01$) and 1999 ($P < 0.0001$), when compared pairwise to the spray year 1997 (Table 1). The decline in diversity of the non-target butterfly community immediately following the spray suggests that the *Bt* application negatively impacted the local butterfly community. Butterfly species richness throughout the four-year study period was also substantially lower in the two years following the *Bt* spray (Appendix). In 1997 there were 22 species detected in the spray area, but in the two following postspray years species richness had dropped to only eight species (Appendix). In the year 2000, three years following the initial spray, species richness was 19, a level similar to that measured in the spray year (Appendix). Furthermore, butterfly density was also substantially lower in the two postspray years compared to the spray year. In 1997 there were 5.49 individuals/ha, while in 1998 and 1999, there were only 1.27 and 2.68 individuals/ha respectively. However, there were more butterflies per hectare in 2000, 8.18 individuals/ha, than there were in the spray year, suggesting that the assumption of a no treatment effect on the adult butterfly community during the spray year was violated with some species.

Butterfly diversity, species richness, and density all showed similar reductions in numbers following the year following the *Bt* spray, but the lack of a suitable control area to statistically account for stochastic effects on an untreated butterfly community is a drawback to the this study. Despite a lack of a suitable control area, fair numbers of *Mitoura grynea* (6 individuals), *Boloria epithore chermocki* (8 individuals), *Euphydryas chalcedona colon* (18 individuals), *Everes comyntas* (10 individuals), *Parnassius clodius claudianus* (4 individuals), and *Papilio rutulus* (5 individuals), were detected just outside the spray boundary in 1998 during one sampling day. However, no individuals of the same butterfly species were found within the *Bt* treatment area (Appendix), suggesting the detrimental effects of *Bt* on the local butterfly populations.

Further evidence for the effects of *Bt* on adult populations are demonstrated by comparing the yearly population size and lifehistory of the two dominant butterfly species, *Ceononympha tullia eunomia* and *Glaucopsyche lygdamus columbia*. *Ceononympha t. eunomia* was in the larval life stage during the *Bt* spray, so the small adult population in the spray year was likely the effect of the *Bt* treatment. The abundance of *C. t. eunomia* was lowest in the spray year and steadily increased with time following the *Bt* spray (Appendix). Comparing *C. t. eunomia* to another dominant species, *Glaucopsyche lygdamus columbia*, that was in the pupal stage when the spray occurred (adults would not be affected in the spray year), a different trend in adult abundance occurred. *Glaucopsyche l. columbia* adult populations were high in the spray year and low the two following years (Appendix), indicating that the *Bt* treatment did affect the competitively dominant species of the butterfly community and likely the competitively inferior species as well.

The data from the *Bt* spray (Appendix) evince that an overall depression in butterfly abundance occurred in the years following the spray. Moreover, the decreasing trend among the majority of the butterfly species was synchronized, implying that a large scale disturbance event affected the community. Outside of seasonality, synchronized patterns in species abundance are not expected to occur unless some catastrophic ecological event affects the entire community. Butterfly community data from a five year study in Ecuador demonstrate the asynchronous nature of the component species in a butterfly community under natural conditions (DeVries & Walla 2001). Pollard (1984) collected butterfly species abundance data for numerous sites throughout England for seven years, a Temperate Zone with a butterfly community similar to western Oregon, and the butterfly species did not all follow the same pattern of relative abundance increases and declines between years. The only known factor that had the potential to cause a community wide concurrent decrease in species abundance at Schwarz Park was the *Bt* spray.

Miller (1990, 1992), Peacock et al. (1998), and Whaley et al. (1998) predicted local species extinction of non-target Lepidoptera in response to repeated *Bt* application events. Locally distributed, monophagous, and rare Lepidoptera species are the ones most likely to experience a resultant *Bt* induced extinction because they occupy narrow ecological niches. It appears that a localized extinction of three non-target butterfly species transpired within Schwarz Park following the spray. *Mitoura grynea*, *Parnassius clodius claudianus*, and *Phyciodes p. pratensis* were found in 1997, but were not detected inside Schwarz Park the three years following the spray (Appendix). Larvae of *M. grynea* use *Calocedrus deccurens* (Torr.) Florin. (Cupressaceae), *P. clodius claudianus* uses *Dicentra formossa* (Andr.) Walp. (Papaveraceae), and *P. pratensis* uses *Aster hallii* (Gray) Cronq. (Asteraceae) as their sole larval host plants at the study site, which are locally restricted within the treatment area. Two migratory butterfly species, *Danaus plexippus* and *Vanessa cardui* (Scott 1986), were found in 1997 but have not be seen since, perhaps indicating that they too were extirpated from the treatment area. However, it is not known if there were local populations of these species in Schwarz Park before the spray or the individuals were observed migrating through the area. Considering the relatively small size of the spray area (32.4 ha), local butterfly extinction should not transpire because there is ample edge habitat for recolonization. However, the isolated nature of the spray site and the relative blanketing of butterfly habitat with *Bt* spores suggests how even common species can be at risk of local extinction when they are subject to pest control measures.

