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LACK OF EFFECTS OF *BACILLUS SPHAERICUS* (VECTOLEX®) ON NONTARGET ORGANISMS IN A MOSQUITO-CONTROL PROGRAM IN SOUTHEASTERN WISCONSIN: A 3-YEAR STUDY

RICHARD W. MERRITT,[1] JOANNA L. LESSARD,[1] KELLY J. WESSELL,[1] OSVALDO HERNANDEZ,[1] MARTIN B. BERG,[2] JOHN R. WALLACE,[3] JOHN A. NOVAK,[4] JOHN RYAN,[5] AND BRETT W. MERRITT[1]

ABSTRACT. A 3-year study (2000–2002) in southeastern Wisconsin was conducted to assess the effects of *Bacillus sphaericus* applied for mosquito control on nontarget wetland invertebrates. The experimental design consisted of control and treatment sites (that were applied by helicopter with Vectolex® CG), each in 2 vegetation habitat types: reed canary grass marsh (*Phalaris arundinacea*) and cattail marsh (*Typha* spp.). In each of these areas, a predetermined number of timed (30-sec) D-frame aquatic net samples containing vegetation, detritus, and invertebrates were collected 1 day before spraying and 72 h after spraying to detect for effects. We examined and compared 5 bioassessment measures to determine if there was an effect of *B. sphaericus* on nontarget organisms during each of the sampling years. The metrics tested were 1) mean taxa richness (the mean number of all taxa), 2) mean diversity (combines taxa richness and abundances in a summary statistic; i.e., Shannon Index [H']), 3) Diptera richness (minus mosquitoes) as a proportion of all other taxa richness (Diptera/others richness), 4) Diptera abundance (minus mosquitoes) as a proportion of all other invertebrate abundance (Diptera/others abundance), and 5) functional group changes in percent collector–gatherers, collector–filterers, scrapers, shredders, and predators. When Vectolex was applied during 6 treatments at the labeled dosage rate in the above habitats in Brookfield, WI, no detrimental effects to nontarget organisms could be attributed to this microbial insecticide. Much of the variation in the control vs. treatment and pre vs. post plots was attributed to factors other than the effects of *B. sphaericus* on nontarget organisms, such as the time of sampling, natural variation that occurs in such diverse habitats as canary grass and cattail marshes, and water depth, which varied among years.

KEY WORDS Nontarget, *Bacillus sphaericus,* invertebrates, mosquito control, biological control

INTRODUCTION

Numerous field efficacy trials have been conducted using different formulations of *Bacillus sphaericus* Neide as a microbial larvicide against mosquitoes (see review by Yap 2003). Most of these trials have been carried out against species of *Culex* (e.g., Davidson et al. 1984; Lacey et al. 1984; Mulla et al. 1984a, 1984b; Walton and Mulla 1991); however, trials also have been conducted against *Anopheles* (Davidson et al. 1981, 1984; Kramer 1984), *Aedes* (Wraight et al. 1982, Mulla et al. 1985), and *Mansonia* (Yap 1985, Yap et al. 1988). *Bacillus sphaericus* offers some distinct advantages over its commercially produced counterpart, *B. thuringiensis* ssp. *israelensis* (*Bti*) in that there is an increased duration of larvicidal activity against certain species of mosquitoes, especially in organically enriched habitats, and the bacterium may recycle within mosquito cadavers (Des Rochers and Garcia 1984, Nicolas et al. 1987, Karch et al. 1990, Lacey 2003). Also, tests with *B. sphaericus* against nontarget invertebrate species under laboratory and field conditions have confirmed their culicid specificity and lack of effect on a variety of mosquito predators, Chironomidae, and other species of nematoceran Diptera (Ali and Nayar 1986, Aly and Mulla 1987, Rodcharoen et al. 1991, Walton and Mulla 1991).

In 1998, the City of Brookfield, WI, proposed controlling nuisance mosquitoes with the larvicide *B. sphaericus* (Vectolex® CG). Vectolex is the first biological larvicide based on the *B. sphaericus* bacterium to be registered for use in North America. While first registered for control of *Cu.,* Vectolex uses have been expanded to include control of several *Aedes, Ochlerotatus, Anopheles, Psorophora.* and *Coquillettidia* species (Valent BioSciences Corp., Libertyville, IL). The major pest mosquitoes of concern to the residents of Brookfield were temporary woodland pool and floodwater species (*Aedes vexans* [Meigen], *Ochlerotatus stimulans* [Walker], *Oc. trivittatus* [Coquillet]) but also included various summer species of *Cu., An.,* and *Ps.* In order to approve the annual use of *B. sphaericus* in Brookfield, the Wisconsin Department of Natural Resources (DNR) required a study lasting 3 years (at the same time a control program was underway) to answer the question as to whether *B. sphaericus* could reduce the mosquito population without affecting the nontarget invertebrate community.

