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Effects of a Microbial Insecticide, *Bacillus thuringiensis kurstaki*, on nontarget Lepidoptera in a Spruce Budworm-infested Forest

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Abstract. Species in a guild of nontarget leaf-feeding Lepidoptera on tobacco brush, *Ceanothus velutinus* Dougl. were monitored in the field to assess ecological effects of one application of the microbial pest control agent, *Bacillus thuringiensis* Berliner var. *kurstaki* [BTK]. The Lepidoptera were sampled to compare species richness, species evenness, species diversity, larval abundance, and a dominance index between an untreated and BTK treated site over a period of two years. The guild of leaf-feeding Lepidoptera on *C. velutinus* consisted of 32 species. No statistically significant differences were observed in overall species richness, although the number of species in the untreated site was 30% higher two weeks after treatment. However, species richness among uncommon species was significantly reduced in the treated site. Also, no statistically significant differences were observed in species evenness or species diversity but the indices were lower in the untreated site in three of the four post-treatment samples. A dominace index was consistently higher in the untreated site. The total number of caterpillars per 100 sec sampling was significantly higher (5.4-fold) in the untreated site in the early summer sample, two weeks after treatment. Also, larval abundance in the early summer sample was significantly higher (3.5-fold) one year later. No differences were noted in larval abundance in the late summer sample in either year.

Introduction

Microbial pest control agents [MPCAs] are a primary means of biological control for insect pests. In general, the use of MPCAs is targeted for a particular pest species. The insect pathogen, *Bacillus thuringiensis* [BT] Berliner var. *kurstaki* [BTK], is a bacterial MPCA used for suppression of pest Lepidoptera. For instance, large-scale use of BTK against the gypsy moth and spruce budworm has been commonly employed over forested habitats (Brookes et al. 1987, Doane and McManus 1981, Dreistadt and Dahlston 1989). However, nontarget species that are taxonomically related to the target pest may also be adversely effected (Laird 1973, Lighthart et al. 1988, Pimentel et al. 1984, Podgewaite 1986). Miller (1990) noted that BTK treatments (three in a single season) for the gypsy moth in western Oregon reduced species richness and larval abundance for up to two years within a guild of native, nontarget Lepidoptera feeding on oak. Franz and Krieg (1967) observed that other Lepidoptera decreased in number when BT was applied to control *Tortrix viridana* L. in Europe.

Many concerns need to be addressed regarding the use of MPCAs, particularly in large-scale programs and in the advent of genetically altered organisms. Among these are: (1) the impact of an MPCA on nontarget populations that have important functions in food webs; (2) the fate of species of special interest like the monarch butterfly (Brower 1986); (3) population trends in endangered species; and (4) conflict with other biological control agents, such as Lepidoptera on weeds (Miller 1990). Also, studies on ecological effects of MPCAs that are not genetically altered are needed to compare to those ecological effects involving genetically engineered MPCAs (Kirschbaum 1985, Flanagan 1989, Tiedje et al. 1989).

The objective of the current study was to determine if the use of BTK resulted in significant differences in the abundance of individuals and species composition of immature Lepidoptera between treated and untreated sites.

Materials and Methods

The data presented here come from an investigation into the effects of a single BTK treatment targeted for the spruce budworm (*Choristoneura occidentalis* Freeman) on a guild of native, nontarget leaf-feeding Lepidoptera. The study was conducted from June 1989 to August 1990. The field samples focused on the guild of immature Lepidoptera (caterpillars) that feed on the foliage of tobacco brush (*Ceanothus velutinus* Dougl.). This plant was selected because of its general abundance within the plant community where the spray zone was located. Also, earlier studies (author, unpubl. data) indicated that the species richness and abundance of Lepidoptera on tobacco brush was relatively high in the Oregon Cascade Mountains.

The study site was 50km (31mi) south of Estacada, Clackamas Co., Oregon. The area is on the western slope of the Cascade mountain range at 1000-1200m (ca. 3000-4000ft) elevation where the mean maximum temperature in July is 24-28°C (75-81°F) and precipitation ranges from 160-200cm (63-79in) per year, mostly between December and March (Franklin and Dyrness 1988). The plant community is dominated by Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Also present were alders (*Alnus* spp.), willows (*Salix* spp.), maples (*Acer* spp.), and many species of shrubs, such as, blueberry (*Vaccinium* spp.), rhododendron (*Rhododendron macrophyllum* G. Don), ocean spray (*Holodiscus discolor* (Pursh) Maxim.), and *C. velutinus*.

