Long Term Evaluation of the Effects of *Bacillus thuringiensis kurstaki*, Gypsy Moth Nucleopolyhedrosis Virus Product Gypchek®, and *Entomophaga maimaiga* on Nontarget Organisms in Mixed Broadleaf-Pine Forests in the Central Appalachians

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EXECUTIVE SUMMARY

Gypsy moth (*Lymantria dispar* (L.)) may be considered the most economically and environmentally important hardwood forest defoliator in the eastern United States. Since its early introduction into Massachusetts, it has extended its range despite great efforts to eradicate or slow its spread. Various control methods have been developed and applied through the years, including integration of these methods. Most current approaches emphasize use of pheromones, growth regulators, and biopesticides. Though the origins of the biopesticides may be natural, only when they are introduced into an ecosystem is their true impact realized.

In 1994, a USDA-funded project was initiated to evaluate potential impacts of two registered biopesticides, *Bacillus thuringiensis kurstaki* (*Btk*) and the nucleopolyhedrosis viral product, Gypchek®, on nontarget arthropods, and selected vertebrate predators. Also addressed were data gaps in the biology, life history, and nontarget effects of the gypsy moth fungal pathogen, *Entomophaga maimaiga* (Humber, Shimazu & Soper) and its interaction with the above biopesticides.

Because of the known mode of action of *Btk*, potential nontarget effects are more likely to occur to susceptible Lepidoptera larvae (caterpillars) as a direct impact, whereas indirect impacts may occur potentially to arthropod predators and parasitoids of caterpillars and farther up the food chain to vertebrates such as songbirds and salamanders that prey upon caterpillars. Including studies of indirect or secondary impacts allows for an ecosystem approach that examines interactions of a wide diversity of arthropods and their vertebrate predators.

To pursue these evaluations, eighteen 200 ha study plots were set up in the George Washington and Monongahela National Forests in Virginia and West Virginia, respectively. An initial two years of study (1995, 1996), using a variety of sampling methods, collected baseline pretreatment data including richness, abundance, and productivity of Lepidoptera, selected other insects and arthropods, songbirds, and salamanders. For the next two consecutive years (1997, 1998), *Btk* and Gypchek were aerially applied, each to six of the l8 plots; the remaining six plots served as untreated controls. Throughout the treatment years, and for the subsequent three post-treatment years (1999-2001), sampling and evaluation of nontarget impacts continued.

An earlier report (FHTET-2003-06) provided details of the methodology used in this project. Included in that document were expanded descriptions of the study plots and the survey and sampling methods. We present here the results from that project.

ACKNOWLEDGEMENTS

A project of this duration and magnitude is carried out only through the efforts of numerous individuals to whom we are indebted. Most notably, the Forest Health Technology Enterprise Team's logistic support and advisory activities have been crucial. Our main contact, Richard C. Reardon, has been available whenever needed and took the lead in coordinating Monongahela National Forest treatment applications on the study plots. Input and support from Allan T. Bullard and John D. Stein have been most helpful. An early review of the project by a team assembled by the Forest Service provided valuable input. This review team included John Hazel (USDA-FS), Jeffrey Miller (Oregon State University), William Schneider (US-EPA), and William Laudenslayer (USDA-FS).

Logistic support on the national forest study plots came from David Rhodes, Tom Lail, and Mike Gallegly from the Deerfield Ranger District, VA; Pat Sheridan from the Greenbrier Ranger District, WV, and Cindy Schiffer and Oreana White from the Marlinton Ranger District, WV. Treatment applications on the George Washington were supported by Jeffrey Witcosky, Dee Dee Sellers, and Teddy Mullins of the USDA-FS. David Curry performed the gypsy moth egg mass surveys. Early statistical guidance was provided by Edwin Townsend (West Virginia University) and Sylvia Mori (USDA-FS).

Graduate students in many ways have been the backbone of the project, and we thank them for their hard work and leadership. For the entomological work, vegetation and soil sample site studies and *Entomophaga maimaiga* field sampling we thank Toby Petrice, Christine D. Plaugher, Terry Carrington, Michael Whitman, Rachel Braud, Karen Kish, Sandra Raimondo, Kenneth E. Rastall, and Changlu Wang, all of West Virginia University. For the ornithological and plot level vegetation work we thank University of Georgia graduate students Carrie A. Straight, Lars Pomara, and Tim S. Keyes as well as W. Russ McClain, University of Memphis. For the salamander portion of the study we thank Marshall University graduate students Nancy Dickson, Zachary Felix, Robert Fiorentino, Jeffrey Humphries, Keith Johnson, Sandra Kilpatrick, Brian Lindley, Andrew Longenecker, Adam Mann, and Beth Anne Pauley.

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CHAPTER 1. PROJECT DESCRIPTION

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INTRODUCTION

Background for Project

From its point of introduction in Massachusetts, gypsy moth (*Lymantria dispar* (L.)) spread over a wide area of the United States despite control efforts. By the initiation of this project in 1994, infestations were established through all of New England, as far south as Virginia, and west into Michigan (Figure 1).

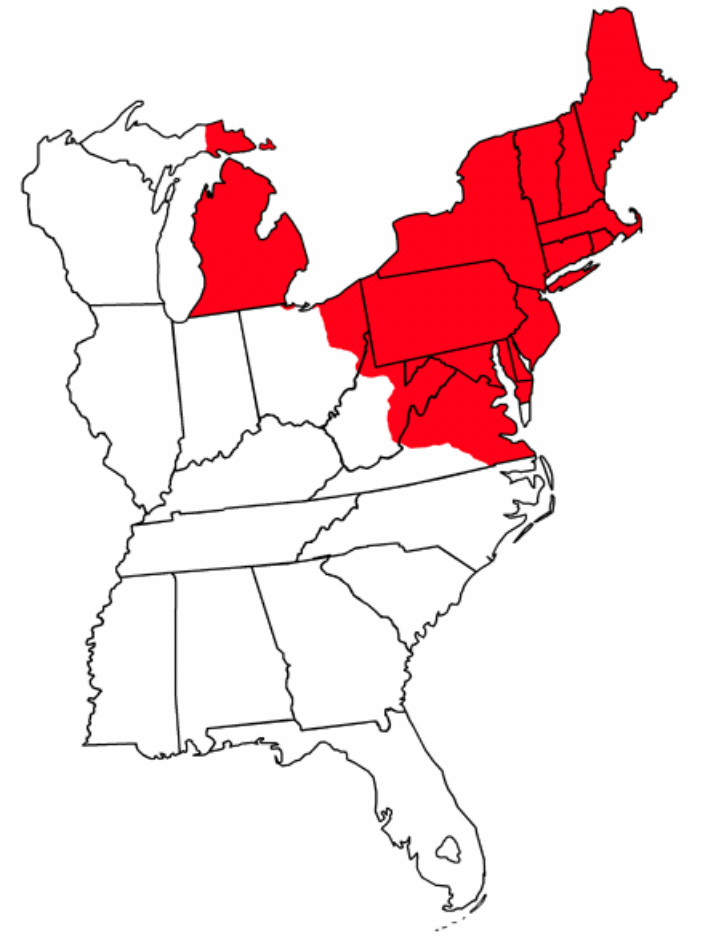

Figure 1. Area of gypsy moth infestation in 1994 (after Liebhold et al. 1997a).

During the 1970's, as an integrated approach to pest control began to gain momentum, various agencies initiated research to develop tactics other than chemical insecticides. In 1983, the Maryland Gypsy Moth Integrated Management Pilot Project was begun by the USDA Forest Service to continue the development and field evaluation of these non-chemical tactics across a several county area in Maryland (Reardon et al. 1993). The encouraging results of this study

supported a broader initiative that began in 1987, the Appalachian Integrated Pest Management (AIPM) project for gypsy moth (Reardon 1996). This demonstration project was congressionally mandated to involve larger geographical areas including more mountainous terrain.

The environmental impact statement prepared for the AIPM project identified a number of data gaps concerning the impacts of treatments on nontarget organisms (USDA 1989). The direct and indirect effects of the bacterium *Bacillus thuringiensis kurstaki* (*Btk*)(Foray® 48F) and the insect growth regulator diflubenzuron (Dimilin®) on nontarget organisms ranked highest in needing additional documentation. In fact, of the 11 data gaps listed in the AIPM Environmental Impact Statement, five related to nontarget impacts. In 1991 the USDA Forest Service created a review team comprised of scientists from government agencies, universities, and environmental groups to assist in defining the broad outline of a nontarget project to address some of these data gaps.

Based on the review team's recommendations, funds were appropriated for a project to determine nontarget impacts of *Btk* over a larger geographical area and with adequate pretreatment, treatment, and multiple years of post-treatment evaluation. A solicitation for proposals was advertised in the Commerce Business Daily as well as in the Newsletter of the Entomological Society of America. In 1993, the review team selected from 15 formal proposals the one from a team led by Linda Butler of West Virginia University.

In the original study plan (1994) equal emphasis was placed on studying the potential nontarget impacts of *Btk* and gypsy moth defoliation. In 1995 during the first year of fieldwork, gypsy moth populations within some study plots and the surrounding area began declining due to the gypsy moth fungal pathogen, *Entomophaga maimaiga* (Humber, Shimazu & Soper). Gypsy moth populations continued to decline in 1996 with only localized defoliation occurring which necessitated modification and diversification from the original study plan. This population decline of gypsy moth in the mid-Atlantic region occurred on the southern edge of the fungal epizootic that was first recorded in the northeast in 1989 and had rapidly spread throughout much of the contiguous gypsy moth infested area (Hajek et al. 1995a).

A revised study plan was submitted and approved by the review team in 1996 that incorporated the original objective of determining the potential nontarget impacts of multiple year applications of *Btk* and substituting other objectives for the infeasible defoliation treatment. Now included was a more detailed examination of potential nontarget impacts of the gypsy moth nucleopolyhedrosis virus product Gypchek® and a detailed field study of *E. maimaiga*. Gypsy moth continued to be present on study plots for the duration of fieldwork, but no significant defoliation occurred to confound the design of the revised study plan.

The development of this project, further details of its study design, and the study site characteristics are described by Strazanac et al. (2003).

Gypsy Moth, *Lymantria dispar*

Gypsy moth is the most important hardwood forest defoliator in eastern North America (Doane and McManus 1981), causing significant mortality to forest and ornamental trees (Campbell and Sloan 1977). Its caterpillars prefer some of the most widely distributed trees and shrubs in North America including oaks (*Quercus* spp.), aspens and poplars (*Populus* spp.),

birches (*Betula* spp.), larches (*Larix* spp.), hawthorns (*Crateagus* spp.), and alders (*Alnus* spp.) to name a few. Hundreds of other host species are acceptable by late instar caterpillars or at dense caterpillar population levels (Leonard 1981). Liebhold et al. (1997b) estimated the potential distribution of gypsy moth in the United States based on host distribution and proportion of favored host species in forest stands (Figure 2).

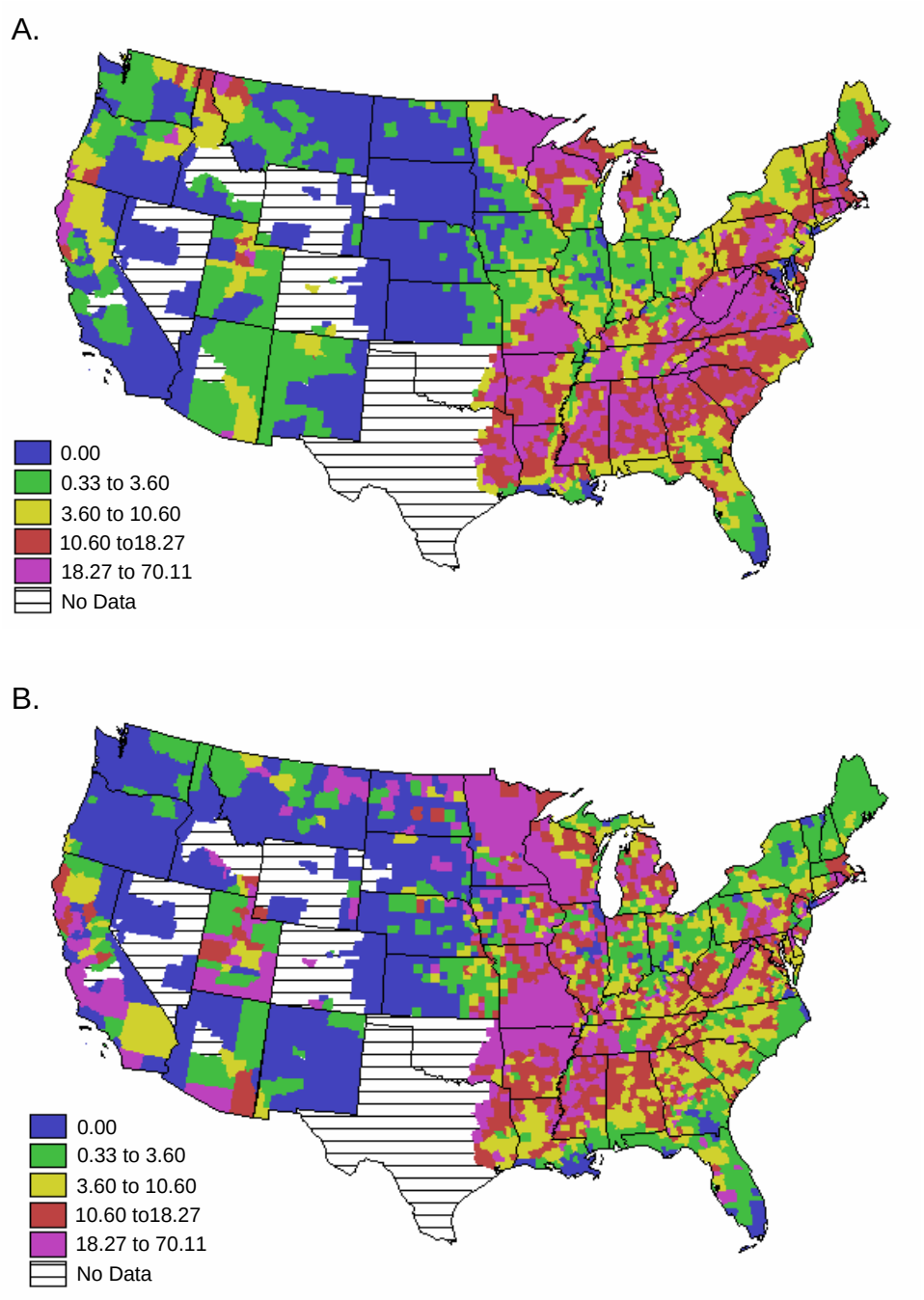

Figure 2. A. Total basal area of preferred hosts of gypsy moth. B. Proportion of basal area of trees preferred by gypsy moth caterpillars for feeding (after Liebhold et al. 1997b).

Gypsy moth goes through one generation per year with eggs laid during the previous mid-summer and hatching in April to May. First instar larvae may disperse through ballooning in the wind, and then settle and feed for several weeks. In the mid-Atlantic region larvae mature by late June or early July, and pupate. Adult moths emerge in about two weeks (Doane and McManus 1981).

Gypsy moth population dynamics tended to be bimodal with an innocuous mode having low populations and an outbreak mode (Campbell and Sloan 1978). The innocuous mode is of indefinite length, possibly kept in check by a number of direct or indirect factors including natural enemies, weather, host availability and condition, or possibly mast availability (Liebhold et al. 2000). During outbreaks, runaway population growth can cause multi-year defoliation resulting in significant tree mortality which is often the result of weakened trees being more susceptible to secondary pest attacks (Wargo 1977, Muzika et al. 2000).

Over time, methods to control or eradicate gypsy moth have included a wide array of inorganic, synthetic organic, and biologically based insecticides, introductions of natural enemies, and mating disruption with sex pheromones. In more recent years, suppression efforts have emphasized the aerially applied biopesticides *Bacillus thuringiensis kurstaki* and nucleopolyhedrosis virus, and the insect growth regulator diflubenzuron. The latter has been documented to produce broader nontarget impacts than those of microbial insecticides (Butler et al. 1997, Reardon 1995). The most recently documented gypsy moth natural enemy that has produced broad regional impacts is the entomopathogenic fungus, *Entomophaga maimaiga*. It has been very effective in reducing gypsy moth numbers, however its interaction with other control techniques is only now beginning to be thoroughly studied.

Bacillus thuringiensis kurstaki

Bacillus thuringiensis is a common, naturally occurring entomopathogenic bacterium found associated with numerous insect species worldwide (Martin and Travers 1989). More than 30 varieties (serovars) have been identified and most of these are pathogens of Lepidoptera (de Barjac and Frachon 1990). The *B. thuringiensis* serovar *kurstaki* (*Btk*) was first isolated by Kurstak in 1962 and noted to be effective primarily against Lepidoptera (de Barjac and Lemille 1970). Dulmage (1970) isolated a much more potent strain of *Btk* and coded it HD-1. This is the strain used in most formulations for the control of lepidopteran pests. The formulation used in this study was selected from the HD-1 strain for its higher activity to control gypsy moth and is available as Foray® 48F. Developed and produced by Abbott Laboratories Incorporated, Foray® 48F is now marketed by Valent Biosciences Corporation.