In the case of Schwarz Park, the spray area was relatively small, but non-target Lepidoptera populations in larger spray areas may not recover as quickly as the Schwarz Park area. Historical *Bt* sprays in western Oregon have been large, a 120,000 ha block was repeatedly sprayed in 1985, 1986, and 1987 (Johnson et al. 1989). With increasing evidence for the negative consequences of *Bt* on non-target Lepidoptera communities and the species that depend on Lepidoptera for food, the ecological impacts associated with broadscale use of *Bt* for pest control should be questioned. Other alternatives that are pest species specific are the best option for minimizing the mortality and largescale disruption of native Lepidoptera communities in pest eradication areas.

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LITERATURE CITED

BAI, B. & K. JOHNSON. 1997. Environmental Assessment: Gypsy moth eradication program, Lane Co., Oregon. Oregon Department of Agriculture.

BELLOCQ, M. I., J. F. BENDELL & B. L. CADOGAN. 1992. Effects of the pesticide *Bacillus thuringiensis* on *Sorex cinereus* (masked shrew) populations, diet, and prey selection in a jack pine plantation in northern Ontario. Canadian Journal of Zoology 70:505–510.

BROCK, T. D. 2000. Biology of microorganisms. Ninth Ed. Prentice Hall. Upper Saddle River, New Jersey.

DEVRIES, P. J. & T R. WALLA. 2001. Species Diversity and community structure in neotropical fruit-freeding butterflies. Biological Journal of the Linnean Society 74:1–15.

GADDIS, P. K. & C. C. CORKRAN. 1986. Secondary effects of *Bt*. spray on avian predators; the reproductive success of Chestnut-backed Chickadees. Oregon Department of Agriculture, Salem.

HANSEN JESSE, L. C. & J. J. OBRYCKI. 2000. Field deposition of *Bt* transgenic corn pollen: lethal effects on the monarch butterfly. Oecologia 125:241–248.

HERSHEY, A. E., A. R. LIMA, G. J. NIEMI & R. R. REGAL. 1998. Effects of *Bacillus thuringiensis israelensis* (BTI) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. Ecological Applications 8:41–60.

HINCHLIFF, J. 1994. An atlas of Oregon butterflies: the distribution of the butterflies of Oregon. The Oregon State University Bookstore, Inc., Corvallis, Oregon.

HOFMANN, C., P. LUTHY, R. HUTTER & V. PLISKA. 1988. Binding of the delta-endotoxin from *Bacillus thuringiensis* to brush-border membrane vesicles of the cabbage butterfly (*Pieris brassicae*). European Journal of Biochemistry 173:85–91.

HOLMES, S. B. 1998. Reproduction and nest behavior of Tennessee warblers *Vermivora peregrina* in forests treated with Lepidoptera-specific insecticides. Journal of Applied Ecology 35:185–194.

JOHNSON, K. J. R., A. D. MUDGE & L. C. YOUNG. 1989. The gypsy moth in Oregon. Plant Division, Oregon Department of Agriculture.

JOHNSON K. S., J. M. SCRIBER, J. K. NITAO & D. R. SMITLEY. 1995. Toxicity of *Bacillus thuringiensis* var *kurstaki* to three *non-target* lepidoptera in field studies. Environmental Entomology 24(2):288–297.

LOSEY, J E., L. S. RAYLOR & M. E. CARTER. 1999. Transgenic pollen harms monarch larvae. Nature 399:214.

MAGURRAN, A. E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, New Jersey.

MARTIN, P A. W. & R. S. TRAVERS. 1989. Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. Applied Environmental Microbiology 55:2437–2442.

MILLER, J. C. 1990. Field assessment of the effects of a microbial pest control agent on *non-target* lepidoptera. American Entomologist 36:135–139.

———. 1992. Effects of a microbial insecticide, *Bacillus thuringiensis kurstaki*, on *non-target* lepidoptera in a spruce budworm-infested forest. Journal of the Research on the Lepidoptera 29(4):267–276.

NAGY, L. R. & K. G. SMITH. 1997. Effects of insecticide-induced reduction in lepidopteran larvae on reproductive success of Hooded Warblers. The Auk 114(4):619–627.

PEACOCK, J W., D. F. SCHWEITZER, J.L. CARTER & N.R. DUBOIS. 1998. Laboratory assessment of the effects of *Bacillus thuringiensis* on native lepidoptera. Environmental Entomology 27(2):450–457.