Nontarget aquatic organisms are important components of wetland ecosystems and often occupy the same habitat as mosquitoes. Therefore, it was important to make sure that this microbial agent did not significantly affect those beneficial organisms

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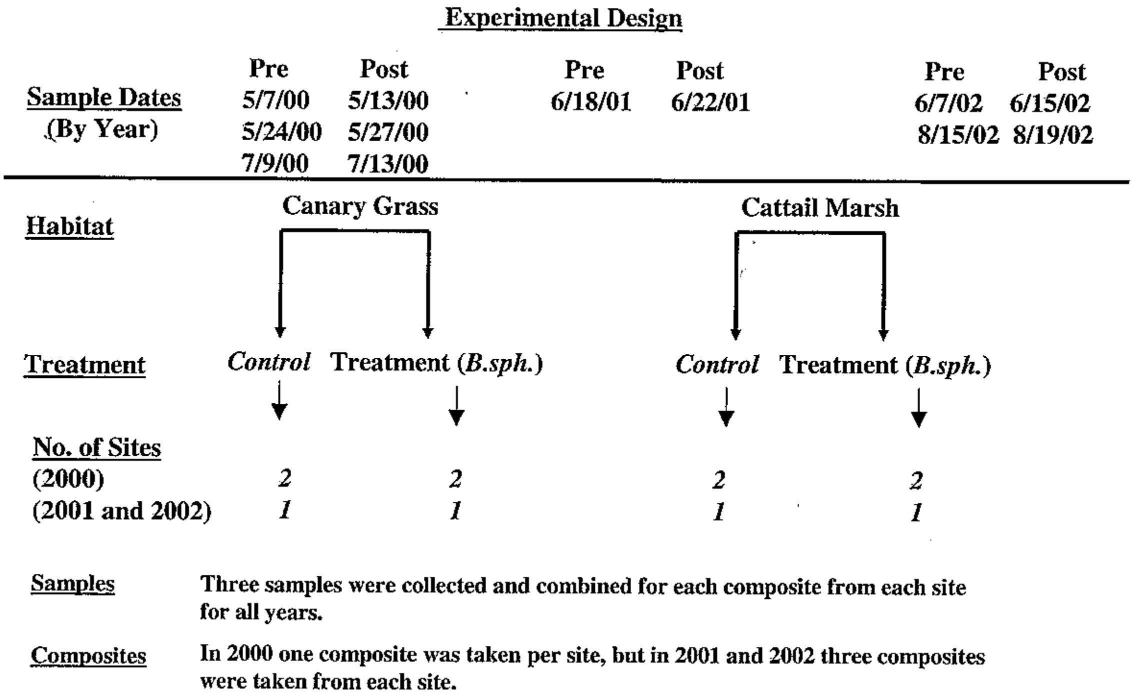
[1] Department of Entomology, Michigan State University, East Lansing, MI 48824.

[2] Department of Biology, Loyola University of Chicago, Chicago, IL 60626.

[3] Department of Biology, Millersville University, Millersville, PA 17551.

[4] Department of Biology, Colgate University, Hamilton, NY 13346.

[5] Clarke Mosquito Control, Roselle, IL 60172.



Samples Three samples were collected and combined for each composite from each site for all years.

Composites In 2000 one composite was taken per site, but in 2001 and 2002 three composites were taken from each site.

Fig. 1. Experimental design for Brookfield, WI, nontarget macroinvertebrate study using *Bacillus sphaericus* for mosquito control.

that play a direct or indirect role in the overall food web in nature.

MATERIALS AND METHODS

Experimental design

The experimental design in this study (Fig. 1) consisted of control and treatment sites (that were treated by helicopter with *B. sphaericus* [Vectolex CG]), each in 2 wetland types: reed canary grass marsh (*Phalaris arundinacea*) and cattail marsh (*Typha* spp.). These reed canary grass and cattail marshes were hydrologically unconnected wetlands with sufficient separation so that control areas would not be affected by flow from adjacent treated areas. The treated and untreated sites represented 44% of the 2,250 potential mosquito-breeding acres within the city (treated: 750 acres; untreated or control: 250 acres). A total of 6 applications of Vectolex CG were made throughout the 3-year study, with 3 in year 2000, 1 in 2001, and 2 in 2002 (Fig. 1). Differences in sampling dates and number of treatment applications among years were due to natural variation in rainfall and marsh water levels across years. Samples from the 2 habitat types were collected 1 day prior to spraying and 72 h after spraying to detect for effects on nontarget fauna due to *B. sphaericus*.