The toxicity of *Btk* lies in the spores and unique bipyramidal shaped crystalline proteins referred to as the crystal. The proteins that make up the crystal are called delta-endotoxins and are formed along with a newly formed spore during sporulation. When ingested, the crystal proteins dissolve in an alkaline insect gut system causing the lysis of gut cells and eventual rupture of the gut walls (Reardon et al. 1994).

Unlike the following two pathogens, *Btk* has never been observed to naturally cause epizootics (Reardon et al. 1994) because once an infected caterpillar dies it typically drops to the ground

spreading spores or crystals only into the soil when it decomposes. Thus, *Btk* must be applied as a conventional stomach-poison insecticide annually (Dubois et al. 1988). *Btk* is broader in its effect on native lepidopterans (Peacock et al. 1998) compared with the following viral and fungal pathogens.

Gypchek® (Gypsy Moth Nucleopolyhedrosis Virus)

A member of the virus genus *Baculovirus*, the gypsy moth nucleopolyhedrosis virus has been known since the beginning of the last century (Glaser and Chapman 1913). It is often referred to as "wilt" because the caterpillars killed by the virus are soft and limp. Virus collected from a Hamden, Connecticut gypsy moth population (Hamden strain) is the active ingredient in the Gypchek product used in this study. Limited testing of the gypsy moth nucleopolyhedrosis virus indicates a narrow host range with no known direct adverse effects on beneficial insects or vertebrates (Barber et al. 1993, Reardon et al. 1996).

Under natural conditions, within a dense population of gypsy moth the nucleopolyhedrosis virus may become virulent producing an epizootic. Whereas *Btk*-infected caterpillars die and generally drop to the ground taking potential inoculum with them, caterpillars infected by the virus tend to remain in the trees and die at their resting spots. Viral inclusion bodies are released by these dead, infected caterpillars, onto canopy foliage where they are consumed, along with the foliage, by other gypsy moth larvae. Once ingested they dissolve in the gut releasing rod-shaped virus particles or virions. The virions attack the gut wall, eventually entering the hemocoel infecting other tissues and organs, creating a general infection. As the virus quickly multiplies, many of the internal organs of the caterpillars break down causing death. In dense populations, epizootics will usually reduce gypsy moth populations to an innocuous level.

After epizootics the virus may persist in the soil, on bark, and leaf litter for at least one year (Podgwaite et al. 1979). Therefore, the virus has the potential to re-infect gypsy moth populations from year to year but more likely follows a host density-dependent model (Woods and Elkinton 1987). Transmission can occur when eggs are laid on contaminated bark (Doane 1975), or directly through injection by gypsy moth parasitoids (Lautenschlager and Podgwaite 1979, Raimo et al. 1977), or can be passed and dispersed by birds and mammals (Lautenschlager and Podgwaite 1979).

Along with the following fungal pathogen, the gypsy moth nucleopolyhedrosis virus is highly host specific and is not considered closely related to any known human pathogen (USDA 1995). Gypchek is the preferred treatment in environmentally sensitive areas because of its selectivity.

Entomophaga maimaiga

The Asian fungus, *Entomophaga maimaiga* (Humber, Shimazu & Soper), was first found to be infecting North American gypsy moth caterpillars in several northeastern states in 1989 (Andreadis and Weseloh 1990, Hajek et al. 1990). The source of the fungal strain has never been determined (Hajek et al. 1995a, Hajek 1999). Aided by releases in several states (Hajek et al. 1996a), but primarily through the natural movement of wind borne spores (conidia), the fungus spread rapidly through established gypsy moth populations in many northeastern, north central,

and mid-Atlantic states. In many areas, this resulted in dramatic epizootics, collapsing existing or building gypsy moth populations.

Throughout the life cycle of *E. maimaiga*, two different types of spores are produced, conidia (asexual spores) and resting spores (azygospores). When gypsy moth caterpillars die from the fungal infection, the conidia are actively ejected from the host cadavers (Hajek and Shimazu 1996). These conidia are short-lived but are capable of moving considerable distances on the wind, spreading infection (Hajek 1999). Resting spores are produced within host cadavers and are the resistant form of the fungus, facilitating overwintering of the fungus, and capable of persisting in soil and litter for many years in the absence of a host (Hajek and Shimazu 1996).

To produce infection in the caterpillar, infective fungal spores must contact the host cuticle and by some method, not fully determined for this group of fungi (Hajek 1999), penetrate into the host body. As the infection progresses, the density of *E. maimaiga* cells increases within the host and nutrient depletion results. While infected caterpillars may appear to be normal until a short time before death, they have been found to eat less in their last two days of life (Hajek 1989). The type of spore formed after host death is determined by various host-related factors and environmental conditions. Host instar, host molting status, fungal dose, temperature, and humidity have all been found to influence spore type (Hajek 1999).

While another pathogen, the gypsy moth nucleopolyhedrosis virus, tends to follow a density-dependent model (Woods and Elkinton 1987) in which the virus produces an epizootic and collapses host populations when they are at high densities, *E. maimaiga* appears to have no association, or only a weak association, with gypsy moth density (Hajek 1999). Accordingly, an epizootic may be produced in a small, building population of gypsy moth as long as infective spores are sufficiently abundant and environmental conditions (i.e., humidity, rainfall) are suitable (Hajek 1999). Following a fungal epizootic, high numbers of resting spores are found in soil and litter (Hajek 1999) where they may persist for many years (Weseloh and Andreadis 2002).

As *E. maimaiga*, recognized as a highly virulent pathogen, was spreading rapidly in this country and affecting population dynamics of gypsy moth, concern was expressed as to its host specificity (Reardon and Hajek 1993) and several studies were conducted. A laboratory study challenging 78 species of nontarget caterpillars with high doses of spores found that about a third of the species could become infected (Hajek et al. 1995b). Subsequent preliminary studies indicated that actual abundance of infected nontarget caterpillar species collected in the field was extremely low, whether the caterpillars were sampled from foliage and tree bands (Hajek et al. 1996b) or from litter beneath trees (Hajek et al. 2000).

This fungus is now a part of the natural history of forests within contiguous gypsy moth-infested states of eastern U. S. and the Great Lakes region. It has dramatically influenced gypsy moth populations and will certainly have an influence on populations of gypsy moth natural enemies and, potentially, on populations of some nontarget Lepidoptera. When *E. maimaiga* moved into our study plots in 1995 and 1996, we were provided an opportunity to collect data to help answer some outstanding questions.

NONTARGET PROJECT

Broad Null Hypothesis: Neither consecutive multiple applications of *Bacillus thuringiensis kurstaki* and Gypchek, nor the naturally occurring *Entomophaga maimaiga* fungus, nor the interaction of the three microbials will cause negative impacts on arthropods, birds, or salamanders on study plots.

Objectives

1. To collect baseline data on Lepidoptera and other selected herbivorous, predaceous, and parasitic arthropods, songbirds and terrestrial and aquatic salamanders on plots representing gypsy moth susceptible forest type in the George Washington and Monongahela National Forests.

2. To evaluate the impact of two sequential yearly applications of *Bacillus thuringiensis kurstaki* (as Foray® 48F) and Gypchek and their interactions with the fungus *Entomophaga maimaiga* on the herbivorous, predaceous, and parasitic arthropod communities and selected insect pollinators and to evaluate the impact of arthropod perturbations on selected species of songbirds and terrestrial salamanders.

3. To identify the best indicator communities or species among the herbivorous, predaceous, and parasitic arthropods, and pollinating insects for evaluation of impacts of *Btk* and Gypchek.

4. To evaluate the relationship between humidity/temperature factors, abundance of *E. maimaiga* resting spores and gypsy moth populations, and to evaluate impact of the fungus on nontarget Lepidoptera.

3. To identify the best indicator communities or species among the herbivorous, predaceous, and parasitic arthropods, and pollinating insects for evaluation of impacts of *Btk* and Gypchek. To develop recommendations for Federal and State Cooperative Suppression, Eradication, and Slow-the-Spread projects to minimize impacts to nontargets.

Study Design

In 1994, eighteen 200 ha (500 acres) study plots were established, equally divided between two locations in the George Washington and Monongahela National Forests representing gypsy moth susceptible habitat. The projected time-line for the study on these plots included two years of baseline data collection, two consecutive years of application of microbial insecticides, and a minimum of three years of post-treatment data collection. Based on surrounding gypsy moth population densities, it was estimated that the insect would enter the plots and begin producing defoliation within two years after the study was initiated. The study design planned for application of *Bacillus thuringiensis kurstaki* (as Foray® 48F) and Gypchek, each to six of the 18 plots with the remaining six plots serving as controls with no treatment. The treatments were to be applied in a randomized block design based on vegetation type (see Chapter 2 for vegetation analysis) determined when the plots were first established. The 18 plots were divided

into three blocks in each of the National Forests, each block containing the three treatments randomly assigned. This design was recommended by the U. S. Forest Service and supported by E. C. Townsend, West Virginia University Experiment Station statistician. The total number of plots was based on availability of funds.

Within each plot a 30 ha (75 acre) subplot was established for the monitoring of arthropods, birds, and salamanders, and other data acquisition. These subplots were 600 m by 500 m and along their length six 600 m parallel transects spaced 100 m apart were flagged at 25 m intervals to conduct bird, vegetation, and gypsy moth egg mass surveys (Figure 3). The Universal Transverse Mercator (UTM) coordinates for all points along the transects were determined using a Global Positioning System and SONIN® electronic distance recorders (SONIN, Brewster, New York, USA). Additional sample sites and transects were established within each plot for intensive arthropod, bird, and salamander monitoring.

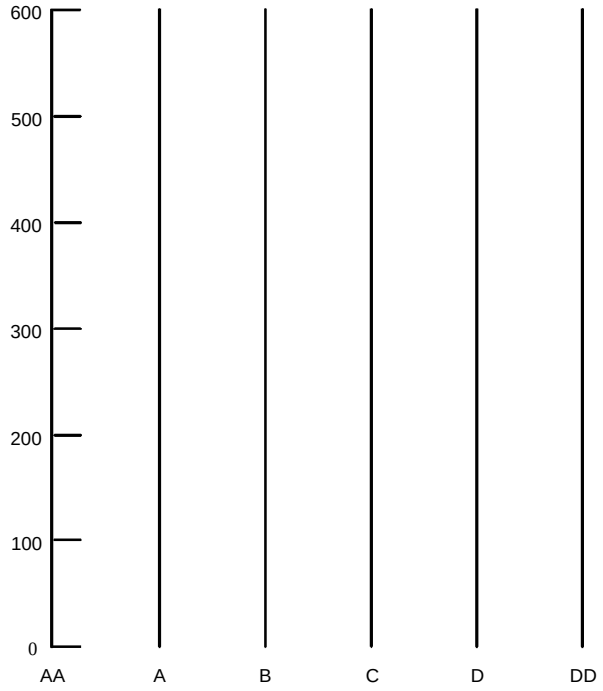

Figure 3. Layout and designations of transects on 600 m by 500 m subplots.

Study Location

Locations for study sites were selected in the George Washington National Forest in Virginia, and the Monongahela National Forest in West Virginia (Figure 4). The nine plots located in the George Washington National Forest were in the Deerfield Ranger District, on the southeast portion of Great North Mountain. The George Washington National Forest plots represent a xeric forest of mixed oak and pine. The greatest distance between the subplots is 16.4 km with a midpoint of 38° 6' 17" N and 79° 22' 8" E.

The remaining 9 plots were located in the Greenbrier and Marlinton Ranger Districts in the Monongahela National Forest. All sites are predominantly mixed oak forests with some pine, and

are more mesic than the George Washington National Forest plots. The greatest distance between subplots is 27.5 km with a midpoint of 38° 18' 12" N and 79° 51' 54" E. The distance between the midpoints of the study plots in the George Washington and Monongahela National Forests is 49.5 km.

Figure 4. Study plot locations and numbers on the George Washington and Monongahela National Forests.

Priority Nontarget Organisms

The selection of nontarget organisms for inclusion in this study was based on their likelihood of having observable primary (direct) or secondary (indirect) effects as the result of the *Btk* and Gypchek treatments. Primary or direct nontarget impacts may result if exposure to the treatments produces either a positive or negative effect. Based on what is known of these biopesticides and their aerial application for target gypsy moth caterpillar control, nontarget caterpillar populations feeding on foliage shortly after treatment may receive some type of direct negative impacts.

Secondary or indirectly impacted nontarget organisms may experience either a positive or negative effect as the result of the direct impact on foliage feeding caterpillars. A positive secondary effect may be felt by other primary herbivores of canopy foliage at the same trophic level, as competition from susceptible herbivores is reduced. Release from competition with caterpillars may not be noticeable however, since most caterpillars tend to be widely and sparsely distributed on foliage, so much so that defoliation is usually not readily noticeable except during outbreaks.

A positive effect at a higher tropic level is possible if weakened caterpillars become more vulnerable to predators and parasites. Also, caterpillars that are killed by treatments could create a temporary positive effect as a "windfall" of food items benefiting opportunistic organisms like carrion feeders and omnivores. These benefits may be felt especially by the secondarily impacted organisms that have a single year life cycle and have a critical feeding period shortly after spray treatments. Depending on the impact of the first year treatment on univoltine (one generation per year) caterpillar species and the recovery of multivoltine (multiple generations per year) caterpillar species, the second year of treatment may or may not produce a "windfall" of food items.

Treatments for gypsy moth are applied in early spring when their caterpillars are young. Many nontarget caterpillars are also early instars, and therefore small, at the time of treatment. Predators that utilize small prey or parasitoids that only attack young caterpillars may suffer immediate secondary negative impacts. Treatments affecting young animals not only reduce their abundance but their ultimate contribution to biomass. Removing caterpillars from the food web early in the season obviously prevents them from growing into more sizable food items. A reduced abundance of multivoltine caterpillars may also limit their contribution in later generations to biomass within the same year. The two consecutive years of treatments may have cumulative negative secondary impacts also.

A negative secondary or indirect impact may be most noticeable in organisms that require live, healthy caterpillars on which to feed and/or depend on caterpillars as their primary food resource over a period of time. Forest canopy caterpillars are a primary food source for many other forest animals; and these are the secondary nontargets of greatest emphasis in this study.

Priority nontarget organisms emphasized in this study include Lepidoptera larvae, that may receive a direct negative affect; competing, potentially non-sensitive, herbivore sawfly larvae; non-sensitive predators such as ground beetles, spiders, selected songbirds and salamanders; and wasps and flies parasitic on caterpillars that may receive indirect negative affects.

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CHAPTER 2. SITE CHARACTERISTICS

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PHYSICAL

Topography

The centers of the George Washington (GWNF) and Monongahela National Forests (MNF) plots were 49.5 km apart. The GWNF plots and the MNF plots are in the Allegheny Mountain section and in the Northern Ridge and Valley section, respectively (Keys et al. 1995). These sections are geographically adjacent. The GWNF plots are on the southeastern slope of Great North Mountain (Figure 5), while the MNF plots are in groups of threes on separate mountains (Figures 4 & 6). The two study areas were in different watersheds; the GWNF plots in the James River watershed and the MNF plots in the Greenbrier watershed. The main surface water drainages are somewhat different in that the GWNF has a more parallel network of drainage, while the MNF is deeply dissected. All plots had perennial streams on or near the subplots and were of a medium gradient. Seasonal streams and small washes were common on the plots.

Figure 5. Great North Mountain (foreground) in the George Washington National Forest; location of the Virginia study plots.

Figure 6. View towards Marlin Mountain (background) in the Monongahela National Forest; location of some of the West Virginia study plots.

The subplots in the MNF were generally higher in elevation than those in the GWNF, and based on 28 evenly spaced points on each plot along the four central transects, the MNF terrain is more variable in slope and aspect. The MNF plots ranged in elevation from 800 to 1300 m, whereas the GWNF plots ranged in elevation from 400 to 900 m. Slopes were generally steeper on the MNF subplots with a mean slope of 22°, ranging from 1° to 90°, compared to the GWNF subplots with a mean slope of 20°, with a range of 0° to 54°. The MNF and GWNF subplots had a similar range of aspects, 4° to 364° and 3° to 354° respectively. Points along the transects on the MNF plots most often faced a southerly direction, and, least often towards an easterly direction (Figure 7). The GWNF transect points most often faced a southerly direction, and, least often, in a northerly direction. The above description can be applied to the subplots, but also indicate the general topography of the plots.

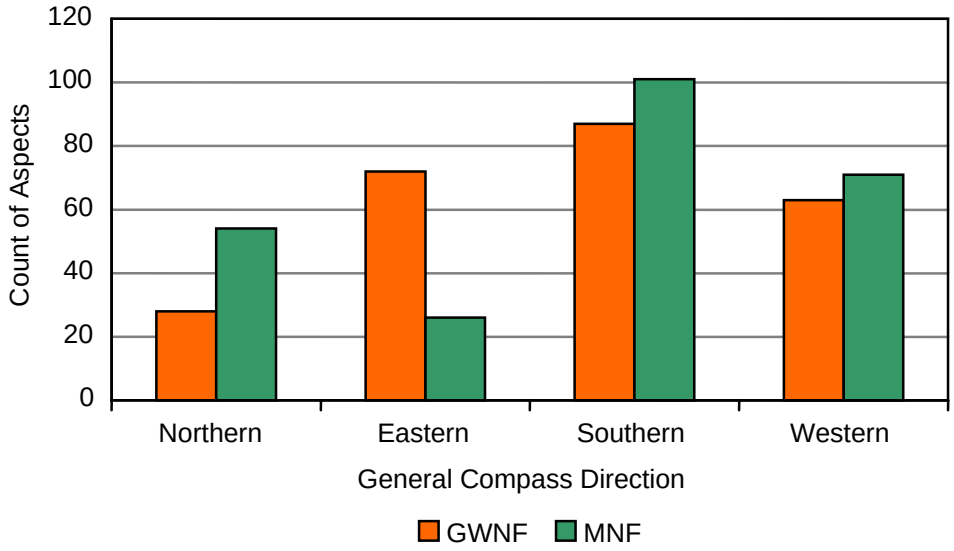

Figure 7. Counts of aspects taken at 25 m points along subplot transects, grouped by general direction on study subplots for George Washington (GWNF) and Monongahela (MNF) National Forests.

