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Palatability of Leaf Material Contaminated with *Bacillus thuringiensis* var. *kurstaki,* to *Hydatophylax argus,* a Detritivorous Aquatic Insect

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The microbial insecticide *Bacillus thuringiensis* Berliner var. *kurstaki* (B.t.k.) is widely used for control of forest lepidopteran pests in Canada and the United States (van Frankenhuyzen 1990). Preference for this material over chemical insecticides in pest management strategies is based largely on its relatively specific insecticidal properties, and its purported environmental safety (Melin and Cozzi 1990). Because applications of B.t.k. for forest protection are often made from aircraft over large tracts of land, there is potential for contamination of aquatic ecosystems. Although B.t.k. is a natural and ubiquitous soil bacterium (Addison 1993), aerial spraying may cause unnaturally-high concentrations of B.t.k. spores and toxin crystals in aquatic systems. This warrants investigation into the potential for harmful effects on aquatic organisms.

A few published studies have evaluated the effects of B.t.k. on aquatic macroinvertebrates by direct exposure in laboratory tests and stream channels (Eidt 1985; Kreutzweiser et al. 1992). Community-level investigations have also been conducted to measure direct and indirect (food-chain) effects on aquatic invertebrates (Richardson and Perrin 1994; Kreutzweiser et al. 1994). Results from these studies indicated that significant adverse effects on macroinvertebrates were not likely to result from contamination of water bodies with B.t.k. However, none of these studies determined functional effects on aquatic invertebrates in terms of efficiency in processing particulate organic matter. Because of the tendency for B.t. spores to accumulate on organic material (Ohana *et al.* 1987), the potential for indirect effects on macroinvertebrates that process organic matter may be quite high.

The decomposition of allocthonous organic matter, particularly deciduous leaf material, is the primary energy source of forested headwater streams, and macroinvertebrates play a crucial role in this process (Anderson and Sedell 1979; Cuffney *et al.* 1990). Microbial conditioning of leaf surfaces is essential for efficient decomposition by macroinvertebrates. “Shredder” invertebrates will pref-

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erentially select the most microbially colonized (conditioned) material (Cummins and Klug 1979). Reice and Wohlenberg (1993) called this detritivore feeding on microbially conditioned organic matter, “the principal energy pathway in streams”. We speculated that accumulation of B.t.k. particles and formulation components on organic substrates could affect the microbial colonization of substrate surfaces. This in turn may reduce the palatability or acceptability of organic substrates to detritivorous invertebrates. Reduced palatability is likely to result in reduced consumption and processing of organic material. Such an effect could have significant ecological consequences if this major energy pathway of organic matter processing was disrupted.

MATERIALS AND METHODS

The effect of B.t.k. on the palatability of leaf material to a detritivorous caddisfly (*Hydatophylax argus* Harr.) was determined by measuring the preference for, or avoidance of, treated leaf disks in a flow-through preference test. The disks (23 mm diameter) were cut from yellow birch leaves (*Betula alleghaniensis* Britton) that were collected just before leaf fall, leached in running water for 2 d, then air dried and held at room temperature. To facilitate colonization by natural aquatic microbial communities, the leaf disks were individually pinned to a wooden rack that was placed in a laboratory flow-through trough for 4 d under standard fluorescent lighting at 16:8 hr light/dark conditions. Water in the flow-through trough was drawn from the upper St. Mary’s River. Water temperature during the incubation period was 12-13° C. Results from concurrent experiments determining aquatic microbial activity on leaf disks in respiration chambers demonstrated that the 4-d incubation period under these conditions was sufficient to establish viable microbial communities on the leaf material (unpublished data).

The leaf disks were randomly allocated among controls, and two concentrations of treated groups. Those in control groups were held in river water for an additional 2 d, while those in treated groups were placed in recirculating river water treated with B.t.k. at the maximum expected environmental concentration (EEC) and 1000 X the EEC (20 and 20,000 IU/mL, respectively) for 2 d. By the end of the 2-d treatment in B.t.k., leaf disks in the 1000 X EEC groups were noticeably covered with a whitish residue. Microscopic examination of this material indicated an accumulation of B.t.k. spores and formulation materials. A proliferation of the biofilm, notably other rod-shaped bacteria and fungi, was also observed.

The B.t.k. was applied as the aqueous flowable formulation, Dipel® 64AF (Abbott Laboratories, Chicago, Illinois). The expected environmental concentration (EEC) was calculated as the concentration that would occur in a 15-cm deep body of water that was directly oversprayed at an application rate of 30 BIU/ha. The 1000-fold increase over the EEC is the maximum challenge concentration currently suggested by Canadian regulatory agencies for conducting first-level screening of microbial pesticides for adverse effects on non-target organisms.