Field methods

In each treatment and control area, random sites within these areas were chosen for sampling using a prenumbered quadrat with a random-number table generator. In each of these sites, 3 timed (30-sec) D-frame aquatic net samples containing vegetation, detritus, and invertebrates were collected and combined into one composite sample. In 2000, we sampled 8 sites (2 in each of the control and treatment habitat types), and in 2001 and 2002, the number of sites was cut in half and the number of samples per site was tripled (Fig. 1). Therefore, for the last 2 years of the study, a total of 9 samples (3 composites) were taken per site each year. Thus, the total number of composited samples collected for each round of application (pre and post) in treatment and control areas on each sample date equaled 48 in 2000 and 24 in 2001 and in 2002 (Fig. 1). As part of the overall program, but not included in this study, larval and adult mosquitoes were sampled on a weekly basis throughout the season, and plankton samples were taken (using a 125-μm mesh tow net) to assess further the effects on target and nontarget organisms.

Sampling invertebrates consisted of 1 person repeatedly placing the D-frame net on the sediments at the bottom of the rooted plants (either canary grass or cattails) and moving it vigorously back and forth and up and down while drawing it to the surface along the plant stems for 30 sec. This method collects animals in the upper several centimeters of sediments and on submerged stems and leaves of plants, associated periphyton, and detritus (Merritt et al. 1996). This technique, using timed samples in emergent vegetation, was semiquantitative and has been successfully used in the Kissimmee River/Marsh floodplain restoration study in south central Florida (Merritt et al. 1996, 1999; Cummins and Merritt 2001) and in a study of the Caloosahatchee River oxbows (Cummins and Merritt 2001, Merritt et al. 2002). Water temperature and depth were taken at each site when collections were made.

The net contents containing invertebrates, vegetation, detritus, and some sediments were emptied into 14-liter buckets, field sorted to remove large debris and any large macroinvertebrates, and then washed several times through a 250-μm mesh sieve to separate smaller invertebrates and remaining coarse debris from silt and fine detritus. The remaining material in each sample was then placed in Whirl-Pak® bags, labeled, and preserved with 95% ethanol to a final concentration of 70% before the bags were sealed.

Laboratory methods

Samples from the first application (May 7 and 13) in 2000 were sorted in the laboratory under a dissecting microscope and all invertebrates were picked and sorted from detritus, and then identified and enumerated. Because of the large size of these first samples, all subsequent collections were subsampled following the method of Waters (1969). The validity of subsampling these samples using this technique was tested and evaluated in the laboratory and shown to be statistically representative of what was present in terms of numbers and richness.

After samples were picked, taxonomic identifications of all taxa, except chironomids, were made to the lowest taxonomic level possible using Wiederholm (1983), Pennak (1989), Thorp and Covich (1991), Merritt and Cummins (1996a), and other taxonomic references given in Merritt and Cummins (1996a).

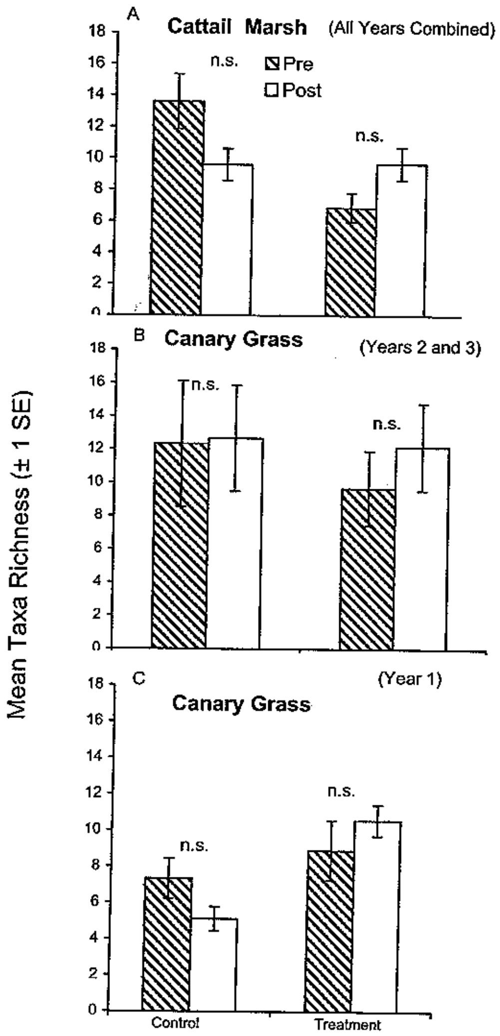


Fig. 2. Mean taxa richness (±1 SE) of nontarget fauna in control and Vectolex®-treatment plots of canary grass and cattail marsh during pre- and postapplication to control mosquitoes. Plots combine all years in the cattail marsh and are separated in canary grass (see text). n.s., nonsignificant.

Analysis

As set forth by the Wisconsin DNR, we examined and compared 5 bioassessment measures in control or treatment plots before and after spraying in canary and cattail marsh habitats to determine if there was an effect of *B. sphaericus* on nontarget organisms during each of the sampling years. Due to the difficulty of obtaining truly quantitative samples in these wetland habitats, the following semiquantitative metrics were evaluated: 1) mean taxa richness (the mean number of all taxa), 2) mean diversity (combines taxa richness and abundances in a summary statistic; i.e., Shannon Index [H′]), 3) Diptera richness (excluding mosquitoes) as a proportion of all other taxa richness (Diptera/others richness), 4) Diptera abundance (excluding mosquitoes) as a proportion of all other macroinvertebrate abundance (Diptera/others abundance), and 5) functional feeding group changes in percent collector–gatherers, collector–filterers, scrapers, shredders, and predators (Merritt and Cummins 1996b). This latter metric was added in 2001 and 2002 at the request of scientists from the Wisconsin DNR.