Soil Characteristics

The formation of soils, or pedogenesis, in moist temperate deciduous-coniferous forests where our study plots were located produces distinct layers or horizons by intense leaching of soluble salts and metals, clays, and organic material. The mineral soil thus formed has some level of acidity. The MNF and GWNF share some soil characteristics (Flegel 1998, Hockman et al. 1979). The formation of clay and its translocation by leaching forms a lower soil horizon enriched in clay. The upper horizons of these forest soils in their natural state are generally not considered rich in nutrients for plant growth compared with other soils, especially those for agricultural purposes. Though typically moist, the soil can dry out in the upper horizons during summer months, especially along ridges and upper south facing slopes. A perpetual layer of leaf/needle litter guarantees a distinct humus layer in forests.

A survey of the upper soil was performed at the arthropod pitfall sites. This consisted of taking six 3-inch deep and 3-inch diameter soil cores and analyzing them in the laboratory. These samples confirmed the general acidity of the soils, clay content, and abundant humus as expected. The pitfall sites represented a variety of soils. The main soil series encountered in the MNF were Weikert, Macove, and Calvin (Flegel 1998) and in the GWNF, Monongahela, Berks, Craigsville, Hazleton, and Leetonia (Hockman et al. 1979).

CLIMATE & WEATHER

Regional Climate

The Appalachian Mountains are about 2,400 km in length and parallel the Atlantic coastline. Because of their great length and relatively low elevation, their climate is generally more influenced by latitude than elevation as indicated by the fact that their northern and southern regions are mostly impacted by weather systems from different origins (Whiteman 2000).

Elevations in the Appalachian Mountains are high enough to create a slight rain shadow effect. This is a result of weather systems in eastern North America usually moving in a general southeasterly direction, cooling and losing precipitation as they go over the mountains and then being drier and warmer as they drop down over the lower eastern ridges. As a result, the western ridges leading to the highest elevations of the Appalachian Mountains tend to be more mesic than the eastern ridges. This can be seen in monthly precipitation normals (Owenby and Ezell 1992) averaged from a 30 year period from NOAA cooperative weather stations near the two study areas (Figure 8). Although the two study areas share similar precipitation patterns, the MNF study area which is west of the highest elevations receives more precipitation than does the GWNF study area to the East.

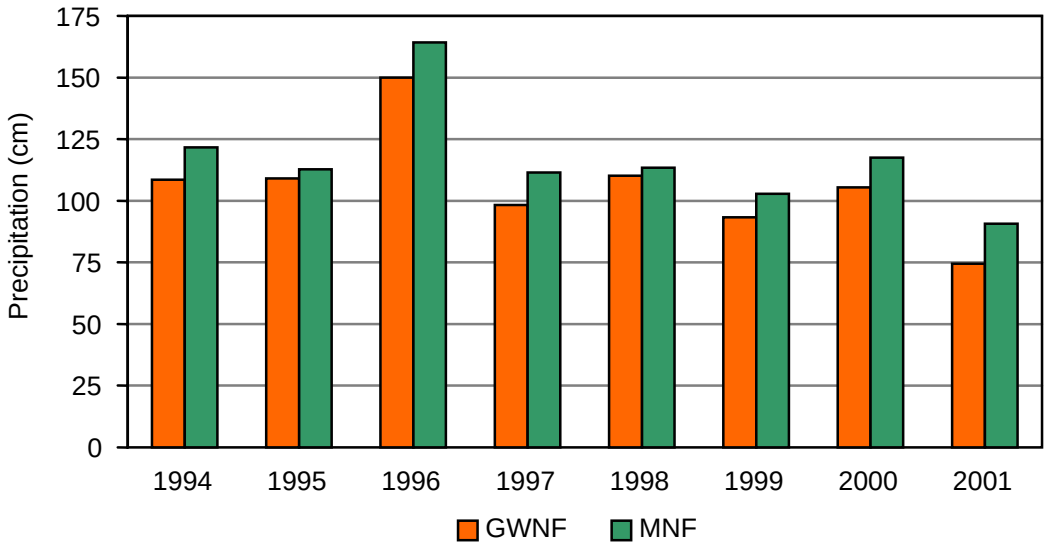

Figure 8. Precipitation normals near the George Washington (GWNF) and Monongahela (MNF) National Forests study areas based on NOAA weather station 30-year normals data. MNF data is from Buckeye, WV and GWNF data is an average of four stations.

Monthly maximum and minimum temperature normals give smooth curves representing the transitions between winter and summer climates (Figure 9). As expected the higher MNF study area is cooler than the GWNF study areas throughout the year, but the difference within a given month is only a few degrees.

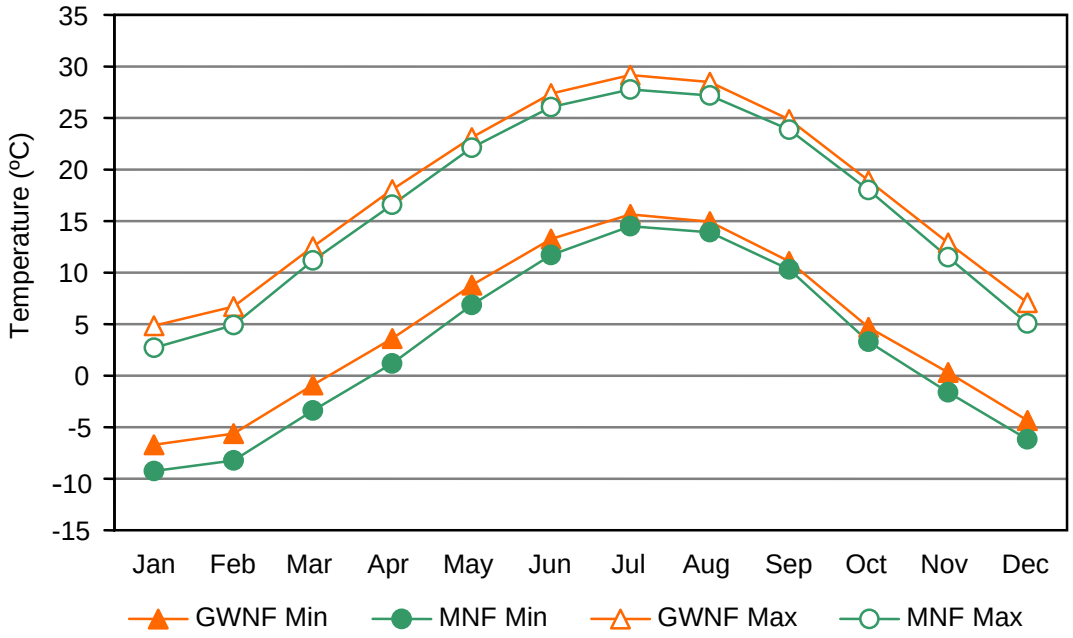

Figure 9. Temperature minimum and maximum normals near the George Washington (GWNF) and Monongahela (MNF) National Forests study areas. MNF data is from Buckeye, WV and GWNF data is an average of three stations.

Regional Weather

Data on daily precipitation and temperature extremes were obtained from NOAA cooperative weather stations near the plots. These data supplemented that collected on-plot during the field collection season and for the year prior to the study.

Data from NOAA cooperative weather stations was used to monitor daily precipitation and temperature extremes during the periods when our weather stations were not in the field. NOAA stations near the study areas were selected to represent various elevations and also to obtain recorded data for the entire period of fieldwork. The on-plot weather stations had a rain gauge and a minimum/maximum thermometer and were monitored weekly for 15 weeks starting in mid-May each year.

Annual precipitation was consistently higher near the MNF study area as would be expected (Figure 10). The highest amount of annual precipitation was experienced in 1996 and the least in 2001. With the exception of 1996, annual precipitation appears fairly consistent from year to year.

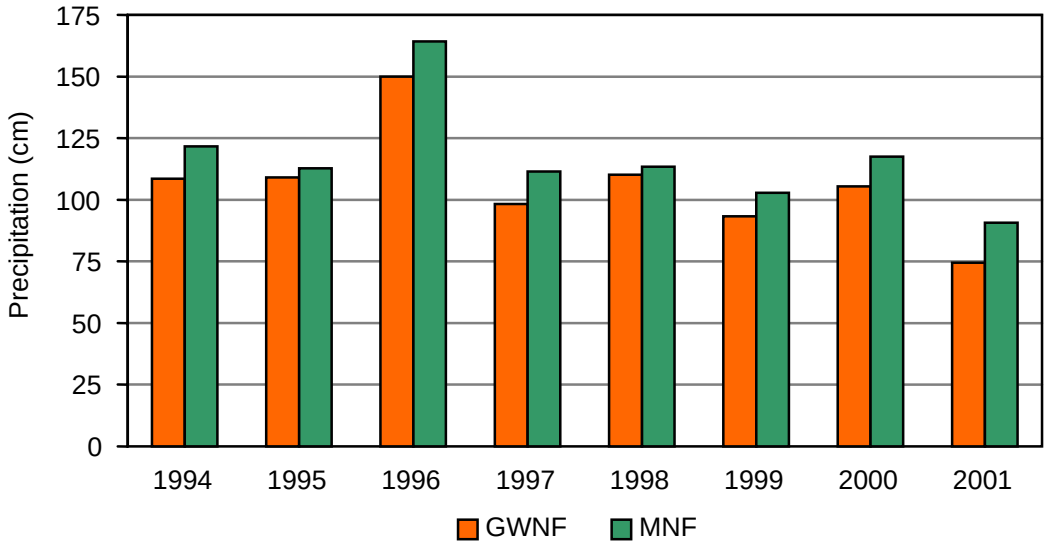

Figure 10. Annual precipitation based on NOAA cooperative weather stations data for the George Washington (GWNF) and Monongahela (MNF) National Forests study areas.

During the 15-week sampling periods each year the highest precipitation totals occurred in 1995 and 1996, while the lowest totals occurred in 1999 (Figure 11). With the exception of 1995, the MNF study plots received more or similar amounts of precipitation compared to the GWNF study plots. The higher level of precipitation in 1995 for the GWNF study plots is mostly the result of a severe storm that released 10 cm more rain on the GWNF plots than the MNF plots within a two day period (Figure 12). Without this storm, the rainfall for the 1995 15-week period on the GWNF study plots would be similar to that of other years. In contrast, the relatively higher combined 15 weeks of precipitation in 1996 was spread out more evenly over the season. Although 1999 had similar annual precipitation compared to other years based on NOAA weather data, the combined and weekly precipitation readings taken on the plots indicate a relatively much drier period during sampling.

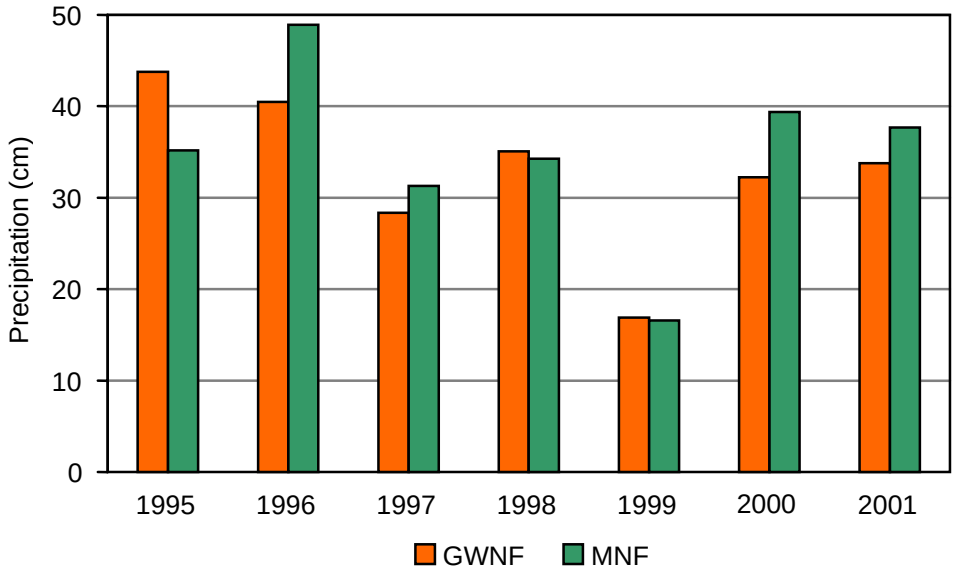

Figure 11. Combined 15-week precipitation for 1995 to 2001 on the George Washington (GWNF) and Monongahela (MNF) National Forests.

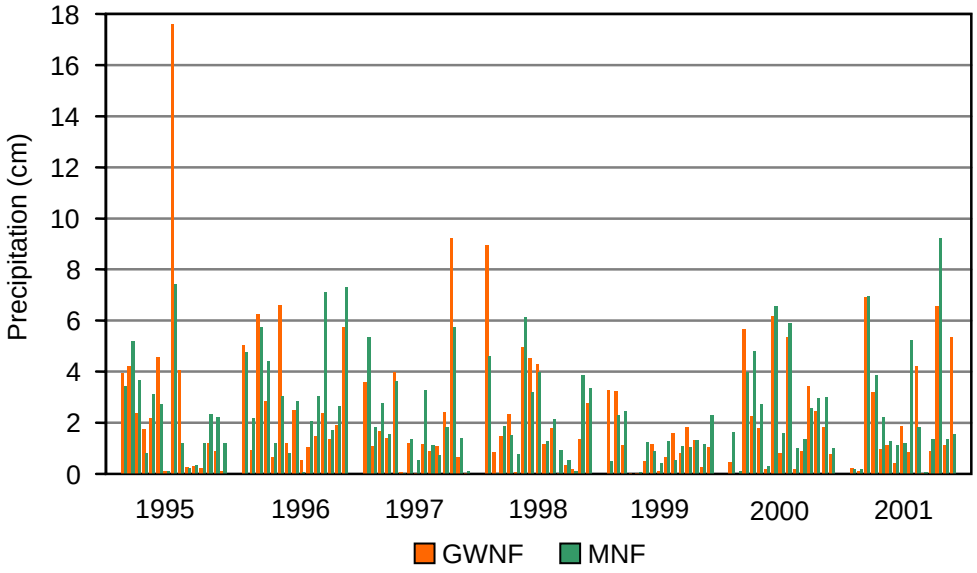

Figure 12. 15-weekly precipitation observations for 1995 to 2001 on the George Washington (GWNF) and Monongahela (MNF) National Forests.

The two study plot areas shared similar fluctuation patterns of minimum and maximum temperature readings during the 15-week monitoring period each year (Figure 13). Minimum temperature reading patterns shared a general warming trend each year in both study areas typical of temperate summers in the northern hemisphere. This seasonal trend is not apparent in the maximum temperature readings. In fact, there is no obvious trend or pattern between seasonal maximum temperature readings year to year. As expected though, the GWNF study plots were regularly warmer than the MNF study area, but only by a few degrees.

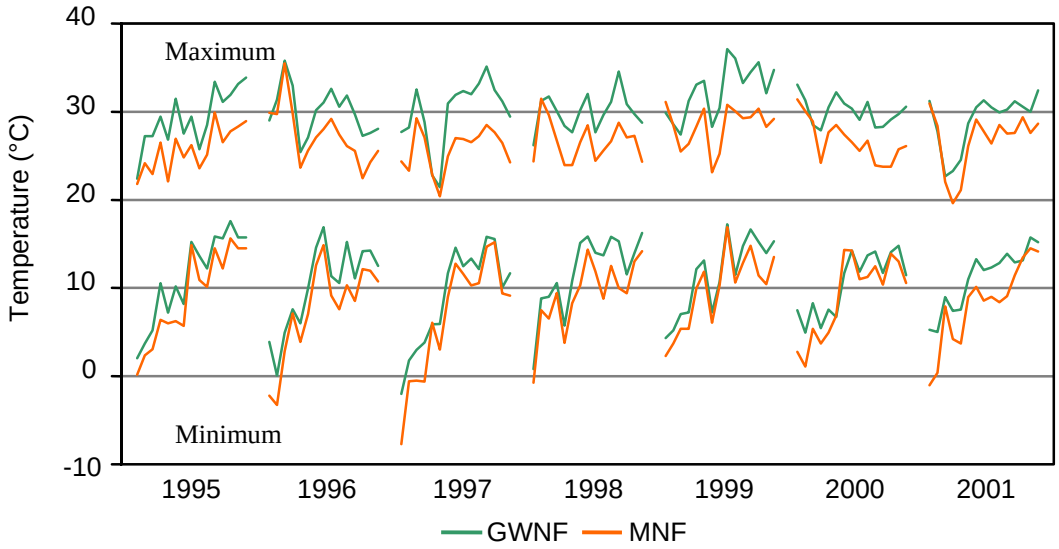

Figure 13. Temperature by week during the 15-week sampling periods for 1995 to 2001 on the George Washington (GWNF) and Monongahela (MNF) National Forests.