Two transects for sampling caterpillars were established within each treatment. Thus, a total of four transects were established. Each transect was 100m (330ft) long and 2m (6ft) wide. The transects located in the treated site were 0.5km (0.3mi) within the boundary of a 4000ha (ca. 10,000ac) region that was sprayed in late June 1989. The area where the transects occurred was treated on June 26. The BTK was applied from a helicopter at the rate of 8 billion international units (BIU) per 2.8 liters (3qt) of water per 0.4ha (1.0ac). The transects in the untreated site were located outside the spray zone by at least 2km (1.3mi). Because of topographical and floral heterogeneity the transects were matched between treated and untreated sites using: (1) physical aspects of the habitat (south facing slope, open canopy, elevation of 1000-1200m (3000-4000ft);

(2) similarly sized plants of *C. velutinus* that were 1-2m (3-6ft) tall; and 3) the presence of 30-40 plants of *C. velutinus* within an area of 100x100m (330x330ft). In 1989, each transect was sampled on June 21 (pre-spray), July 11 (early summer), and August 25 (late summer). In 1990, each transect was sampled on July 11 and August 20.

The caterpillar fauna was sampled by shaking foliage over a 75x75cm (30x30in) sheet for a timed interval of 30-45 sec. Sampling was continued until a total of 180-220 sec of sampling effort was achieved along each transect. Typically, 4-5 plants along each transect would be sampled at each date to attain the 180-220 sec sampling effort. A particular plant was only sampled once within each year. Caterpillars were collected alive and reared to adults in the laboratory on clean tobacco brush foliage to determine viability and verify identifications. The number of larvae collected per 100 sec of sampling was used to compare population density among transects and between treatments.

The samples were analyzed to determine: (1) the individual abundance of all immmature leaf-feeding Lepidoptera; (2) species richness (s); (3) species evenness (J'); (4) species diversity (H')(Pielou 1974); and (5) a dominance index (d) (Berger and Parker 1970, Southwood 1978)(Table 1). Also, a novel method for classifying and comparing common and uncommon species was developed for this study. This index was used to measure proportional abundance of individuals to species richness. A rating of a species as common was given if the proportional abundance of individuals of that species was equal to or larger than its proportion of species richness. A species was considered uncommon if the proportional

Table 1. Indices involving species composition among leaf-feeding caterpillars.

Variable	Equation
Species evenness	$(J') = (H')/\ln s$
Variance of (J')	$\mathrm{var}\,(H')/(\ln s)^2$
Species diversity (H')	$(-Ep_i \ln p_i)-[(s-1)/2N]$
Variance of (H')	$[(Ep_i \ln^2 p_i)-(Ep_i \ln p_i)^2/N]+[(s-1)/2N^2]$
Dominance index (d)	N_{max} / N_T
Proportional abundance-richness index	N_T/s

s is species richness; p_i is the proportion of the i^{th} species; N_{max} is the number of individuals of the most abundant species; and N_T is the total number of individuals of all species (see Berger and Parker 1970, Pielou 1974, Southwood 1978).

abundance of individuals of that species was less than its proportion of species richness. The data on species richness and larval abundance were analysed by using a G-test for independence (Sokal and Rohlf 1981).

Results and Discussion

A total of 32 species belonging to nine families of Lepidoptera were collected during the study. The most commonly encountered taxa were in the Geometridae, Noctuidae, Lycaenidae, and Gelechiidae (Table 2). The most abundant species were; two geometrids, *Drepanulatrix falcataria* (Pack.) and *Eupithecia* sp.; a lycaenid, *Satyrium saepium saepium* (Boisduval); and a gelechiid, *Chionides* sp.

Larval abundance. A total of 1389 immature Lepidoptera was collected from all sites during the two year study. Prespray equality in larval abundance between sites was demonstrated by the recovery of 75.2 and 75.5 larvae per 100 sec of sampling in the untreated and treated sites, respectively (Table 3).

Table 2. Some of the more common species of Lepidoptera collected on *Ceanothus velutinus* in and near an area sprayed with BTK for control of the spruce budworm, Estacada, Oregon, USA. 1989-1990.

FAMILY Genus species	Peak abundance*
GELECHIIDAE	
Chionides sp.	23.7
GEOMETRIDAE	
Drepanulatrix sp. (mostly *D. falcataria*)	38.5
Eudrepanulatrix sp.	14.1
Eupithecia sp.	54.6
Hesperumia sulpharia	14.4
Nematocampa sp.	10.6
LYCAENIDAE	
Satyrium saepium saepium	18.5
NOCTUIDAE	
Orthosia hibisci	6.1
TORTRICIDAE	
Choristoneura sp.	12.3

*Number of larvae collected per 100 sec sampling effort with a 75x75cm beating sheet.