In analyzing the 2 habitats separately, an analysis of variance (ANOVA) on each metric was performed using SYSTAT to determine if there was significant enough variation among years to require separate analyses or if years could be combined.

Treatment effects were tested using a *t*-test to compare pre- and posttreatment data from the sites for each metric. Control sites were used as a reference to help explain differences encountered in the treatment sites (i.e., natural variation, emergence periods, etc.). A repeated measures ANOVA was also applied to the 2000 and 2002 data to determine whether samples within years and given wetlands could be pooled. This analysis compared the response to treatment (for treated sites) or treatment period (for control sites) within each habitat type over time for the years that had multiple treatment periods. The *P*-value used to denote level of significance in this study was $P \leq 0.05$.

RESULTS

Water temperatures ranged from 10 to 23°C, generally increasing from May to August during the study, except in 2002, when cooler temperatures prevailed late in the season. Water depth in canary and cattail sites for the study period ranged from 7 to 60 cm (mean = 17 cm), which made sampling with the D-frame net more difficult at certain times during pre- and postapplication periods.

Weekly larval mosquito sampling and light trap survey results during pre- and postapplication throughout the 3-year study (2000–2002) showed a 90–99% reduction in target mosquito species due to *B. sphaericus* (Vectolex CG) (J. Mathwig, unpublished data).

Both canary grass and cattail marsh habitats supported an abundant and diverse nontarget fauna of invertebrates (Appendix 1). A total of 138 taxa of invertebrates were exposed to *B. sphaericus* treatments over a 3-year time period. The cattail marsh had the highest richness with 115 taxa, whereas the canary grass had 110 taxa. There were 66 taxa common to both vegetation types (Appendix 1), and several groups were widely represented in both habitats. It was not always possible to identify specimens further than family or generic level because of the unavailability of adequate species-level keys. The class Insecta had the greatest diversity of all invertebrates collected with the Diptera (55 taxa) and Coleoptera (35 taxa) having the greatest representation.

All metrics in the cattail marsh (Figs. 2a, 3a, 4a, 5a) and most of the canary grass marsh (Figs. 2b, 3b, 4b, 5b) showed no significant annual variation and thus data from multiple years were combined. Metrics in the canary grass habitat exhibiting significant annual variations were analyzed separately (Figs. 2c, 3c, 5c). We found no significant negative changes in any of the metric values due to *B. sphaericus* effects in either the canary grass or cattail habitats between pre and post samples (Figs. 2a–c to 5a–c). One metric, mean diversity, during years 1 and 3 (2000 and 2002) in canary grass (Fig. 3b) showed a significant reduction between pre- and postapplication period in the control plots ($P = 0.045$), but significant increases in the treatment plots ($P = 0.019$). Mean diversity also was significantly reduced ($P = 0.032$) between pre- and posttreatment periods in the control plots for cattail marshes across all years (Fig. 3a), but there was no significant difference between treatment areas for this habitat type. A similar trend for cattail marshes was seen for mean taxa richness (Fig. 2a), but mean differences for this metric in both control and treatment areas were not significant, but only marginally ($P = 0.06$ and 0.052, respectively; Fig. 2a).

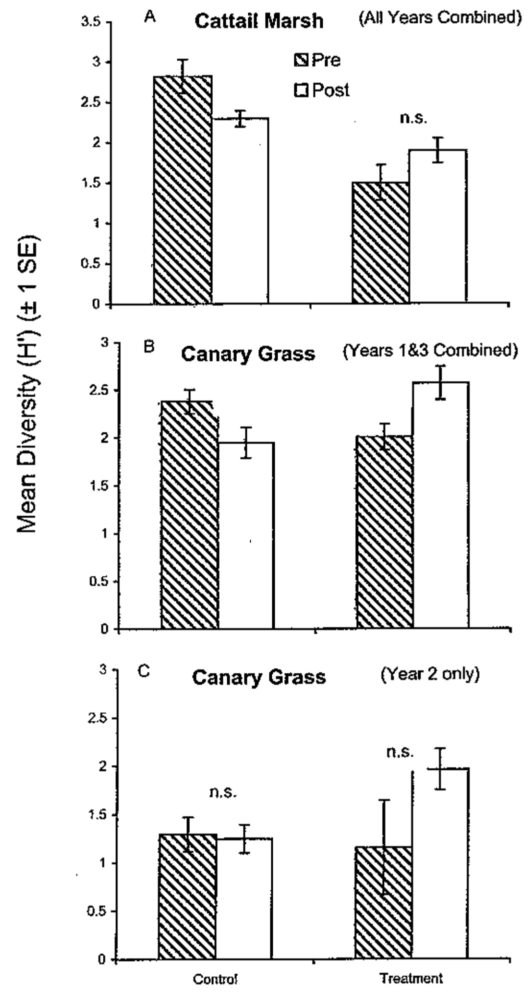


Fig. 3. Mean taxa diversity (Shannon index [H']) (±1 SE) of nontarget fauna in control and Vectolex®-treatment plots of canary grass and cattail marsh during pre- and postapplication to control mosquitoes. Plots combine all years in the cattail marsh and are separated in canary grass (see text). n.s., nonsignificant.