VEGETATION

Regional Overview

The study sites lie within the eastern humid temperate region of North America. Although they are in the hot continental subdivision, the study areas are at higher elevations and are relatively cool for this designation (Bailey 1995). The "cool" hot continental division coincides with the boundaries of the central Appalachian broadleaf and coniferous forests province (McNab and Bailey 1994) (Figure 14). This is prime gypsy moth susceptible habitat near the middle of the central Appalachian hardwood forests dominated by oaks (Beltz et al. 1992). Throughout this region species of hickories, pines, and maples are also common co-dominate trees (Keys et. al. 1995). Spring can bring a ground cover of highly diverse herbaceous plants until the deciduous canopy shades out this understory and hampers the growth of lower canopy trees and shrubs.

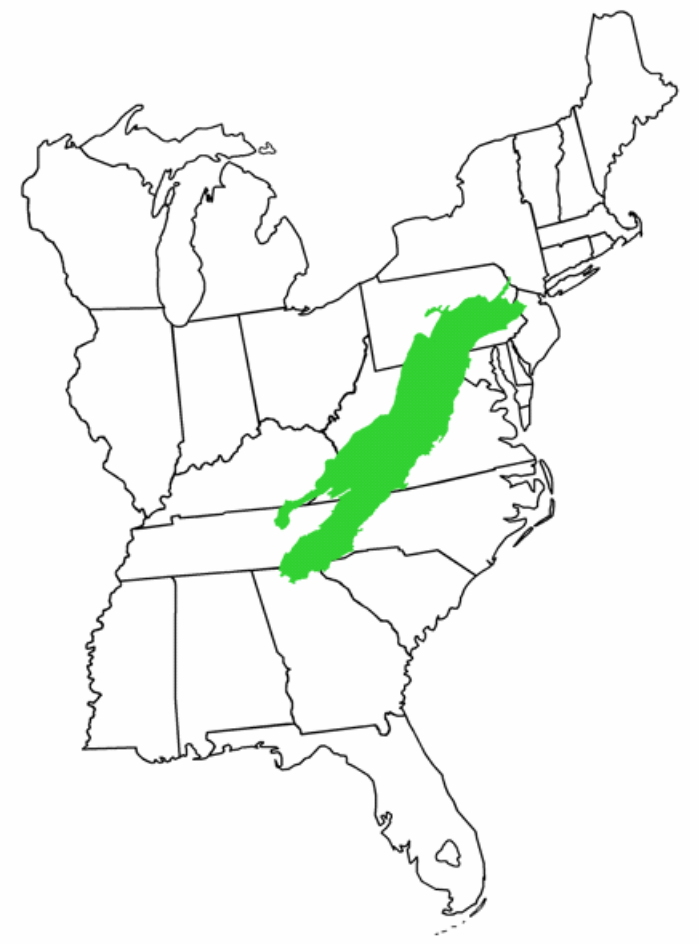

Figure 14. Appalachian broadleaf coniferous forests (after Keys et al. 1995).

The region of the study plots possesses elements of both northern and southern forest types (Skeen et al. 1993, White and White 1996, Stephenson et al. 1993). The transitional aspect applies to other organisms also and may be why the central Appalachians are considered one of the "Hot Spots" of biological rarity and richness in North America (Chaplin et al. 2000). Thirty or more species of trees can be found in some areas, which is unique for this continent.

During the study a number of vegetation surveys were performed on the subplots. The main survey for trees and shrubs was along the transects. Other plant surveys, including herbaceous plants, were associated with the arthropod study sites.

Trees and Shrubs

The MNF plots were of the mixed mesophytic forest type. Chestnut, northern red and white oak, pine, red and sugar maple, and hickory were the dominant canopy species. The GWNF sites also were dominated by chestnut oak and red oak but consisted of a greater proportion of pines than the MNF (Figure 15). The MNF sites contained more variability in vegetation than the GWNF sites. In general, basal area was similar between the two sites but was slightly higher on MNF. Although both of these sites were located within large areas of contiguous forest, the

larger landscape surrounding the GWNF sites contained a greater percentage of agriculture (Keyes 1999).

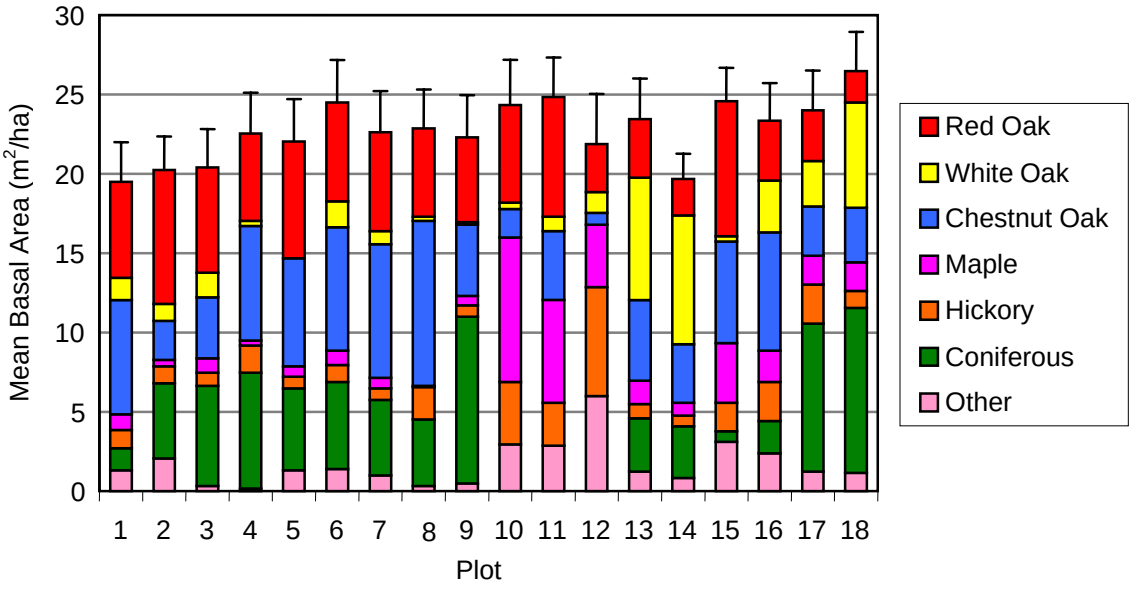

Figure 15. Mean basal area (95% CI) on each of the subplots on the George Washington (plots 1 to 9) and Monongahela (plots 10 to 18) National Forests during 1995 to 1999 broken down into dominant tree species categories.

The shrub layer density and diversity were much higher on GWNF, with the understory dominated by mountain laurel, black gum, witch-hazel, dogwood, "Vaccinium", and maple (Figure 16). The shrub layer on MNF was dominated by maple, witch-hazel, mountain laurel, and pine.

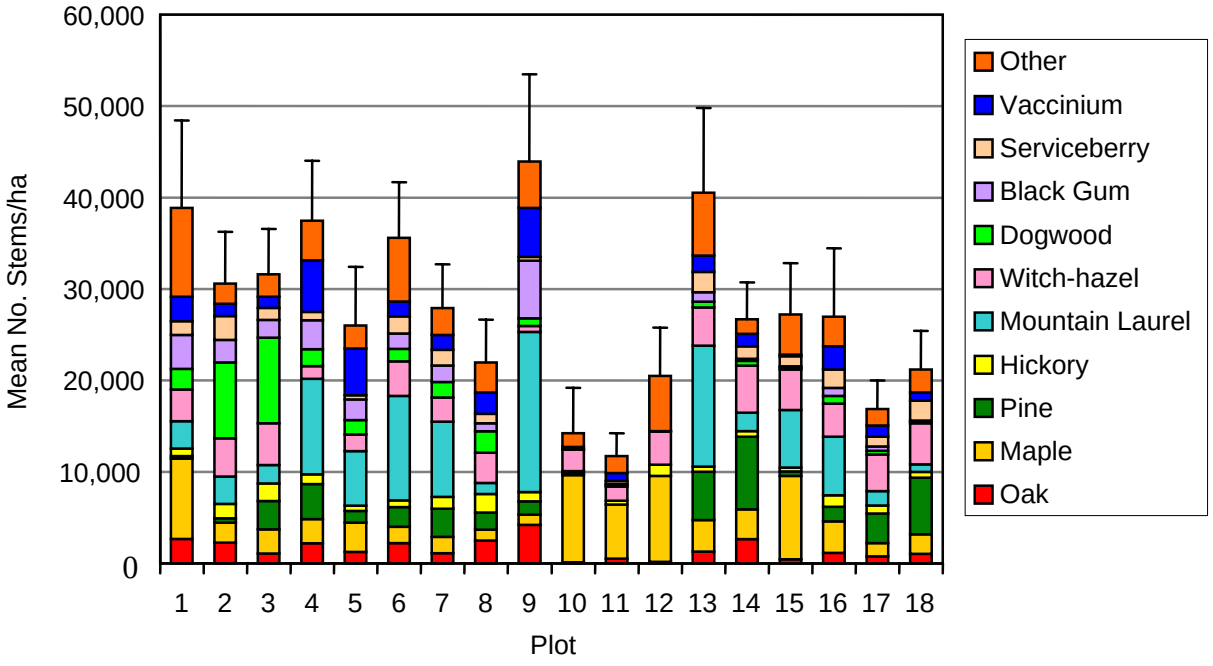

Figure 16. Mean number of stem/ha (95% CI) on each of the subplots on the George Washington (plots 1 to 9) and Monongahela (10 to 18) National Forests during 1995 to 1999 broken down into dominant species categories.

Herbaceous Plants and Mosses

Herbaceous plants were surveyed three times during their growing season in 2000 to 2001 on all subplots near the upper and lower arthropod sampling sites in the vicinity of the Malaise traps. These surveys were performed using a method similar to that of Stephenson and Adams (1986). A 50 m transect was laid with its mid point at the pole that holds up a Malaise trap (arthropod sampler). Along the transect on either side of the Malaise trap were established 5 points 5 m apart starting at the end of the transect. At each of these points within a 1 m area herbaceous plants and bryophytes were counted and vouchers taken for identification.

Plants typical of the region were commonly sampled. Regularly encountered ferns, especially on moist sites, included Christmas fern (*Polystrichum acrostichoides* (Michx.)), hay-scented fern (*Dennstaedtia punctilobula* (Michx.)), and bracken fern (*Pteridium aquilinum* (L.)). Also at wetter sites jack-in-the pulpit (*Arisaema triphyllum* (L.)), trout lily (*Erythronium americanum* Ker), and rattlesnake orchid (*Goodyera pubescens* (Willd.)) were common. Some other common flowering herbaceous plants included Asters (*Aster* spp.), white snakeroot (*Eupatorum rugosa* Houtt.), wreath goldenrod (*Solidago caesia* L.), violets (*Viola* spp.), Solomon's seals (*Polygonatum* spp.), bellworts (*Uvularia* spp.), bedstraws (*Gallium* spp.), flowering wintergreen (*Polygala paucifolia* Willd.), sticktights (*Desmodium* spp.), lion's foot (*Prenanthes trifoliata* (Cass.)), and black cohosh (*Cimicifuga racemosa* Michx.). Various sedges were sampled, with *Carex* species being the most common. Grass taken regularly included *Panicum* species, autumn bent grass (*Agrotis perennans* (Walt.)), and poverty grass (*Danthonia spicata* (L.)). Bryophytes or mosses sampled from the soil surface and rocks included *Leucobryum glaucum* (Hedw.), *Polytrichum* species, *Dicranum scoparium* Hedw., and *Plagiomnium ciliare* (Müll. Hal.).

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CHAPTER 3. APPLICATION OF TREATMENTS

KENNETH E. RASTALL, JOHN S. STRAZANAC AND LINDA BUTLER

TREATMENT APPLICATIONS

Following two years of baseline sampling, treatments were applied in 1997 and 1998. Within each block the three individual plots were randomly designated to be treated with one application of *Bacillus thuringiensis kurstaki* (*Btk*), Gypchek®, or left untreated to serve as a control, giving a total of 6 replicates of each designation for the 18 plots. The *Btk* was applied as Foray® 48F (Valent BioSciences Corporation) with BOND sticker (Loveland Industries, Greeley, Colorado, USA). The Gypchek® was applied as the Hamden strain (USDA Forest Service) with carrier 038 (Omnova Solutions, Inc., Chester, SC).

Aerial applications were applied to the designated plots in each forest during the spring of 1997 and 1998 following leaf bud-break, when white oak (*Quercus alba* L.) leaves had expanded approximately 25% of full size or about 3 cm. Specific application dates are presented in Table 1. The Foray® 48F was applied at a dosage rate of 40 Billion International Units (BIU)/acre, the maximum dose allowable at that time by the EPA. The Gypchek® was applied at a rate of 4 x 10^{11} Polyhedral Inclusion Bodies (PIB)/acre.

Table 1. Block arrangement, plot treatment type, and treatment dates on George Washington (GWNF) and Monongahela (MNF) National Forests.

				Treatment Dates	
	Block	Plot	Treatment	1997	1998
GWNF	1	1	Gypchek	May 23	May 7, 8
		2	Control	-	-
		3	*Btk*	May 17	May 10
	2	4	Control	-	-
		5	*Btk*	May 18, 19	May 10
		6	Gypchek	May 23	May 7, 8
	3	7	*Btk*	May 18	May 10
		8	Control	-	-
		9	Gypchek	May 21	May 7, 8
MNF	4	10	Gypchek	May 23	May 13
		11	*Btk*	May 29	May 15
		12	Control	-	-
	5	13	Gypchek	May 23	May 13, 14
		14	Control	-	-
		15	*Btk*	May 29	May 15
	6	16	Control	-	-
		17	Gypchek	May 23	May 13
		18	*Btk*	May 28	May 15

Spray treatments on the George Washington National Forest plots were applied by helicopters and on the Monongahela National Forest plots by fixed-wing aircraft. Balloons were raised at the corners of the plots to confirm their location and GPS (Global Positioning System) units were used to guide and record the application of treatments.

ANALYSIS OF DEPOSITION

METHOD

To confirm spray coverage of *Btk* and Gypchek® over subplots, water-sensitive spray cards were set out within plots. Once application and settling of airborne spray treatments were confirmed for each treated plot, foliage sampling was performed as the first step in determining the concentration of *Btk* protein toxins in relation to leaf surface utilizing ELISA techniques (Wie et al. 1982).

To determine *Btk* toxin concentrations on the foliage, leaf samples were collected within 2 hours of application. Samples were taken at evenly spaced intervals along subplot transects A and D (Figure 3) on each plot treated with *Btk*. Samples were also taken in *Btk* treated areas where foliage was pruned for the foliage arthropod studies along a transect in a similar fashion. The samples were taken from oak leaf clusters which were clipped from the lower and mid-canopy at a height of approximately 4 to 6 m using pole pruners. Once clipped, the falling cluster was caught by the woody portion before it reached the ground. A single leaf was then removed that had not been touched by the pruner or rubbed against other leaves in the cluster. Once removed by holding its petiole, the leaf was placed into an individual plastic, zipper sealed bag along with some air to cushion it, and the bag was suspended from one edge in an iced cooler for transport to the laboratory. A full sample at each sample point consisted of 20 leaves with approximately 150 cm^2 of total surface area. In the laboratory, while still bagged, the 20 leaves from each sample were individually scanned with a leaf area meter and pooled.

In 1997 a total of 50 samples was taken from the *Btk* treated plots, with eight samples each taken from plots 3, 5, 7, 11, and 15, and ten samples from plot 18. In addition, 13 samples were taken from the foliage pruning sites used in the arthropod studies on the periphery of the subplots, with four samples each taken from plots 3 and 5, and five samples taken from plot 7. During 1998, only four samples were taken on each of the six *Btk* treated plots, and two from each of the foliage pruning sites associated with the *Btk* treated plots, yielding a total of 36 foliage samples.

For *Btk* analysis, toxins were extracted and concentrations of toxin/cm^2 leaf area were determined utilizing DAS ELISA (double antibody sandwiched enzyme-linked immunosorbent assay) techniques. The following is an abbreviated version of the procedure with the first portion conducted at West Virginia University.

The first laboratory steps were performed using a DAS ELISA field research kit for *Btk* endotoxin proteins found on deciduous foliage supplied by Abbott Laboratories. Extraction consisted of placing 1 ml of a buffer per 5 cm^2 of leaf area into a zipped plastic bag with the

pooled leaves and removing the air before closing the bag. This was incubated for 1 hour at room temperature. After incubation, the leaves were gently rubbed without opening the bag. From the solution within each bag, 0.5 ml was pippetted and added to individual vials containing 0.5 ml of a neutralizing buffer. These samples were then frozen.

The final steps of the endotoxin analyses were conducted at the USDA-Forest Service Center for Forest Health Research Laboratories at Hamden, Connecticut, under the supervision of the late Norman Dubois, Research Microbiologist. The frozen samples were allowed to thaw. A subsample was then placed into a well with anti-*Btk* proteins (perox enzyme conjugate) and allowed to incubate for 1 hour. The wells were then rinsed six times with tap water and drained. A buffer (MEB) was then added and after 3 minutes the wells were drained again. A colorizing solution (TMB peroxidase substrate) was added and after 15 to 30 minutes the wells were analyzed using an automated plate reader to measure optical densities from which toxin concentrations were calculated.