Table 3. Effects of BTK on nontarget Lepidoptera on *Ceanothus velutinus*: Larval abundance. Estacada, Oregon, USA. 1989-1990.

Date	Site untreated	Site treated	G	df	P
1989					
June 21	75.2	75.5	0.00	1	0.999
July 11	80.1	14.9	49.16	1	0.001
August 25	29.2	29.0	0.00	1	0.999
1990					
July 11	29.8	8.6	12.39	1	0.001
August 20	20.9	19.4	0.04	1	0.800

values are number of larvae per 100 sec sampling effort with a 75x75cm beating sheet.

Differences in the number of larvae collected between the prespray samples (June 21) and the first postspray samples (July 11) demonstrated the impact of BTK on nontarget Lepidoptera (Table 3). The number of larvae per 100 sec sample effort in the untreated site increased 6%, from 75.2 in the prespray sample to 80.1 in the first postspray sample. This increase in larval abundance was due to natural seasonal cycles of species in the late spring and early summer. In contrast the number of larvae in the treated plots decreased 80%, from 75.5 to 14.9 indivduals per 100 sec sampling time. There was an 81% difference in larval abundance between treated and untreated sites on July 11. The second postspray sample, on August 25, showed no differences in larval abundance between sites.

One year later, the early summer sample (July 11) again showed a significant difference in larval abundance; 71% fewer larvae were found in the treated site (Table 3). Although the BTK application occurred more than one year prior to this sample, the effects were still present because nearly all species involved are univoltine. The second year of the study was in effect monitoring the first generation from individuals present at the time of the treatment. The late summer sample one year after treatment showed no differences in larval abundance between sites.

Species richness. The results on species richness in the prespray samples indicated the treated and untreated sites were similar. A comparison of treated and untreated sites indicated that statistically BTK did not significantly effect the number of species of Lepidoptera on *C. velutinus* (Table 4). Although species richness was 30% higher (6 more

Table 4. Effects of BTK on nontarget Lepidoptera on Ceanothus velutinus: Species richness (s) and species evenness (J'). Estacada, Oregon, USA. 1989-1990.

Date/ treatment	*s*	*J'*	var *J'*	95% CI *J'*
1989 June 21				
untreated	15	0.76	0.01	0.64-0.89
treated	15	0.82	0.01	0.70-0.94
1989 July 11				
untreated	19	0.67	0.01	0.58-0.76
treated	10	0.92	0.02	0.67-1.00
1989 August 25				
untreated	15	0.48	0.01	0.35-0.60
treated	15	0.44	0.01	0.33-0.55
1990 July 11				
untreated	10	0.61	0.01	0.52-0.70
treated	12	0.85	0.03	0.52-1.00
1990 August 20				
untreated	6	0.52	0.01	0.32-0.72
treated	8	0.59	0.01	0.42-0.75

species) in the untreated site in the first early summer sample, the difference was not significant ($G=1.064$, $df=1$, $P>0.25$).

Species evenness. The results on species evenness in the prespray samples indicated the treated and untreated sites were similar (Table 4). In both years a lower value for species evenness occurred in the late summer samples. Thus, a seasonal trend in species evenness was evident. No significant differences in species evenness were observed between treatments within respective sample dates. However, in both early summer samples species evenness was consistently higher in the treated sites. Higher values for species evennness can be interpreted to indicate a decreased degree of numerical dominance by any given species.

Dominance index. An index indicating the degree of numerical dominance by the most abundant species was higher in untreated sites in all samples (Table 5). A difference of 13% was detected in the prespray samples compared to a range of 45 and 54% between treatments in the early summer samples. The late summer samples exhibited a difference of 8 and 10%. These data suggest that the samples from untreated plots tended to be dominated by certain species. The application of BTK had

Table 5. Effects of BTK on nontarget Lepidoptera on Ceanothus velutinus: Dominance index (d) and species diversity (*H'*). Estacada, Oregon, USA. 1989-1990.

Date/ treatment	*d*	*H'*	var *H'*	95% CI *H'*
1989 June 21				
untreated	0.30	2.07	0.03	1.72-2.41
treated	0.26	2.22	0.03	1.88-2.57
1989 July 11				
untreated	0.35	1.96	0.02	1.68-2.25
treated	0.16	2.12	0.10	1.49-2.75
1989 August 25				
untreated	0.63	1.29	0.03	0.98-1.61
treated	0.58	1.19	0.02	0.91-1.47
1990 July 11				
untreated	0.51	1.41	0.01	1.21-1.61
treated	0.28	1.96	0.17	1.16-2.76
1990 August 20				
untreated	0.71	0.93	0.03	0.58-1.28
treated	0.64	1.22	0.05	1.00-1.44

the effect of evenning the numbers of caterpillars among species, an observation consistent with the indices on species evenness.