Functional feeding group changes between pre- and postapplication for each specific habitat are shown in Figs. 6 and 7. There were no significant differences in functional group composition (percent collector–gatherers, collector–filterers, scrapers, shredders, and predators) due to *B. sphaericus*

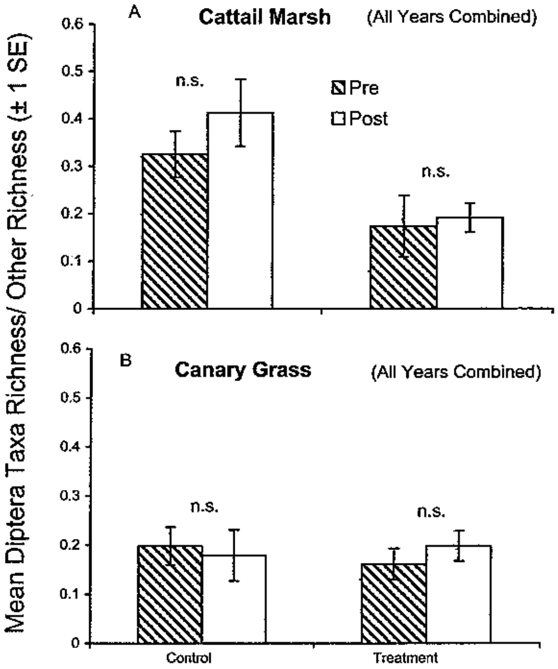


Fig. 4. Mean Diptera taxa richness (minus Culicidae)/ total other taxa richness (±1 SE) in control and Vectolex®-treatment plots of canary grass and cattail marsh during pre- and postapplication to control mosquitoes. Plots combine all years of application (see text). n.s., nonsignificant.

for either canary grass or cattail marsh habitats (Figs. 6, 7). The greatest percentage of functional feeding groups in the canary grass habitat were scrapers (mainly gastropods), followed by collector–gatherers (isopods, oligochaetes, and sphaeriid clams), and then predators (odonates and dytiscid beetles) (Fig. 6, Appendix 1). In cattail marshes, the major functional groups were similar to the canary grass habitat, but collector–gatherers (isopods and oligochaetes) comprised the largest proportion, followed by scrapers (mainly gastropods), and then predators (dytiscid beetles and Diptera larvae) (Fig. 7, Appendix 1).

DISCUSSION

Results of our analysis testing the effects of *B. sphaericus* on nontarget invertebrates did not show any negative effect from this microbial larvicide. If there was a deleterious effect of Vectolex, one would expect to see most of the metrics, except for functional feeding group percentages, significantly decrease in posttreatment compared with pretreatment periods based on responses to stressors in wetlands (Lillie et al. 2003). As shown in Figs. 2–5, except for 1 instance described earlier, this did not occur in any trial, and when significant differences were detected, there was a general pattern of increase in each metric after treatment rather than a decrease. Therefore, there were no significant negative effects of Vectolex on taxa richness, diversity, dipteran richness/total richness for all other taxa, or Diptera abundance/total abundance for all other taxa. Although there were some similarities in pre and post samples for all metrics, most habitats showed considerable variation between sampling periods (Figs. 2–5). However, there were considerable differences between control and treatment plots overall, with control plots having more taxa and a greater diversity in the cattail marsh habitats. This could be attributed to factors such as the time of sampling, natural variation that occurs in such diverse habitats as canary grass and cattail marshes,