RESULTS

Individual concentrations of *Btk* toxins analyzed from collected leaf samples are presented in Table 2. Site locations are identified with P or T referring to pruning areas or transect areas, respectively. Point locations indicate different samples for pruning areas and different locations along the transects which were previously established in the subplots. Histograms generated from this data appear as Figure 17. Additional analyses have been described by Rastall (1999). Concentrations varied between non-detectable levels and 171 ng/cm^2. Only 11 samples (9.9%) were less than 20 ng/cm^2. Above this level, approximately 100% of gypsy moth caterpillars would be expected to receive a lethal dose (Dubois 1998 unpublished experimental data).

Table 2. *Btk* toxin concentrations of each sample collected during the 1997 and 1998 treatment years from the George Washington and Monongahela National Forests. P = pruning area. T = transect areas.

George Washington N. F.

Plot	Site	Point	1997 (ng/cm^2)	1998 (ng/cm^2)
3	P	.1m	36	
3	P	.25m	27	38
3	P	.35m	32	54
3	P	.45m	27	
3	T	A00	57	
3	T	A200	39	28
3	T	A400	50	56
3	T	A600	26	
3	T	D00	28	
3	T	D200	120	69
3	T	D400	22	15
3	T	D600	75	

Plot	Site	Point	1997 (ng/cm^2)	1998 (ng/cm^2)
5	P	1	0	
5	P	2	51	75
5	P	3	160	80
5	P	4	69	
5	T	A00	57	
5	T	A200	20	30
5	T	A400	46	32
5	T	A600	17	
5	T	D00	74	
5	T	D200	27	34
5	T	D400	93	75
5	T	D600	106	

Plot	Site	Point	1997 (ng/cm^2)	1998 (ng/cm^2)
7	P	1	138	
7	P	2	150	
7	P	3.1	171	53
7	P	3.2	77	39
7	T	4	93	
7	T	A00	97	
7	T	A200	150	9
7	T	A400	146	14
7	T	A600	99	
7	T	D00	120	
7	T	D200	43	22
7	T	D400	20	30
7	T	D600	140	

Monongahela N. F.

Plot	Site	Point	1997 (ng/cm^2)	1998 (ng/cm^2)
11	P	1	38	
11	P	2	42	35
11	P	3	100	52
11	P	4	50	
11	T	A00		38
11	T	A200	74	37
11	T	A400	79	42
11	T	A600	65	
11	T	D00	101	
11	T	D200	96	70
11	T	D400	78	32
11	T	D600	109	

Plot	Site	Point	1997 (ng/cm^2)	1998 (ng/cm^2)
15	P	1	40	
15	P	2	39	67
15	P	3	40	45
15	P	4	43	
15	T	A00	44	
15	T	A200	54	48
15	T	A400	89	36
15	T	A600	117	
15	T	D00	81	
15	T	D200	59	71
15	T	D400	51	91
15	T	D600	96	

Plot	Site	Point	1997 (ng/cm^2)	1998 (ng/cm^2)
18	P	1	0	
18	P	2	6	13
18	P	3	66	48
18	P	4	27	
18	T	A00	39	
18	T	A200	21	62
18	T	A200R	13	39
18	T	A400	50	
18	T	A600	8	
18	T	D00	38	
18	T	D200	18	20
18	T	D200R	50	
18	T	D400	33	42
18	T	D600	35	

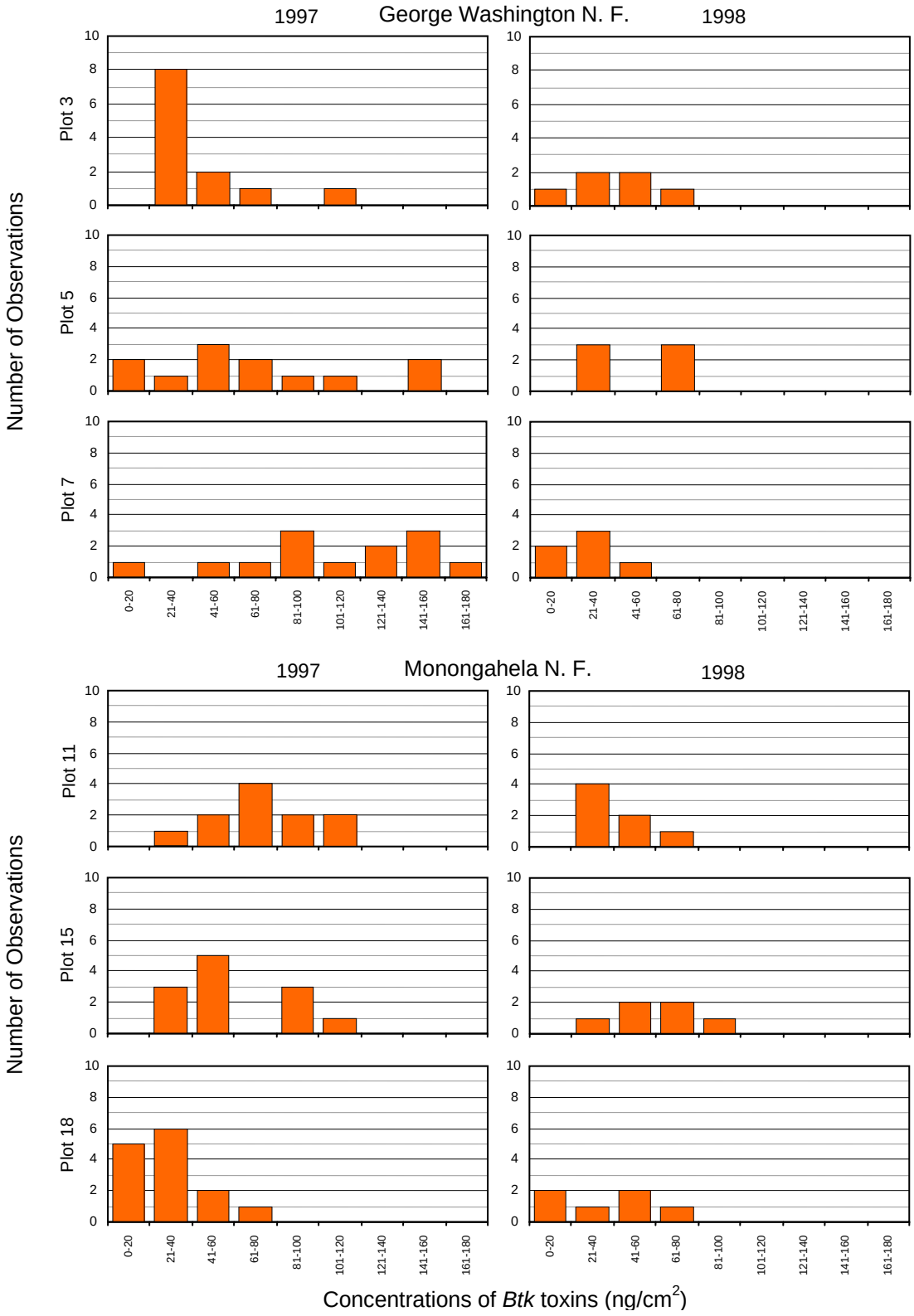

Figure 17. *Btk* toxin concentrations from foliage samples collected during the 1997 and 1998 treatment years from the George Washington and Monongahela National Forests.

DISCUSSION

The data indicated that not only was coverage adequate, but that pesticide concentrations were of sufficient magnitude to be lethal to gypsy moth. As photodegradation of the *Bt* toxin proceeds rapidly (as reviewed by Reardon et al. 1994), fixing the leaf samples should be done soon after pesticide application. Such loss of *Btk* toxicity was demonstrated by bio-assay analyses conducted at West Virginia University where second instar gypsy moth larvae were fed *Btk* treated leaves collected from the plots two weeks after application. Significant lethal effects were not observed. With regard to analysis of *Btk* toxin on treated plots, the Abbot Laboratories DAS ELISA field research kit for preparing leaf samples and the subsequent analysis of these samples provided data integral to this study. For treatment application, spray cards are typically used to confirm coverage, indicate droplet size, and spread. With the DAS ELISA techniques, additional data on toxin concentrations supplemented this information.

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CHAPTER 4. ARTHROPODA STUDIES

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INTRODUCTION

Arthropods are the most abundant and diverse group of organisms in our forest environment and they play an essential role in its ecosystems. They are probably only second to trees in terms of total forest biomas. Typically found at lower trophic levels, arthropods are a primary food source for other forest animals. Many serve as pollinators and in nutrient recycling while a few species have dramatic negative impacts such as tree defoliators. Some arthropds, as parasites and predators of other invertebrates, play a critical role in keeping our forest ecosystems in balance (Kidd and Jervis 1997).

In forested environments, Lepidoptera (moths and butterflies), are commonly encountered and are usually the most abundant of canopy chewing herbivores. They served a central role in this study because of the potential direct impact their caterpillars may receive from feeding on foliage treated with *Btk* and Gypchek. *Btk* is known to broadly affect lepidopteran caterpillars, especially those that feed alongside gypsy moth caterpillars and have similar biologies (Figure 18) (Sample et al. 1996, Wagner et al. 1996, Peacock et al. 1998). Gypchek is considered to be specific to gypsy moth, but there is some evidence that other tussock moth caterpillars may be impacted (Barber et al. 1993). Within the Lepidoptera the families most emphasized in this study are the ones referred to as the macrolepidoptera. The macrolepidoptera group is made up of highly speciose families that are typically “large” as adults and caterpillars compared to other lepidopterans, and are usually exposed on foliage on which they feed. Some of the macrolepidopteran families regularly sampled in Appalachian forests include members of the Hesperiidae, Papilionidae, Lycaenidae, Nymphalidae, Thyatiridae, Drepanidae, Geometridae, Epiplemidae, Mimallonidae, Apatelodidae, Lasiocampidae, Saturniidae, Sphingidae, Notodontidae, Arctiidae, Lymantriidae, and Noctuidae.

Figure 18. *Orthosia rubescens* (Walker) (Noctuidae) is a spring defoliating caterpillar that feeds at the same time as gypsy moth caterpillars on the same host trees.

The long term design of this study allowed us to monitor biopesticide impact and subsequent recovery of the directly impacted macrolepidopterans. Equally important was determining the secondary or indirect impact of the reduction of macrolepidoptera in the food web. Lepidoptera as adults and caterpillars are an important link in food chains in forest ecosystems, serving as hosts or prey for many forest arthropods, songbirds and other vertebrates. The reduction in their populations may cause some type of adjustment in their parasitoid and predator complexes.

Understanding parasitoid preferences is another important aspect of the study design. To this end throughout each season, selected species of macrolepidopteran-type caterpillars were collected and reared in the laboratory to establish relationships with parasitic Hymenoptera (Figure 19) and Diptera (Figure 20) (Butler 1990, 1993). Parasitoids were identified to species with the assistance of specialists at the USDA Systematics Laboratory and Agriculture Canada and by comparison with specimens in the West Virginia University Arthropod Collection. Percent parasitism was determined for gypsy moth and nontarget caterpillars under different treatments. Frequency of parasitism of nontarget caterpillars by introduced gypsy moth parasitoids was also determined. The study was designed to establish if parasitism of nontarget species changes as gypsy moth and nontarget caterpillar populations decline due to the *Btk* and Gypchek applications, or due to impact of the *Entomophaga maimaiga* fungus. In addition, the study includes an analysis of the recovery of parasitoids that attack nontarget caterpillars.

Figure 19. *Therion* sp. (Ichneumonidae) commonly attack macrolepidoptera caterpillars.

Figure 20. *Leschenaultia fulvipes* (Bigot) (Tachinidae) regularly attacks canopy macrolepidopteran caterpillars.

Arthropod predators also play important roles in moderating the abundance of caterpillars (Montllor and Bernays 1993). Two groups of the most diverse and abundant predators found in forest ecosystems are included in the study design: carabid beetles (Figure 21) and spiders (Figure 22). We identified these predators to species to determine fluctuations of species within the groups and to use their known prey preferences in guiding the nontarget analyses. Pentatomid stinkbugs (Figure 23) with predatory habits were also identified to species, and are treated in a similar fashion.

Figure 21. *Sphaeroderus lecontei* Dejean (Carabidae) is a commonly collected carabid beetle in central Appalachian forests.

Figure 22. *Hogna frondicola* (Emerton) (Lycosidae) is a common wolf spider.

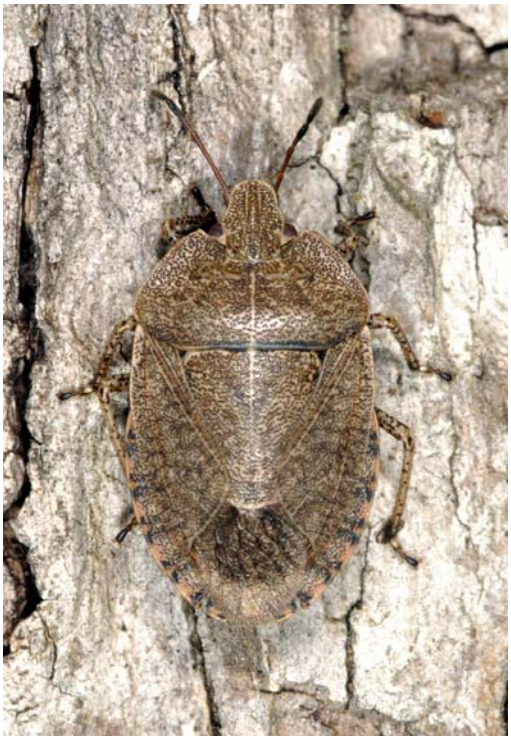

Figure 23. *Menecles insertus* (Say) (Pentatomidae) is found regularly in leaf litter.

Another diverse and abundant group in forest environments that merited special attention in this project were sawflies (Figure 24). They possess a number of similarities with the macrolepidopterans. Many sawfly larvae resemble caterpillars in appearance and in their foliage feeding habits, and in fact are second only to caterpillars as the most commonly encountered foliage chewing insect group. With the exception of one family, the Orussidae, all sawflies are phytophagous, the majority feeding externally on foliage, including species feeding on tree canopy foliage (i.e., *Acordulecera* spp., *Periclista* spp., *Nematus* spp.). Sawfly larvae are also attacked by many of the same parasitoid and predator species that attack caterpillars (Strazanac 2004, Krombein et al. 1979). Some ornithologists collectively refer to larval sawflies and lepidopterans as caterpillars when recording dietary habits of songbirds (Rodenhouse and Holmes 1992). There is evidence that some sawflies may also be directly impacted by *Btk* treatments (Smirnoff and Berlinguet 1966, Gorske et al. 1976). Treatment analyses of sawfly are interpreted with their similarities to caterpillars in mind.

Figure 24. *Pamphilus semicintus* (Norton) (Pamphiliidae) adult on serviceberry flower; larvae feed on the foliage.

Species level identifications of arthropods potentially affected by treatments guide us in designing and interpreting analyses. Arthropod groups less likely to be affected by treatments were tallied in samples, but were generally treated at a level above species, usually at a family level. For analysis, sample counts were grouped by ecological niche or taxonomic group. For example, syrphid flies and bees can be analyzed together as pollinators or based on order or family designations.

The arthropod portion of the study compares sample counts among treatments. Sampling was performed by the use of traps and standardized hand collecting. With the exception of gypsy moth egg mass surveys, the sampling methods produce large samples composed of a diverse array of arthropods. Field sampling methods take relatively much less time than that needed to extract and identify specimens from these samples in the laboratory.

METHODS

Arthropods were sampled by six methods (see Strazanac et al. 2003a for trap design and procedures) within or near the established subplots (Table 3). Sampling was performed at two sites designated within each subplot, along the established transects or adjacent to the subplots. The two sample sites were located on each subplot to represent environmental variation as reflected by vegetation analysis (Waring 1989, Matthews and Matthews 1970). Typically this meant that one site was near a ridge and the other near a stream bed at different elevations.

Table 3. Arthropod sampling methods and locations on subplots.

Sampling Method	Location on each Subplot
Malaise Traps	at both sampling sites
Pitfall Traps	at both sampling sites
Canvas Band Traps	near both sampling sites
Light Traps	between sampling sites
Foliage Sampling	periphery of subplot
Gypsy Moth Egg Mass Surveys	along subplot transects

During each of the 7 years of arthropod sampling, 15 weekly samples were taken from the subplots. This sampling period included weeks before and after gypsy moth caterpillars and adults were present. Gypsy moth egg mass surveys were the only method that targeted a single species. Light traps were employed mostly to sample nocturnal moths.

Analysis

Data Considerations

Seven years, with fifteen weekly sampling periods each year, using five techniques produced large amounts of arthropod material to sort, identify, database and analyze. Excluding certain highly abundant groups that did not serve a role in this study (e.g., Collembola, mites), more than 2 million insects, spiders and other arthropod groups were sorted to some taxonomic level. With samples of this size and diversity, much of the work was separating and identifying taxa thought to most likely be directly or indirectly impacted by treatments.

For direct impact, caterpillars with known phenologies most similar to gypsy moth larvae were important to identify to species. The best candidate taxa for studying indirect impacts have an intimate relationship with the caterpillars likely to be directly impacted by treatments. Tachinid flies, for example, would be such a group since most species parasitize caterpillars. The need for species identification still remains though because some attack an array of species of caterpillars, others parasitize caterpillars not present during treatments, and some of the most commonly collected may not attack caterpillars at all.