Species diversity. The results on species diversity in the prespray samples indicated the treated and untreated sites were similar (Table 5). In both years, species diversity was lower in the late summer samples regardless of treatment. Thus, as with species evenness, a seasonal trend in species diversity was evident. No significant differences in species diversity were observed between treatments in respective sample dates. However, in both early summer samples the index for species diversity was lower in the untreated sites. A lower value for species diversity can be interpreted to indicate a reduction in either species richness, species evenness, or both.

Proportional abundance-richness index. The abundance of individuals in respective species expressed as a proportion relative to the number of species can be used as an index to determine whether a species may be considered uncommon or common (Table 6). This is useful because indices of species richness and species diversity do not provide a means for objectively classifying the relative abundance of individuals among species as common or uncommon. For example, a species repre-

Table 6. Effects of BTK on nontarget Lepidoptera on Ceanothus velutinus: number and proportion of common and uncommon species. Estacada, Oregon, USA. 1989-1990.

Date	No. species		Proportion of species	
	Common	Uncommon	Common	Uncommon
1989 June 21				
untreated	5	10	33	67
treated	6	9	40	60
1989 July 11				
untreated	4	15	21	79
treated	3	7	30	70
1989 August 25				
untreated	2	13	13	87
treated	2	13	13	87
1990 July 11				
untreated	2	8	20	80
treated	4	8	33	67
1990 August 25				
untreated	1	5	17	83
treated	1	7	13	87

sented by one individual in a sample may carry the same weight as a species represented by 50 individuals.

The number of common and uncommon species in the prespray samples was similar (Table 6). The samples were dominated (73% overall) by species considered to be uncommon. Overall, only 27% of the species were categorized as common. In the early summer samples the number of common species was equivalent but the number of uncommon species was reduced by 53% in the treated site, a significant difference ($G=10.66$, $df=1$, $P<0.001$). This effect was not observed in the early summer sample in the second year. These data suggest that certain uncommon species are more likely to be removed from the system than common species and that gross accounts of species richness may mask significant effects on less abundant species. The importance of this observation underscores the need to direct special management needs for rare and endangered species, such as: timing of application, doses, skipping sensitive areas, and mitigation.

Conclusions

The data on larval abundance and richness of uncommon species indicated that one application of BTK reduced the abundance of nontarget Lepidoptera in the guild of caterpillars feeding on leaves of *C. velutinus*. The effects were most notable during the early summer period immediately following treatment. The data regarding species richness, species evenness, and species diversity did not demonstrate a significant effect. However, a statistical analysis of the indices used to describe community composition can be misleading if a biological interpretation is not assessed as well. For instance, species richness declined in the first year of the study following the application of BTK. The decline was not significant but nonetheless six species occurred in the untreated site that were not represented in the treated site. An extrapolation of these data suggests that if any of the species had been limited in its distribution, or a unique genotype of the species was locally endemic, then the population/species would be at high risk of becoming extinct.

Additional studies on the impact of MPCAs on nontarget organisms under field conditions are needed. Many variables contribute to patterns in the data from samples of treated and untreated sites. A comparison of this study to a similar study conducted previously (Miller 1990) shows some of the variables that may influence the impact of a MPCA on nontarget organisms. The dose, frequency of applications, timing of applications, and geometry of the treated site will contribute a strong influence on the immediate impact and overall recovery of a community subjected to a MPCA treatment.

In the present study and that of Miller (1990), BTK was shown to negatively impact certain nontarget organisms. Although no direct comparisons were made with the use of synthetic pesticides, the negative impacts on nontarget organisms would likely be more severe with synthetic pesticides and widespread across a multitude of taxa. Many synthetic pesticides have been shown to exhibit dramatic negative impacts on nontarget organisms (Croft 1990; Ehler and Endicott 1984; Martinat et al. 1988). While the use of microbial pathogens may exhibit certain negative impacts on nontarget organisms, methods for the use of MPCAs can be developed to minimize such impacts and reduce the use of synthetic pesticides in present pest management programs. The development of pathogens as biologically rational pest control agents must involve assessments of nontarget effects for the benefit of prolonging the use of microbes as ecologically and sociologically acceptable pest management options.

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