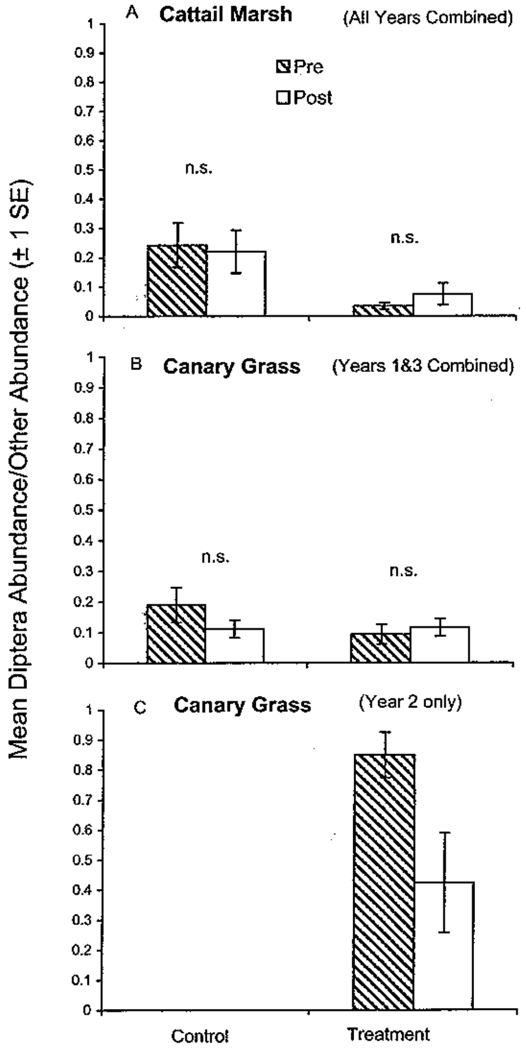


Fig. 5. Mean Dipteran abundance (minus Culicidae)/ total other taxa abundance (±1 SE) in control and Vectolex®-treatment plots of canary grass and cattail marsh during pre- and postapplication to control mosquitoes. Plots combine all years in the cattail marsh and are separated in canary grass (see text). n.s., nonsignificant.

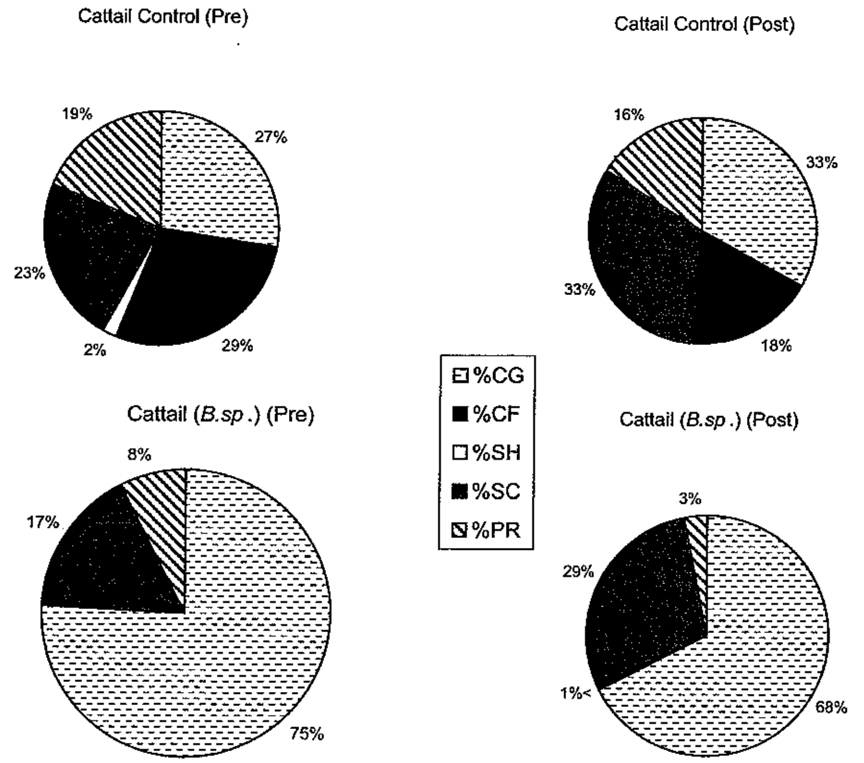


Fig. 6. Changes in mean percentage of taxa belonging to each functional feeding group in control and Vectolex®-treatment plots of cattail marsh during pre- and postapplication to control mosquitoes. (Functional feeding groups: CG, collector–gatherers; CF, collector–filterers; SH, shredders; SC, scrapers; PR, predators.)

and water depth, which varied among years due to snow melt in the spring and annual rainfall and ambient air temperatures during the summer.

Our findings are in agreement with other previously published studies conducted on the effects of *B. sphaericus* on nontarget organisms. Mulla et al. (1984a, 1984b) and Walton and Mulla (1991) found no adverse effects of *B. sphaericus* on larvae of Ephemeroptera, Odonata, and adults of Hemiptera, Coleoptera (also larvae of Dytiscidae and Hydrophilidae), ostracods, and conchostracans in the laboratory and in natural field habitats (i.e., mesocosms, field ponds, both oligotrophic and eutrophic). Studies of the effects of *B. sphaericus* on immature Odonata, Ephemeroptera, Hemiptera, and Coleoptera in lentic environments have been thoroughly reviewed by Lacey and Mulla (1990), Walton and Mulla (1992), and more recently by Lacey and Siegel (2000) and Lacey and Merritt (2003). These studies have shown no adverse effects of *B. sphaericus* on these major nontarget organisms commonly found in freshwater marshes and wetlands. Some toxicity in estuarine invertebrates (grass shrimp) due to *B. sphaericus* has been reported in laboratory assays but at fairly high concentrations (Key and Scott 1992).