Other decisions in such a study must be made purely on a practical basis of timeliness and available resources. Most groups that may have a close relationship with caterpillars were included in this study, with the size of the samples and availability of specialists as factors in

deciding to what taxonomic level to identify material of particular taxa. Some groups having close and highly specific relationships with caterpillars were too uncommon in samples to be statistically analyzed at a species level and were lumped with other species of similar habits.

Our knowledge of insects and related arthropods in forest environments is still quite limited. Regional population differences can also play a role in how one analyzes results. When attempts were made to group caterpillars based on host plants (e.g., oaks), we used published records to assure all species that fed on oak were included. This approach was quickly found to be unsuitable. Published records included too many references to casual observations that could not be verified. There may also be regional differences in host preference or host quality influencing host utilization. For linking caterpillars to hosts and categorizing them as specialists or generalist we used our own sampling data from oaks, maples, and hickories, and published works specific to the central Appalachians. The work of David Wagner (Wagner et al. 1997, Wagner et al. 2001, pers. communications) based mostly on his own research and field experience also was a significant contribution. Where there was doubt or no information was found, no speculation was made based on related taxa. These situations applied to species sampled at low numbers, generally because they were at a limit of their range, not found in the forest types we were sampling, not typically collected with sampling techniques used, or are naturally at low population levels.

For parasitoids, published records served as a guide, but our own extensive rearing was important for verifications and discovering many new host relationships (Strazanac et al. 2001, Petrice et al. 2004).

Method of Analysis

Spray treatment results were analyzed as a randomized complete block design with post-treatment years as repeated measures. Mixed model ANOVA procedures were employed in the analysis of abundance with the pretreatment years combined as a covariate. For any arthropod taxa found to be non-normally distributed, data were log transformed prior to analysis. A typical analysis tested for effects of treatment, year, and the interaction between these factors. Where appropriate, nontarget organisms were grouped by sampling method, by plot, by date, or by year.

For macrolepidoptera caterpillars and adults, selected species representing greater abundance or frequency on the plots, or representing specialized feeding guilds (i.e., Noctuidae subfamily Herminiinae whose caterpillars feed on leaf litter on the forest floor) were analyzed separately. Host preference of macrolepidoptera by arthropod parasitoids and predators guided the analyses of these groups.

In the figures that present total counts grouped by treatment, significant differences between treatments as determined by analyses are signified by a lower case letter. The letter is the first letter of a treatment (b=*Btk*, c=control, g=Gypchek) that resulted in reduced abundance compared to the treatment of the column it is placed above. One standard error is indicated with error bars.

RESULTS

The arthropod section of this report examines three areas of treatment effects. First, those species of caterpillars and other similar arthropods (e.g., sawfly larvae) that appear to be directly impacted by treatments. Second, for these species, how much post-treatment time is required for them to return to pre-treatment abundance levels. Finally, what indirect effects might be inferred, including those on predators, parasitoids, and resource competitors. This last area brings the study more into an ecosystem approach with relationships of different feeding guilds being considered.

Within the scope of this report we can not include in detail the results of all analyses. The order of the discussion begins with groups that are directly impacted by treatments, and then groups most closely connected to these groups, and finally groups with more distant relationships.

As with most field studies, factors outside of the manipulated variables affect sample sizes. In a long term study that includes examining different trophic levels, population cycles influenced by availability of resources generally can be recognized. Weather events may also cause fluctuations, for example extreme cold, heavy rains and drought, all of which occurred during the study. Some of the weather experienced during the study is noteworthy. For example, the unusual cold period at the beginning of the 1997 field season (Figure 13) occurred just when caterpillars were hatching and would be considered the most vulnerable to the *Btk* and Gypchek treatments that were being applied. This cold "snap" with record low temperatures appeared to reduce caterpillar counts on all plots, and thus moth counts later. Another longer event was a drought during most of 1999, which was the first post-treatment year (Figure 11). The negative impact of the drought like the cold period in 1997 can be seen in the results graphs. It should be noted that weather events may have affected caterpillar species differentially based on their cold hardiness or drought tolerances. The assumption is that the factors outside of the variables we manipulate are felt equally among all study plots or at least within treatment blocks. We cannot ignore the fact that the study was performed in highly varied terrain with inherent differences between study plots, at times adding complexity in interpreting results.

Herbivores

Lepidoptera

Foliage and Canvas Band Sampled Caterpillars

Three methods were used to sample macrolepidoptera. Caterpillars were gleaned in the laboratory from pruned oak, hickory, and maple foliage and from under canvas bands wrapped around boles of trees. Moths were sampled using light traps. A total of 608 species of macrolepidopterans were included in the counts. The only microlepidopteran identified to species and analyzed was *Pyromorpha dimidiata* H.-S. (Pyromorphidae), the orange-patched smoky moth, which was collected with Malaise traps as adults. From foliage, larvae of the microlepidopteran families Gelechiidae and Tortricidae were counted.

The application of *Btk* and Gypchek was timed to have maximum impact on gypsy moth populations. The data collected through our sampling of foliage feeding caterpillars is the best

indicator of possible direct impact of these treatments. They have been the most studied of the forest invertebrates with regard to *Btk* and Gypchek (Miller 1990, Wagner et al. 1996, Sample et al. 1996) effects because they are intrinsically most likely to be impacted by the treatment as is gypsy moth based on their shared phenologies, which is in part influenced by taxonomic relationships. The macrolepidoptera, though not a strict taxonomic group, are the focus of the first results presented. Included are species that share host plants with gypsy moth, are exposed when feeding, and are present at the time of treatment application or during the following period of its efficacy.

For analysis of caterpillar counts, species were grouped as present (1) during treatment applications, (2) shortly thereafter, or (3) not at all during the period of treatment efficacy. Light traps produced high counts and species rich samples of macrolepidoptera. The species we did not sample as caterpillars (generally because they feed on host plants not sampled) we also grouped as present or not as caterpillars during treatment applications. These groupings were based on regional publications (Butler 1992, Butler and Strazanac 2000, Wagner et al. 1997, Wagner et al. 2001) and unpublished records taken over 20 years (L. Butler) from previous studies of Appalachian macrolepidopterans. Groupings for bivoltine or multivoltine species were based on potential treatment exposure of the first generation.

The need to group macrolepidopteran species based on their caterpillars being present or absent during treatment is illustrated in the following example. If all macrolepidopteran caterpillars sampled from foliage are included in an analysis it appears that there is support for *Btk* having a negative impact during the second treatment year and again the third post-treatment year (Figure 25). A subset of the data excluding *Hypantria cunea* (Drury), the fall webworm, gives different results (Figure 26). The *Btk* negative impact is again supported in this subset for the second treatment year, but not in the third post-treatment year. This is because the 1,600 fall webworm caterpillars sampled were not well distributed across plots or over the 7 years of sampling. A total of only 7 *H. cunea* caterpillars were collected from the plots treated with *Btk*. From Gypchek plots, about half of the 1,600 total were taken the last year alone and zero the first year. Since the fall webworms are not present during the time of treatment or shortly thereafter, the fluctuations were not directly influenced by treatments.

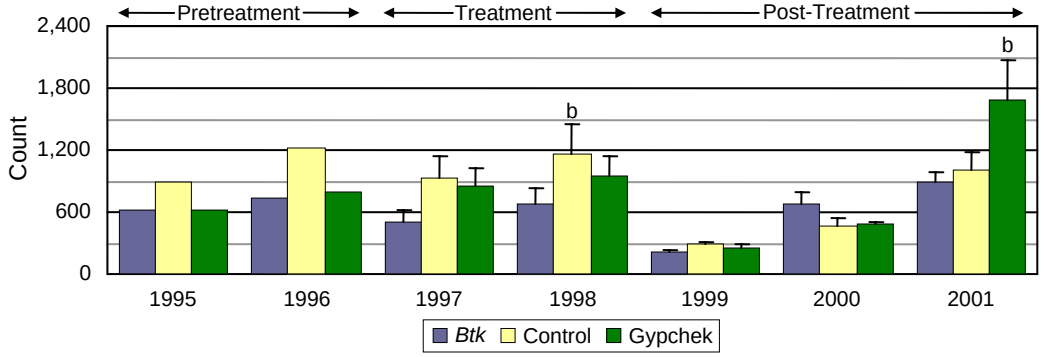

Figure 25. Total counts of foliage sampled caterpillars grouped by treatment. Total count=15,883. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

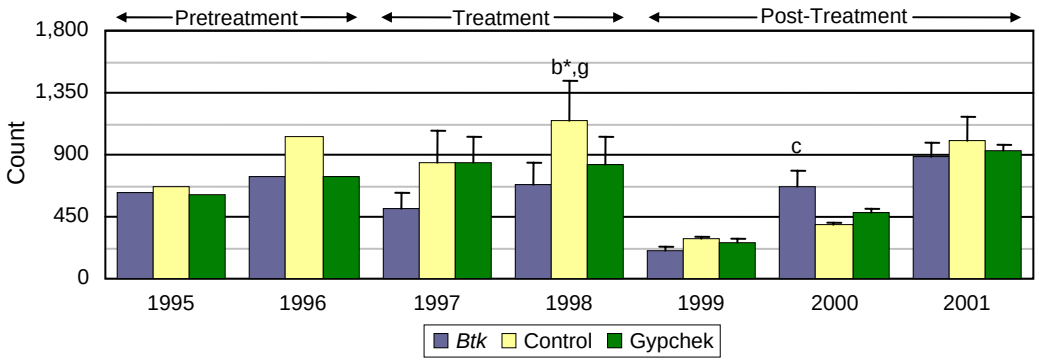

Figure 26. Total counts of foliage sampled caterpillars grouped by plot treatment, excluding the fall webworm, *Hyphantria cunea* (Drury). Total count=14,283. Lower case letters (b=*Btk*, c=control, g=Gypchek) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. An asterisk indicates $p<0.01$. Error bars indicate one standard error.

When fall webworm counts are removed from the analysis, the counts between years are more similar, with the statistically significant decrease in 1998 now on *Btk* plots compared to control plots with $p<0.01$ (Figure 26). But now, Gypchek plots also have a significant decline compared to control plots ($p<0.05$). Removing the fall webworm with its uneven distribution across plots, treatments, and years gives a very different result, but the results remain influenced by similar non-treatment factors. Three noctuid species became very abundant on control plots only during the second treatment year: *Acronicta ovata* Grt., *Hyperstrotia pervertens* (B. & McD.), and *Polia latex* (Gn.) which influenced the significant differences (Figures 25 & 26). Two of these species would be present as caterpillars (*A. ovata* and *H. pervertens*) only long after treatment applications, and one (*P. latex*) would, at most, have a small fraction of its caterpillar population present during treatments or shortly thereafter. These species should not be in a dataset analyzed for the direct impact of *Btk* because of their phenologies. Yet a different species accounted for much of the apparent rebound in 2000 on *Btk* plots (Figures 25 & 26). In one sample from a single *Btk* plot on 18 July 2000, 114 *Anisota virginiensis* (Drury) larvae were collected (of a 115 total collected on all plots that year). These were young caterpillars, which feed gregariously, and would have hatched about 8 weeks after treatments were applied. These and many other species should not be included when studying the direct impact of *Btk* on caterpillars or subsequently as adults.

The number of generations per year also have to be known to interpret recovery. When univoltine species that are considered potentially most sensitive to treatment timing are analyzed separately, *Btk* treatment counts indicate their numbers are significantly ($p<0.05$ or $p<0.01$) reduced during treatment years compared to both control and Gypchek plots (Figure 27). This is true also for the first post-treatment year with *Btk* plots relatively still lower than control and Gypchek plots. In the second post-treatment year there is a rebound of caterpillar populations on *Btk* plots compared to control plots.

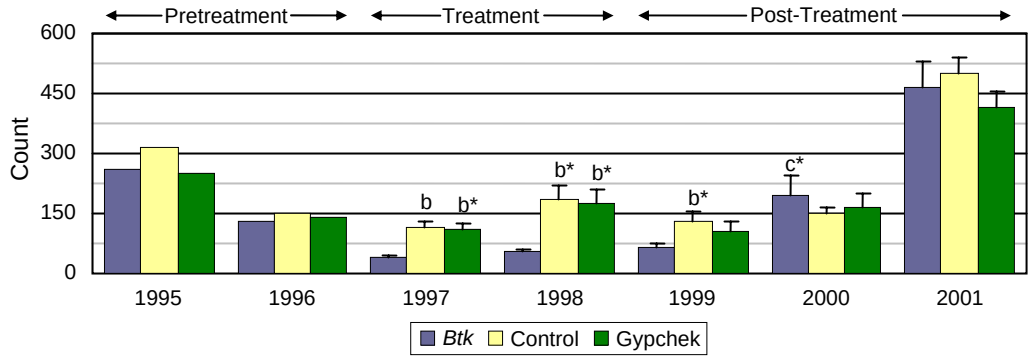

Figure 27. Total counts of foliage sampled univoltine caterpillars considered sensitive to treatment timing, grouped by plot treatment. Total count=4,119. Lower case letters (b=*Btk*, c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. An asterisk indicates $p<0.01$. Error bars indicate one standard error.

Counts of the univoltine caterpillars considered sensitive to treatment timing sampled from under canvas bands indicate possible treatment effects, though not as strongly as with those from foliage (Figure 28). When treatment and post-treatment year counts are combined, *Btk* plots overall had significantly less caterpillars than control ($p<0.05$) and Gypchek (<0.01) plots. However, in only the second treatment year with *Btk* compared to Gypchek, is there a statistically significant difference. There is also a significant decrease on control plots compared to Gypchek that year, making it seem less likely the significant *Btk* decrease was only treatment related. The much lower sample sizes from canvas bands versus foliage sampling may be a factor in the analysis results.

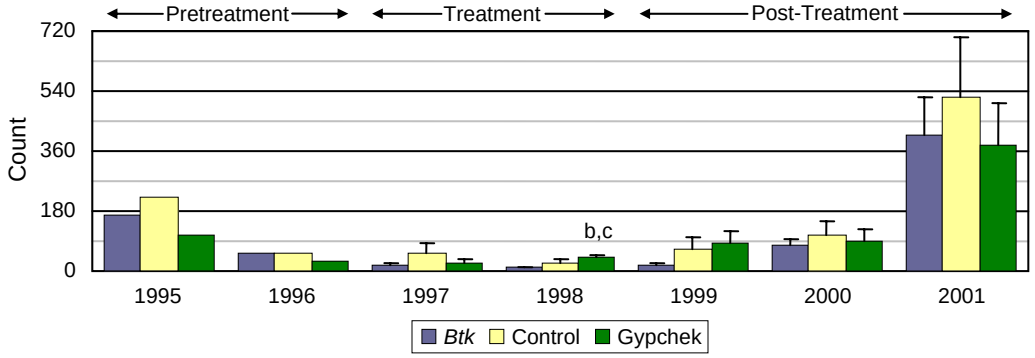

Figure 28. Total counts of canvas band sampled univoltine caterpillars considered sensitive to treatments, grouped by plot treatment. Total count=2,567. Lower case letters (b=*Btk*, c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

Analyses at species level were made difficult by low sample sizes of caterpillars from both foliage and canvas bands. The most abundant caterpillar collected from foliage and considered to be potentially sensitive to treatment timing was *Itame pustularia* (Gn.) (Geometridae), the lesser maple spanworm. Negative impacts of *Btk* seem to occur in the second treatment year and first post-treatment year with strong rebounds in the caterpillars in the second and third post-

treatment years (Figure 29). None of the differences were significant however at a $p < 0.05$. For the other species considered sensitive to treatments, sample counts were around 300 or less for each species with all years combined. Some fluctuations of populations of the lower count species may indicate a significant relative reduction on *Btk* plots, but usually the reduction is for a single year and sometimes may be accounted for by natural increases on control or Gypchek plots. A similar pattern was seen for *Orthosia rubescens* (Wlk.) sampled from foliage, one of the most abundant early season noctuid caterpillars (Figure 30).

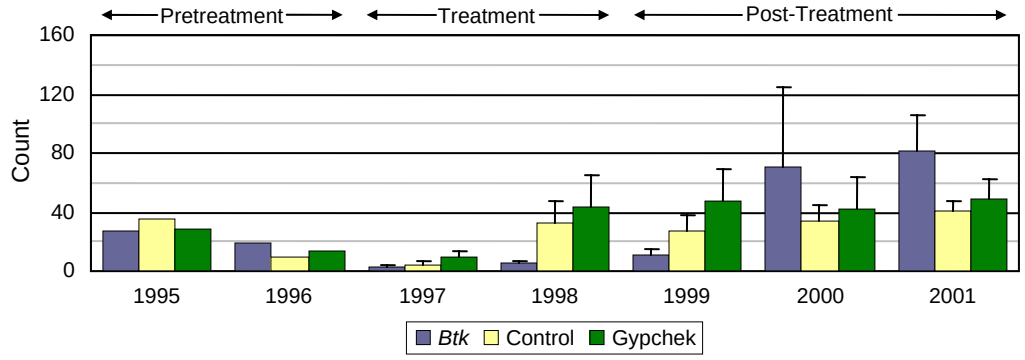

Figure 29. Total counts of lesser maple spanworm, (*Itame pustularia* (Gn.)), considered sensitive to treatment timing sampled from foliage, grouped by plot treatment. Total count=631. Error bars indicate one standard error.