After the 2-d exposure to B.t.k., the leaf disks were placed in 4 replicate flow-through units. These test units were glass containers, 20 X 20 cm square and 8 cm high, and lined on bottom with a 3-mm thick rubber mat. Water flowed into the upper end of the units and out through a screened exit port at the lower end, at an approximate flow rate of 5 cm/sec. Each test unit contained 18 leaf disks, 6 treated at the EEC, 6 treated at 1000 X the EEC, and 6 controls (incubated in river water alone). These were systematically arranged in a 3 X 6 matrix with each treatment level occurring in every third cell, beginning at the upper left comer. The disks were attached to each cell location with a stainless steel pin. When the leaf disks were arranged and attached, 6 caddisfly larvae were placed in the centre of each flow-through unit. Within 1 hr, 79% of the caddisfly larvae (19/24) had moved onto, and were feeding on, leaf disks. After 24 hr, the numbers and treatment levels of leaf disks that had been consumed to some extent were recorded. An estimate of the surface area of the remaining leaf material was made to categorize each disk as <20% of the leaf material eaten, or >20% of the material consumed. This was intended to indicate if the caddisfly larvae initially fed on contaminated leaf material, and subsequently rejected the material as unpalatable.

RESULTS AND DISCUSSION

Exposure to B.t.k. at both 1000X the EEC and the EEC had no apparent effect on the palatability of leaf disks to the detritivore caddisfly larvae. Data from the four replicate flow-through units were tested for heterogeneity among replicates with chi-square analyses of 2 X 3 (consumed, intact X 3 treatment levels) contingency tables (Zar 1984). The tests for heterogeneity were nonsignificant ($p>0.05$), and the replicates were combined to increase the power of the general chi-square test. After 24 hr, there were no differences among the three treatment groups (control, EEC, 1000X EEC) in the numbers of leaf disks that had been partially or entirely consumed (d.f.=3, chi-square=0.1204, $p=0.9416$) (Table 1). Feeding occurred on about 65% of the leaf disks within the 24-hr period. This indicates that the B.t.k. contamination on the leaf disks did not influence the selection or consumption of leaf material. Either the larvae were unable to detect the presence of B.t.k. spores and toxins, or the insecticide did not deter them.

Table 1. Consumption of leaf disks by *Hydatophylax argus* in 24-hr preference tests containing naturally-incubated leaf disks (controls), and disks treated with B.t.k. (Dipel 64AF) at the maximum expected environmental concentration (EEC), and 1000 X the EEC. Results are combined from four replicates, following nonsignificant tests for heterogeneity among replicate preference tests ($p>0.05$).

Measurements	Controls	EEC	1000X EEC
total number of leaf disks	24	24	24
number of disks eaten to some extent	15	15	16
number of disks left intact	9	9	8
number of disks consumed ≤20%	6	5	7
number of disks consumed >20%	9	10	9

There were also no significant differences among treatment levels in the extent to which leaf disks were consumed (d.f.=3, chi-square=0.3598, p=0.8354) (Table 1). Fifty-six to 67% of the leaf disks that were selected by the caddisflies were consumed more than 20%, regardless of the presence or absence of B.t.k. There was no indication that ingestion of contaminated leaf material resulted in adverse effects on larval feeding behavior in 24 hr. Richardson and Perrin (1994) observed less decomposition of leaf packs in stream channels treated with B.t.k. than in control channels, and suggested that invertebrate detritivores may have been preferentially feeding on the B.t.k. particles and formulation products adsorbed to leaf surfaces, rather than the leaf material itself. In our experiment, there was no difference between treated and control disks in the amount of leaf material consumed, even for disks treated at 1000 X the EEC on which a visible residue and biofilm proliferation was evident.

There was no mortality of caddisfly larvae over the 24-hr period. Because this was a short-term preference test (there was insufficient food to sustain the larvae for longer periods), the experimental units were disassembled prior to the 3- or 4-d period normally required for B.t.k. to express lethal toxicity in target organisms (van Frankenhuyzen 1990). However, in previous longer-term experiments (8-15 d observation period), B.t.k. contamination of leaf material was not toxic to detritivorous stonefly nymphs under similar lab conditions, or to caddisfly larvae under field conditions (Kreutzweiser et al. 1994).

The palatability of leaf material to *Hydatophylax argus* larvae was unaffected by B.t.k. contamination. Based on our observations of B.t.k. particle accumulation and biofilm proliferation on leaf disks treated at 1000X the EEC, the microbial community was clearly affected (proliferated) at the high-concentration treatment at least. This did not deter the consumption of leaf material by caddisfly larvae. Results from this experiment indicate that B.t.k. contamination of natural aquatic systems should not interfere with the processing of organic material by macroinvertebrate detritivores. This lends further support to the contention by previous investigators that B.t.k. is unlikely to cause adverse effects on aquatic macroinvertebrates, even in water bodies contaminated by direct overspray (Eidt 1985, Kreutzweiser *et al.* 1992, Richardson and Perrin 1994, Kreutzweiser *et al.* 1994).

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