Nonbiting midges of the family Chironomidae are one of the most abundant and important nontarget organisms occurring in standing-water habitats, both natural and man made. In this family, the lethal concentration to 50% of the population (LC_{50}) of *B. sphaericus* was found to be 10,000-fold the toxic dose for the target mosquito, *Culex quinquefasciatus* (Ali and Nayar 1986). Mathavan and Velpandi (1984) found field-collected *Chironomus* larvae to be totally insensitive to this microbial agent in laboratory tests. At mosquito larvicidal rates in the field, this pathogen did not adversely impact lentic populations of chironomid midges studied over a period of 48 days, during which time 2 applications were made (Mulla 1985). Therefore, it is evident from previous studies and the current one that chironomid midges and other nontarget organisms, several of these being mosquito predators, are unaffected by this microbial larvicide in natural habitats.

When Vectolex was applied at the labeled dosage rate in the above habitats in Brookfield, WI, no detrimental effects to nontarget organisms could be attributed to this microbial insecticide. Much of the variation in the control vs. treatment and pre vs. post plots can be attributed to factors other than the effects of *B. sphaericus* on nontarget organisms. To better understand if there are any detrimental effects over the long term, a better knowledge of the basic ecological interactions between the pathogen

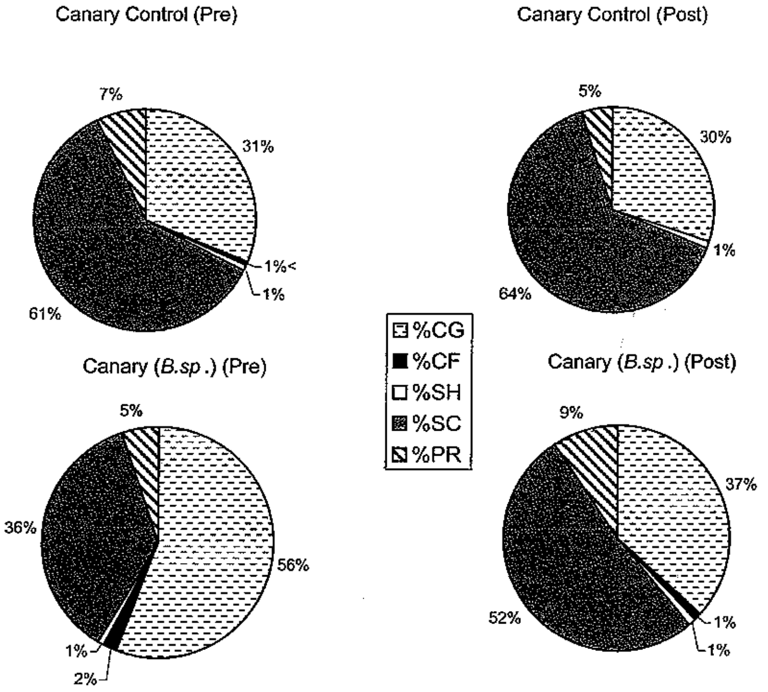


Fig. 7. Changes in mean percentage of taxa belonging to each functional feeding group in control and Vectolex®-treatment plots of canary grass during pre- and postapplication to control mosquitoes. (Functional feeding groups: CG, collector–gatherers; CF, collector–filterers; SH, shredders; SC, scrapers; PR, predators.)

and target and nontarget organisms is required. Long-term studies on the effect of *B. sphaericus* use on food-resource loss (mosquito larvae) for predators and other wildlife that specifically depend on the targeted mosquito host for food or regulation and the effect of sustained host removal on their abundance and diversity are topics for future research (Lacey and Merritt 2003).

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Appendix 1. List of invertebrate nontarget taxa collected and identified from canary grass and cattail marsh habitats in Brookfield, WI, from May 2000 through August 2002.

Taxa	Stage collected			Vegetation	
	Larva	Pupa	Adult	Canary grass	Cattail
Turbellaria	X			X	X
Oligochaeta				X	X
Lumbriculidae					
Lumbriculus terrestrias	X				X
Hirundinea					
Erpobdellidae	X			X	X
Glossiphoniidae	X		X	X	X
Isopoda					
Asellidae					
Caecidotea	X		X	X	X
Amphipoda					
Crangonyctidae					
Crangonyx	X			X	X
Gammaridae					
Gammarus			X	X	X
				X	
Physidae	X		X	X	X
Aplexa elongata	X				X
Physa			X	X	X
Physella			X		X
Lymnaeidae					
Fossaria			X	X	X
Pseudosuccinea	X			X	X
Stagnicola	X			X	X
Planorbidae			X	X	X
Gyraulus			X		X
Planorbula			X		X
Menetus			X		X
Pelecepoda					
Sphaeridae	X			X	X
Corbiculidae	X			X	X
Insecta					
Collembola					
Entomobryiidae	X			X	X
Isotomidae	X		X		X
Sminthuridae	X		X	X	X
Ephemeroptera					
Leptophlebiidae	X			X	X
Odonata					
Aeshnidae					
Aeshna	X			X	X
Anax	X			X	
Boyeria	X			X	X
Gomphaeschna				X	
Lestidae					
Lestes	X			X	X
Coenagrionidae				X	
Ischnura	X				X
Libellulidae	X			X	X
Libellula				X	
Sympetrum				X	
Decapoda					
Cambaridae					
Procambarus	X			X	X
Hydracarina	X			X	X

Appendix 1. Continued.