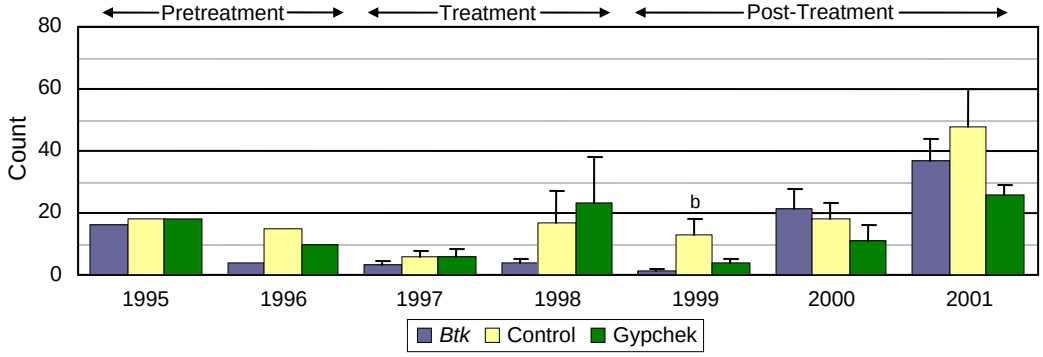

Figure 30. Total counts of *Orthosia rubescens* (Wlk.) caterpillars, considered sensitive to treatment timing sampled from foliage, grouped by plot treatment. Total count=319. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

Only two multivoline species were considered likely sensitive to treatment timing, both sampled from foliage: *Melanolophia canadaria* (Gn.) (Geometridae) and *Besma quercivoraria* (Gn.) (Geometridae). During the seven years of sampling, these two geometrid caterpillars had fairly consistent weekly total counts during the summers. *M. canadaria* was most common in the early season, and *B. quercivoraria* in the later season (Figure 31).

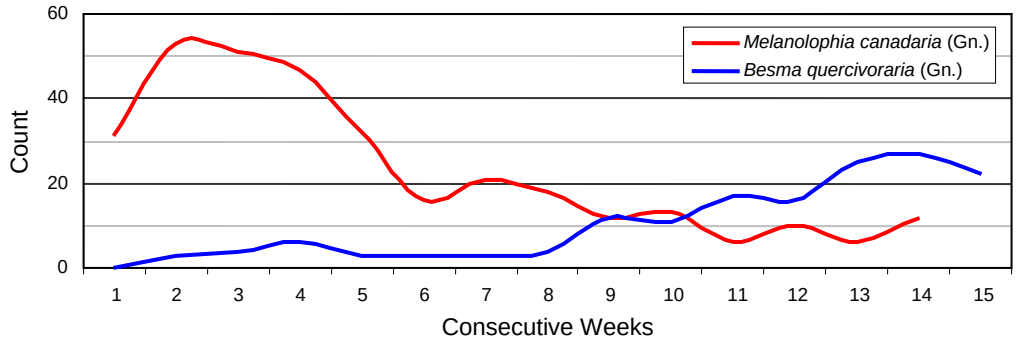

Figure 31. Seven years of combined weekly counts of control and Gypchek treatment plots of foliage sampled *Melanolophia canadaria* (Gn.) and *Besma quercivoraria* (Gn.) caterpillars.

Both *M. canadaria* and *B. quercivoraria* appear to show reduced populations on *Btk* plots, but only *M. candaria*, the species with higher populations during treatment applications, has statistically significant reductions during the 1997 treatment year (Figures 32 & 33). There may also be an indication of a rebound by both species in the second post-treatment year, but the difference is not significant. The low counts of both species should be noted. Both species were collected regularly from oak foliage, but *M. canadaria* was also collected regularly from hickories (*Carya* spp.) and red maple (*Acer rubrum* L.) as well.

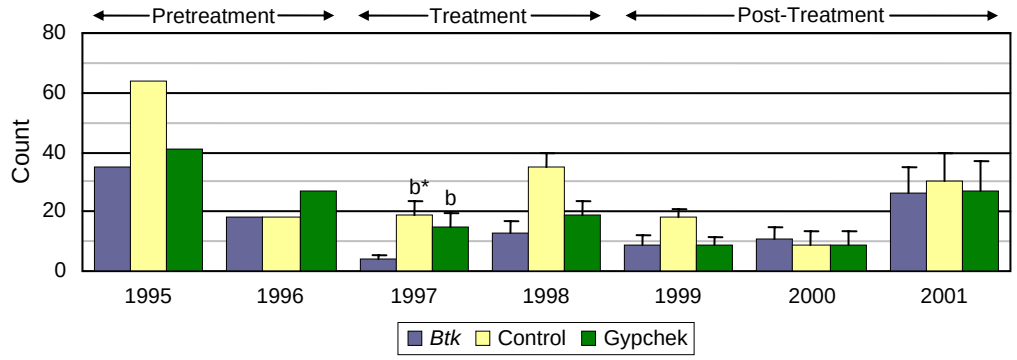

Figure 32. Total counts of *Melanolophia canadaria* (Gn.) caterpillars, a multivoltine species considered sensitive to treatment timing sampled from foliage. Total count=456. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. An asterisk indicates $p<0.01$. Error bars indicate one standard error.

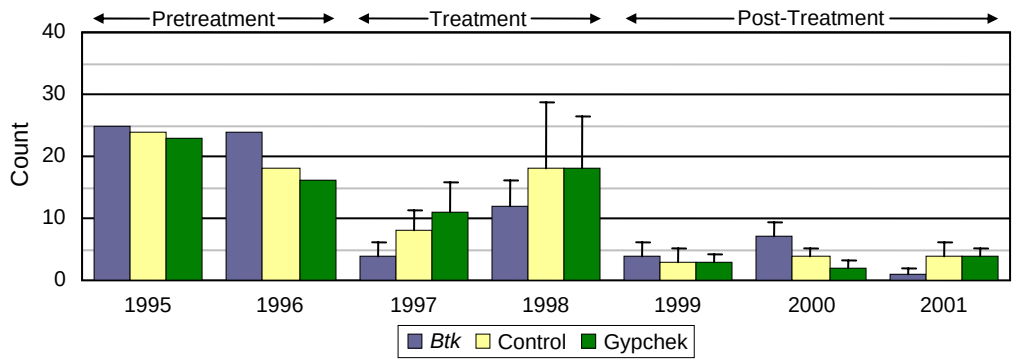

Figure 33. Total counts of *Besma quercivoraria* (Gn.) caterpillars, a multivoltine species considered sensitive to treatment timing sampled from foliage. Total count=233. Error bars indicate one standard error.

Oaks are one of the preferred hosts of gypsy moth. Hickories and maples are generally considered less desirable. There is some evidence that host plant chemistry and/or digestive characteristics of caterpillars may play a role in the impact of *Btk* (Farrar et al. 1996). For the following analyses of host influence on treatment effect only univoltine species sensitive to treatment timing were selected because of possible complications of including multivoltine species.

The caterpillar counts from oak samples decreased on *Btk* plots for the treatment years compared with control and Gypchek treated plots (Figure 34). The reduced counts continued into the first post-treatment year compared to control plots as well. For caterpillars sampled from maple there appears to also be a similar reduction on *Btk* plots during treatment years and the first post-treatment year, but these are not significant ($p<0.05$) (Figure 35). The apparent rebounds of populations on *Btk* treatment plots for the second and third post-treatment years may indicate that reduced counts on *Btk* plots during treatment years altered population cycles (see Figure 29). *Itame pustularia* (Gn.) accounted for 627 of the total 914 count, and this species was most affected. Apparent reductions and rebounds in counts of hickory foliage caterpillars on *Btk* treated plots fall somewhere between oak and maple counts (Figure 36). Some count reductions on *Btk* plots are significant changes, compared to Gypchek plots in the first treatment year, and compared to control plots the second treatment year. What may be population rebounds on *Btk* plots are less for maple counts, and again are not statistically significant. It should be noted that sample sizes are not the same between host groupings, oak having more than twice the caterpillar counts of maple or hickory.

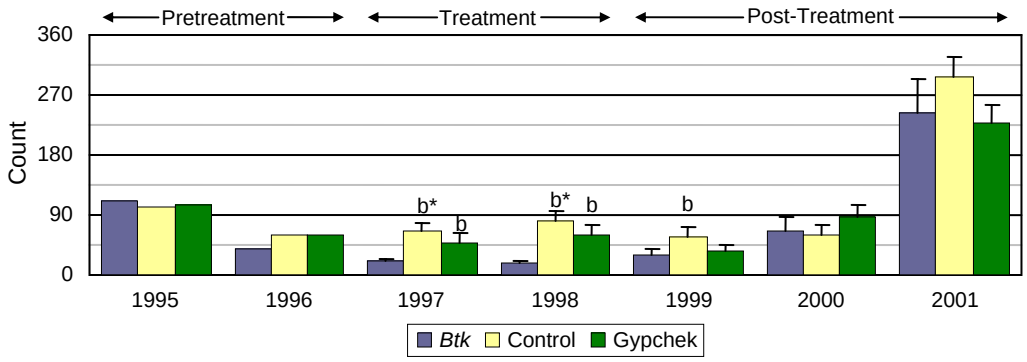

Figure 34. Total caterpillar counts sampled from oak (*Quercus* spp.) foliage that are univoltine and considered sensitive to treatments. Total count=1,869. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. An asterisk indicates $p<0.01$. Error bars indicate one standard error.

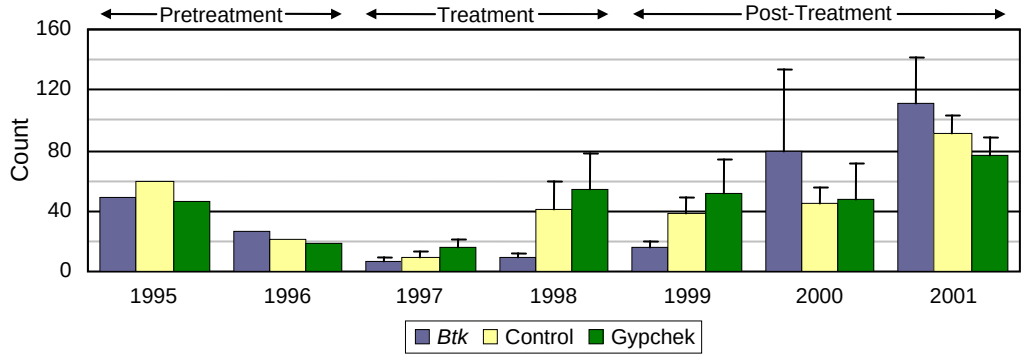

Figure 35. Total caterpillar counts sampled from maple (*Acer* spp.) foliage that are univoltine and considered sensitive to treatments. Total count=914. Error bars indicate one standard error.

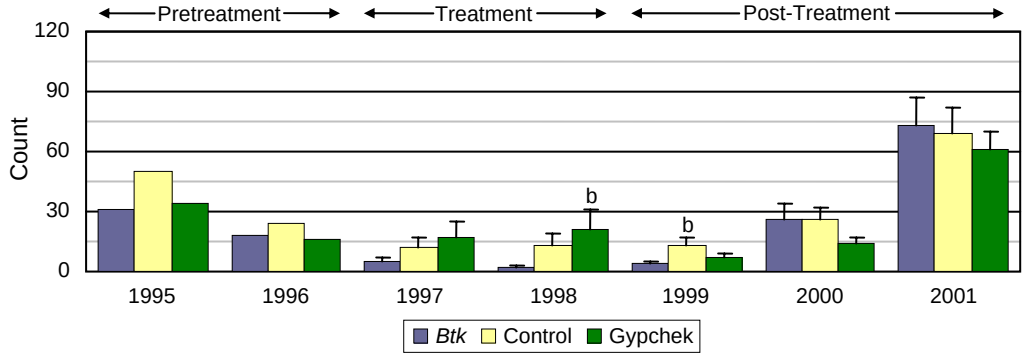

Figure 36. Total caterpillar counts sampled from hickory (*Carya* spp.) foliage that are univoltine and considered sensitive to treatments. Total count=536. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

Gelechiidae and Tortricidae were the only microlepidopteran families collected in large enough quantities from foliage as caterpillars to analyze. The caterpillars of these families typically make some type of retreat by rolling or folding over a portion of the leaf and tying it in place with silk. The caterpillars often feed within these retreats, especially when young. Though the microlepidopteran caterpillars of these families are typically much smaller than caterpillars of the macrolepidopterans, they are potential food sources for small insectivorous birds. Caterpillars of both families show relative *Btk* declines during treatment years and into post-treatment years. For gelechiids there is an overall decline on *Btk* plots though the year to year declines were not significant ($p<0.05$, count=7,143). The tortricid caterpillar declines on *Btk* plots were significant ($p<0.05$) for the second treatment year compared to both control and Gypchek plots (Figure 37). The low counts on *Btk* plots continue into the first post-treatment years, though these were not significant.

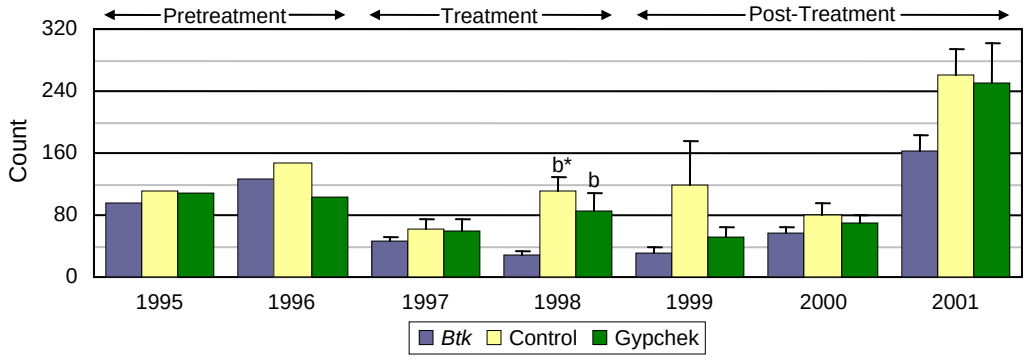

Figure 37. Total counts of Tortricidae caterpillars taken from foliage samples. Total count=2,165. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. An asterisk indicates $p<0.01$. Error bars indicate one standard error.

Light Trap Sampled Moths

Light trapping of Lepidoptera was performed for two primary reasons, to obtain large sample sizes and to increase sampled species richness. A drawback to light trap sampling is that the sample area is not known and this was dealt with in part with the large treatment plots. One must also keep in mind that some univoltine species overwinter as caterpillars or pupae, emerging the following spring as adults. Because of this, if treatment effects are present in adult populations, they will not be recognized in some species until the year after treatments. Like with the caterpillars, using total counts of all species does not reveal any treatment effects (Figure 38). In fact, year to year relative counts between treatments are very similar, which is probably influenced by the high species richness sampled (548 species) and the long sampling period.

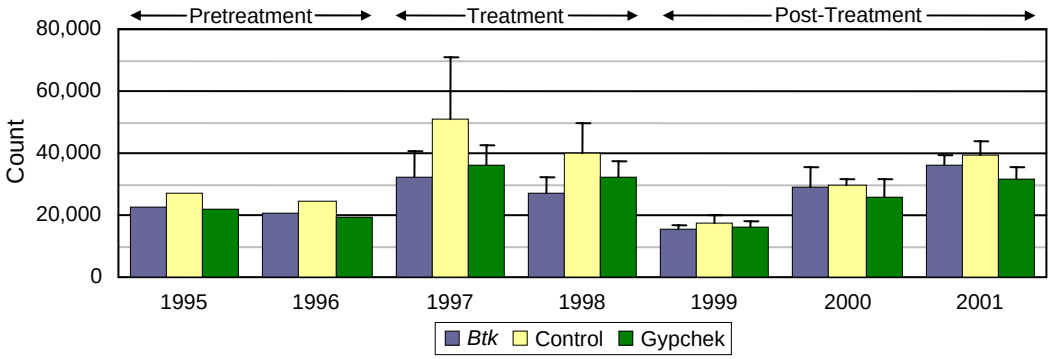

Figure 38. Total counts of light trap sampled moths grouped by plot treatment. Total count=646,874. Error bars indicate one standard error.

By narrowing down the univoltine moth species to include those whose caterpillars we think are susceptible to treatment timing, there is an overall reduction of moths on *Btk* plots during treatment years (Figure 39). Unlike the foliage caterpillars of the same grouping (Figure 27), treatments did not significantly reduce moth counts the first year of treatments or the first post-treatment year. There was also no significant rebound on *Btk* plots the second post-treatment year as seen with foliage sampled caterpillars.