POLLARD, E. 1984. Fluctuations in the abundance of butterflies, 1976–1982. Ecological Entomology 9:179–188.

RODENHOUSE, R. L. & R. T. HOLMES. 1992. Results of experimental and natural food reductions for breeding Black-throated Blue Warblers. Ecology 73:357–372.

SCHNEPF, E., N. CRICKMORE, J. VAN RIE, D. LERECLUS, J. BAUM, J. FEITELSON, D. R. ZEIGLER & D. H. DEAN. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews 62:1092–2172.

SCHWARTZ, J. L., L. GARNEAU, D. SAVARIA, L. MASSON, R. BROUSSEAU & E. ROUSSEAU. 1993. Lepidopteran-specific crystal toxins from *Bacillus thuringiensis* form cation and anion selective channels in planar lipid bilayers. Journal of Membrane Biology 132:53–62.

SCOTT, J. A. 1986. The butterflies of north america: a natural history and field guide. Stanford University Press.

SMITH, R. A. & G. A. COUCHE. 1991. The phylloplane as a source of *Bacillus thuringiensis* variants. Applied Environmental Microbiology 57:311–315.

TOJO, A. & K. AIZAWA. 1983. Dissolution and degradation of *Bacillus thuringiensis* endotoxin by gut juice protease of the silk worm *Bombyx mori*. Applied Environmental Microbiology 45:576–580.

WAGNER, D. L., J. W. PEACOCK, J. L. CARTER & S. E. TALLEY. 1996. Field assessment of *Bacillus thuringiensis* on *non-target* lepidoptera. Environmental Entomology 25(6):1444–1454.

WHALEY, W. H., J. ANHOLD & B. G. SCHAALJE. 1998. Canyon drift and dispersion of *Bacillus thuringiensis* and its effects on select *non-target* Lepidopterans in Utah. Environmental Entomology 27(3):539–548.

WHITMORE, R. C., R. J. COOPER & B. E. SAMPLE. 1993. Bird fat reductions in forests treated with dimilin. Environmental Toxicological Chemistry 12:2059–2064.

ZAR, J. H. 1996. Biostatistical analysis. 3rd ed. Prentice Hall, Upper Saddle River, NJ. pp. 157–159.

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APPENDIX. Adult butterfly data for the Schwarz Park spray site from 1997–2000. "**" = migratory species.

Butterfly Species	Diapause Life Stage	1997	1998	1999	2000
Hesperiidae	Larvae	5	0	0	3
Erynnis propertius (Scudder & Burgess)					
Pyrgus communis (Grote)	Multivoltine	1	0	0	6
Ochlodes s. sylvanoides (Boisduval)	Eggs or 1st instar	0	0	0	1
Amblyscirtes vialis (W. H. Edwards)	Larvae	0	0	0	1
Papilionidae					
Parnassius clodius claudianus Stichel	Eggs	2	0	0	0
Papilio rutulus (Lucas)	Pupae	1	0	0	9
Papilio eurymedon (Lucas)	Pupae	1	0	0	6
Pieridae					
Pieris napi marginalis Scudder	Pupae	1	0	0	3
Pieris rapae Linnaeus	Multivoltine	0	0	0	1
Anthocharis sara flora Wright	Pupae	3	1	1	3
Colias eurytheme Boisduval	Multivoltine	0	2	0	0
Lycaenidae					
Mitoura grynea (Huber)	Pupae	5	0	0	0
Strymon melinus setonia McDunnough	Multivoltine	8	5	5	14
Everes comyntas (Godart)	Multivoltine	5	0	0	15
Celastrina argiolus echo (W. H. Edwards)	Pupae	13	4	4	12
Plebejus acmon acmon (dos Passos)	Multivoltine	0	0	1	0
Glaucopsyche lygdamus columbia (Skinner)	Pupae	97	16	55	108
Nymphalidae					
Phyciodes mylitta mylitta (W. H. Edwards)	Multivoltine	15	6	8	30
Phyciodes p. pratensis (Behr)	Pupae	1	0	0	0
Boloria epithore chermocki (Perkins & Perkins)	4th instar larvae	4	0	0	2
Euphydryas chalcedona colon (W. H. Edwards)	3rd or 4th instar larvae	2	0	0	1
Polygonia satyrus (W. H. Edwards)	Adult	1	0	2	1
Nymphalis antiopa (Linnaeus)	Adult	1	1	0	0
Vanessa cardui ** (Linnaeus)	Adult	1	1	0	0
Vanessa atalanta rubria ** (Fruhstorfer)	Adult	1	0	0	1
Satyridae					
Coenonympha tullia eunomia (Dornfeld)	3rd or 4th instar larvae	8	7	11	48
Danaidae					
Danaus plexippus ** (Linnaeus)	Adult	2	0	0	0