Taxa	Stage collected			Vegetation	
	Larva	Pupa	Adult	Canary grass	Cattail
Gastropoda					
Pleuroceridae					
Psocoptera	X			X	X
Hemiptera					
Corixidae	X			X	X
Notonectidae					
Notonecta			X		X
Pleidae			X	X	
Veliidae					
Microvelia	X				X
Trichoptera					
Polycentropodidae					
Cernotina	X				X
Limnephiilidae					
Eocosmoecus	X			X	
Limnephilus	X			X	X
Ironoquia	X			X	X
Lepidoptera					
Noctuidae	X			X	X
Pyralidae	X			X	X
Acentria	X			X	
Coleoptera					
Dytiscidae					
Agabus			X	X	X
Agabinus	X			X	X
Celina	X			X	X
Dytiscus	X		X		X
Hydaticus	X		X	X	X
Hydrovatus	X				X
Hygrotus			X	X	X
Hydroporous	X			X	
Hydroporina	X			X	
Laccophilus			X	X	
Leodessus	X			X	
Oreodytes			X	X	
Ilybius	X			X	
Rhantus			X		X
Uvarus	X		X	X	X
Gyrinidae					
Gyrinus	X			X	
Haliplidae					
Haliplus	X		X	X	X
Helophoridae					
Helophorus			X	X	X
Hydrochidae					
Hydrocus			X	X	X
Hydrophilidae					
Anacaena			X	X	X
Berosus			X	X	
Crenitus	X			X	X
Dactylosternum			X		X
Dibolocelus	X			X	X
Enochrus			X	X	X
Helochares	X			X	X
Hydrobius	X		X	X	X
Hydrochara			X	X	
Paracymus	X			X	X
Tropisternus	X			X	X

Appendix 1. Continued.

Taxa	Stage collected			Vegetation	
	Larva	Pupa	Adult	Canary grass	Cattail
Coleoptera					
Lampyridae	X			X	X
Scirtidae					
Elodes	X			X	X
Scirtes	X			X	
Curculionidae					
Lixus			X		X
Staphylinidae	X			X	X
Thinobius			X		X
Lepidoptera					
Pyralidae					
Crambus	X				X
Diptera					
Chironomidae					
Acricotopus	X			X	X
Brillia	X			X	
Chaetocladius	X			X	X
Chironomus	X			X	X
Corynoneura	X			X	X
Cricotopus/Orthocladius	X				X
Dicrotendipes	X			X	
Gymnometriocnemus	X			X	X
Larsia	X			X	X
Limnophyes	X			X	X
Orthocladius	X				X
Parachironomus	X			X	
Paramerina	X			X	
Paratanytarsus	X			X	X
Paratendipes	X				X
Phaenopsectra	X			X	
Polypedilum	X			X	X
Psectrocladius	X			X	
Psectrotanypus	X				X
Pseudosmittia	X			X	X
Tanypus	X			X	
Tanytarsus	X			X	X
Thienemannimyia	X				X
Tvetenia	X			X	
Zalutschia	X			X	
Ceratopogonidae					
Bezzia	X			X	X
Forcipomyia	X				X
Chaoboridae	X				
Chaoborus	X			X	
Mochlonyx	X			X	
Dixidae					
Dixella	X			X	X
Dolichopodidae	X				X
Ephydridae					
Scatella	X			X	X
Ephydra	X			X	X
Nostima	X			X	X
Empididae	X				X
Psychodidae	X				
Pericoma	X	X		X	X
Stratiomyidae					
Allognosta	X				X
Caloparyphus	X			X	
Odontomyia	X			X	X
Stratiomys	X			X	X

Appendix 1. Continued.

Taxa	Stage collected			Vegetation	
	Larva	Pupa	Adult	Canary grass	Cattail
Diptera					
Sciomyzidae	X			X	X
Sepedon	X			X	X
Syrphidae	X			X	
Tabanidae					X
Chrysops	X			X	
Hybomitra	X				X
Haematopota	X				X
Tabanus	X			X	X
Silvius	X				X
Tipulidae					X
Helius	X			X	
Tipula	X			X	X
Pilaria	X				X
Prionocera	X				X
Hexatoma	X				X
Limonia	X				X
Ormosia	X				X
Muscidae	X				X