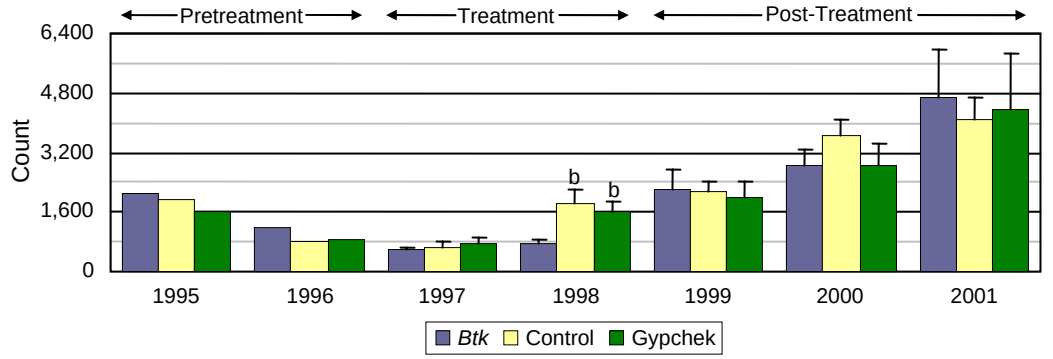

Figure 39. Total counts of light trap sampled univoltine moths whose caterpillars are considered sensitive to treatment timing grouped by plot treatment. Total count=55,523. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

Although there are some similarities in trends between the univoltine and multivoltine species considered sensitive to treatments, including the second treatment year, no reductions in *Btk* compared to control and Gypchek plots were significant (Figure 40). It should be noted that the total number of species considered sensitive to treatment timing were much lower for multivoltine species (n=9) compared with univoltine species (n=44).

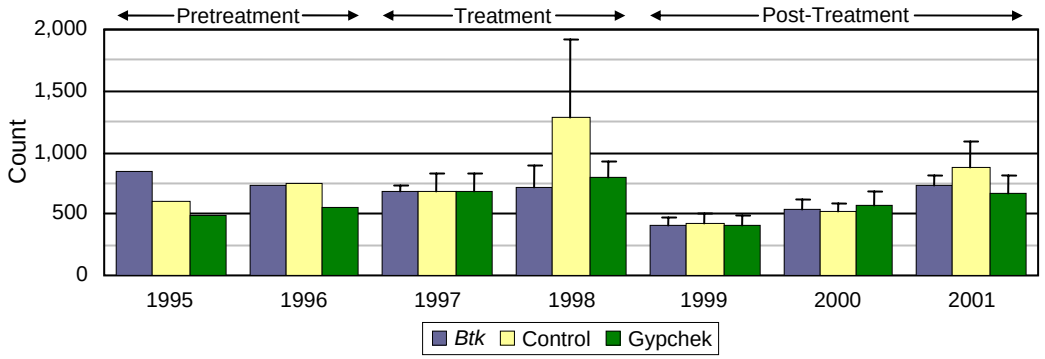

Figure 40. Total counts of light trap sampled multivoltine moths whose caterpillars are sensitive to treatment timing grouped by plot treatment. Total count=13,966. Error bars indicate one standard error.

As earlier mentioned, compared with caterpillar sampling methods, much larger sample sizes are produced by light trapping allowing additional analyzes. This is useful for different taxonomic levels and groupings based on larval host preferences. For species level analyses, we included only species that are well distributed across treatments.

The most abundant nontarget univoltine caterpillar species considered present during treatments, also had the highest counts for this group in light trap samples (Figure 41). *Itame pustularia* (Gn.), the lesser maple spanworm, which emerge as moths the same summer as caterpillars develop, showed a relative reduction in counts during the treatment years on the *Btk* plots as might be expected based on the foliage data, but reductions were not significant ($p<0.05$). There appears to be a rebound beyond pretreatment levels for the first post-treatment year. It should be noted that the increase on control plots during the second post-treatment year was restricted to plots near each other on the Monongahela National Forest. These counts dropped dramatically in the last post-treatment year, partially compensated by a large increase on the George Washington National Forest control plots.

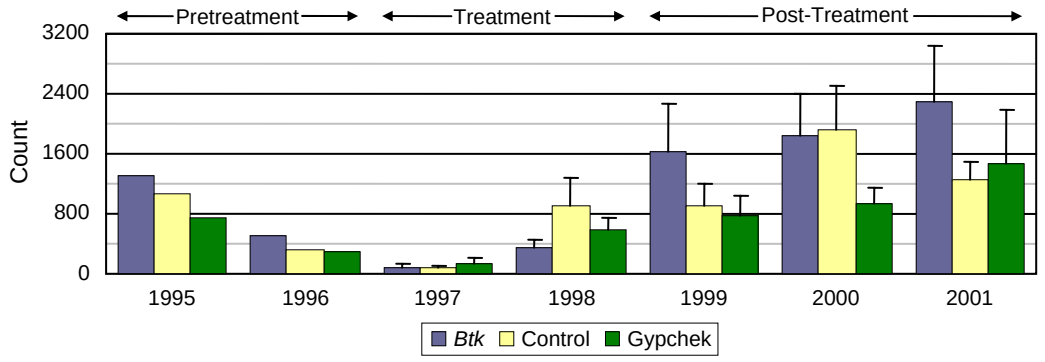

Figure 41. Total counts of *Itame pustularia* (Gn.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=19,382. Error bars indicate one standard error.

The second most commonly sampled univoltine moth with a caterpillar present during treatments was the forest tent caterpillar, *Malacosoma disstria* Hbn. (Lasiocampidae). This species also emerges as moths the same year as the caterpillars develop. There was a significant reduction on *Btk* plots when treatment and post-treatment years are combined, compared to both control and Gypchek plots ($p<0.01$). The reductions can be seen during both treatment years (Figure 42) and perhaps all through the post-treatment years. However, no single year had a reduction that was significant at a $p<0.05$.

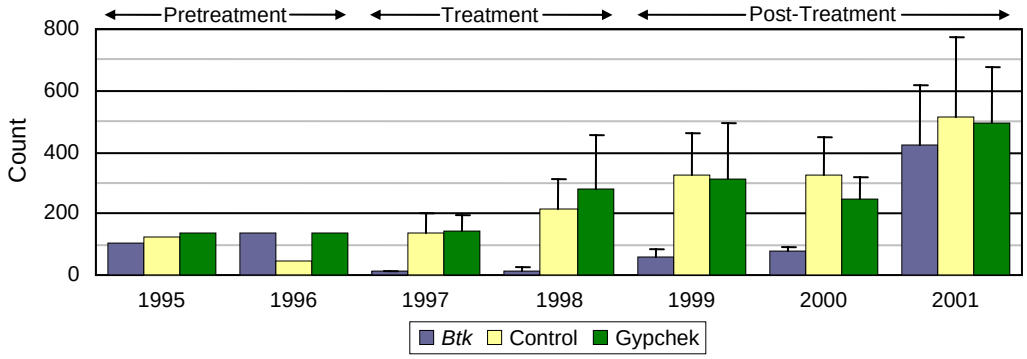

Figure 42. Total counts of *Malacosoma disstria* Hbn. moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=4,258. Error bars indicate one standard error.

The next most often sampled macrolepidoptera moth with caterpillar populations considered sensitive to treatment timing was the lymantriid *Dasychira dorsipennata* (B. & McD.). This species is reported as a generalist feeder on a wide array of woody shrubs and trees. Oaks have been reported as hosts, but we collected only three individuals from oak foliage. A fourth individual was sampled from hickory foliage, but this would be considered unusual. The remaining 20 caterpillars were sampled from under canvas bands and could have been feeding on a large number of woody plants in the area. *D. dorsipennata* offers a different situation in that the caterpillars overwinter as later instar larvae, sometimes pupae, with adults peaking mid-summer. The cold snap in the spring of 1997 very possibly did not affect the overwintered caterpillars that may have sheltered or avoided feeding on *Btk* treated leaves; populations remained fairly high. In addition, the first treatment year seems to be the beginning of a general up swing in population numbers (Figure 43). These factors may be why relative counts on treatments for the first treatment year are similar to the previous year. A relative reduction on *Btk* plots appears in the second treatment year, and is significant ($p<0.05$) the first post-treatment year. The relative counts remain lower in the second post-treatment year, rebounding relative to control and Gypchek plots in the third post-treatment year.

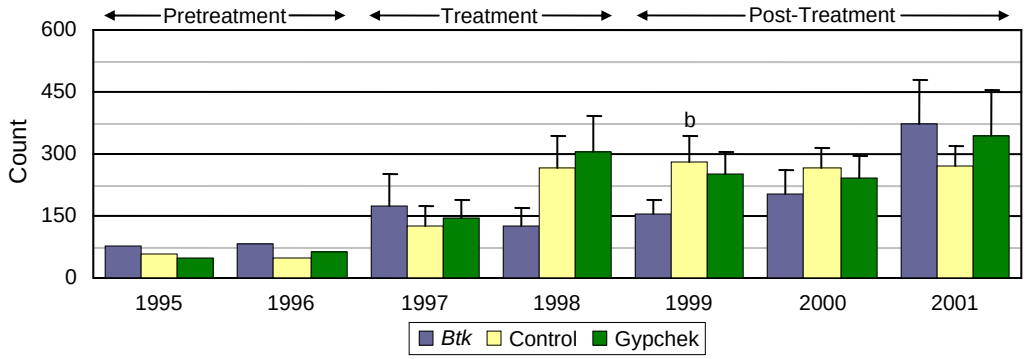

Figure 43. Total counts of *Dasychira dorsipennata* (B. & McD.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=3,892. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

The noctuid *Cosmia calami* (Hardv.), an oak feeder, had the next highest counts. It had very low counts for the pretreatment and treatment years, starting to increase the first post-treatment year (Figure 44). Although caterpillars develop into adults the same year, relative counts only first significantly declined on *Btk* plots the second treatment year. Significantly lower counts on *Btk* plots continue through the second post-treatment year. The third post-treatment year still has relative low counts on *Btk* plots compared to control and Gypchek plots.

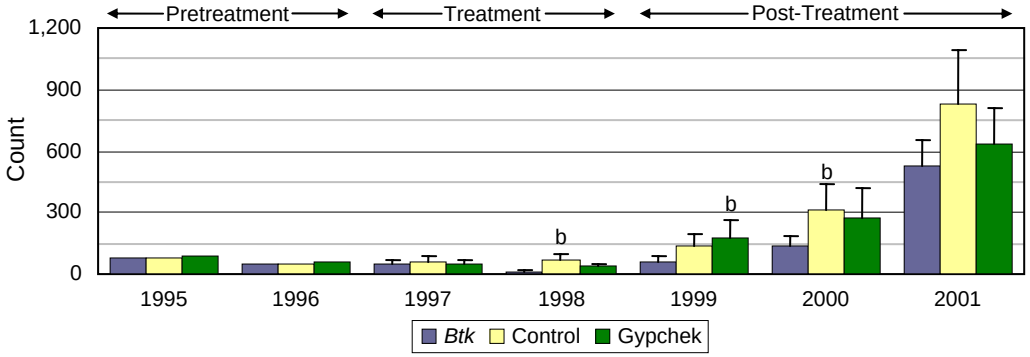

Figure 44. Total counts of *Cosmia calami* (Hardv.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=3,749. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

The eastern tent caterpillar, *Malacosoma americanum* (F.), a species prone to population fluctuations, feeds colonially as larvae on the foliage of cherry trees which were not evenly distributed within study plots. Light trap sampling of this strong flying moth may have compensated for the patchiness of the cherry tree distribution. The eastern tent caterpillar is at times a target of *Btk* treatments, but here it serves as a non-target organism. Although no significant declines in adult counts occurred within a single year, there were strong declines on *Btk* compared with control and Gypchek plots for the treatment years that remained to some

extent through the post-treatment years (Figure 45). There was a significant overall decline ($p<0.05$) on *Btk* plots compared to Gypchek plots when treatment and post-treatment years are combined.

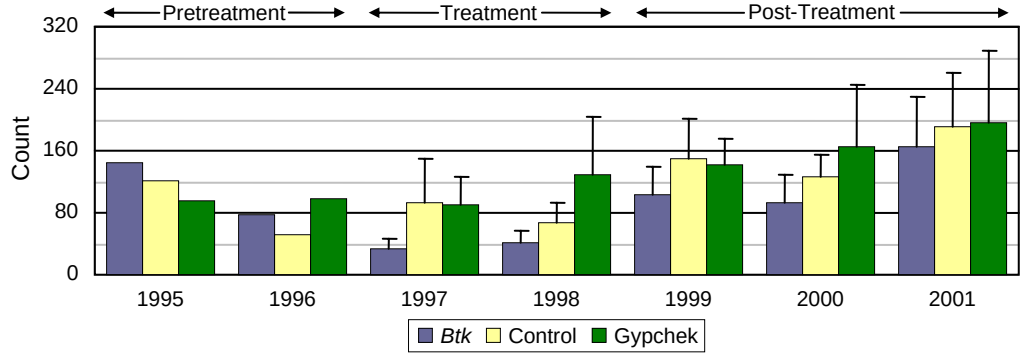

Figure 45. Total counts of eastern tent caterpillar (*Malacosoma americanum* (F.)) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=2,376. Error bars indicate one standard error.

Five noctuid underwing moths, *Catocala micronympha* (Hbn.) (count=2,238), *C. amica* Gn. (count=1,718), *C. palaeogama* Gn. (Drury) (count=880), *C. epione* (Drury) (count=780), and *C. dejecta* Stkr. (count=731) were next in highest counts. These species had similar overall trends with counts not evenly distributed across years. All species had their highest overall counts during the second and third post-treatment years (Figure 46). The two highest count species, *C. micronympha* and *C. amica* also had relatively high pretreatment counts. All species had their lowest counts occur during treatment years.

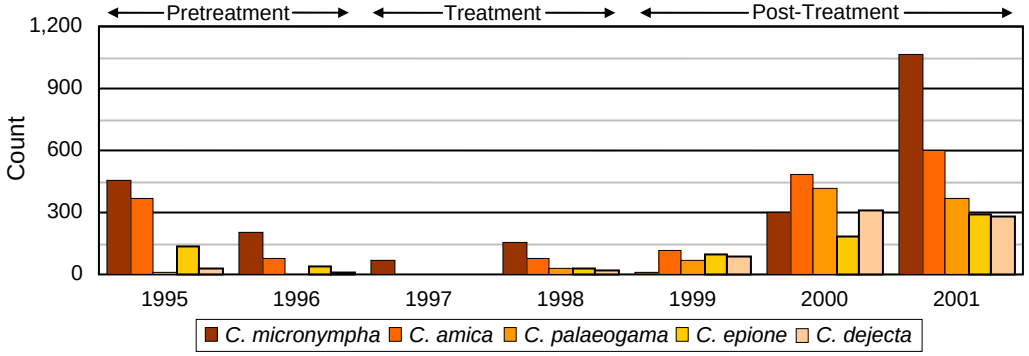

Figure 46. Total yearly moths counts of *Catocala* species sampled with light traps. These are all univoltine and considered sensitive to treatment timing.

When these *Catocala* species counts are broken down by year and treatment they had general trends similar to that of *C. micronympha* (Figures 46 & 47). The very low counts across treatments during the treatment years and first post-treatment year makes interpretation of treatments difficult. The downwards trends may be the results of increasing natural enemy populations and the extreme cold temperatures just prior to treatments. Whatever the cause, one might look at these results as possibly what happens to rare species during treatments. This

scenario is most often played out during the second treatment year when *C. micronympha* had a total of 66 individuals and the other four species a total of 5 individuals sampled across treatments.

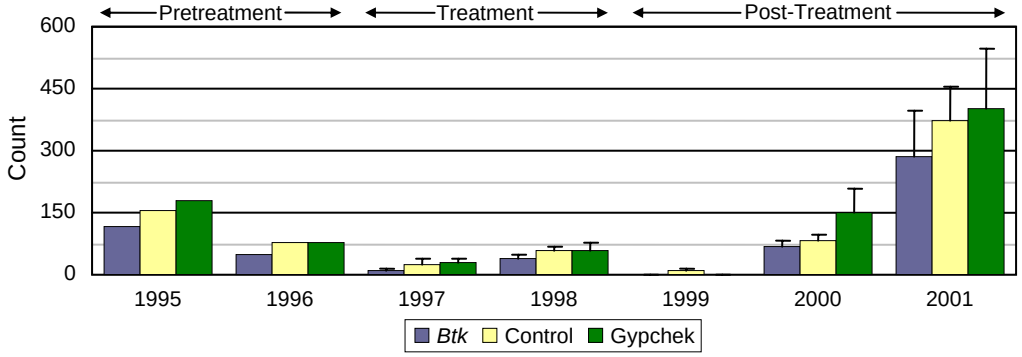

Figure 47. Total counts of *Catocala micronympha* (Hbn.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=2,376. Error bars indicate one standard error.

The next highest total count univoltine species thought to be sensitive to treatment timing was *Euchlaena tigrinaria* (Gn.) (Geometridae) (total count=563), but low counts in the pretreatment years (count=56) made interpretation difficult. The next most commonly collected species *Hypoprepia miniata* (Kby.) (Arctiidae) had a total count of 469 moths, which were fairly well distributed across years and treatments (Figure 48), but almost entirely from the George Washington National Forest. Contrary to results of other species sensitive to treatment time, *H. miniata* had a significant count increase on *Btk* plots compared to Gypchek plots when treatment and post-treatment years are combined. This species emerges as adults the same year as caterpillars develop so a treatment impact if present should be seen the first treatment year; instead a great increase occurred. Two plots accounted for most of the large increase on *Btk* plots, which then were the two *Btk* plots with the greatest declines the following year, possibly indicating that factors other than treatment effects influenced trends. The larvae of *H. miniata* feed on lichens and algae often exposed on the boles of trees, so one still can not totally discount the possibility of direct *Btk* treatment impacts the second treatment year with continuing relatively low counts the first post-treatment year.

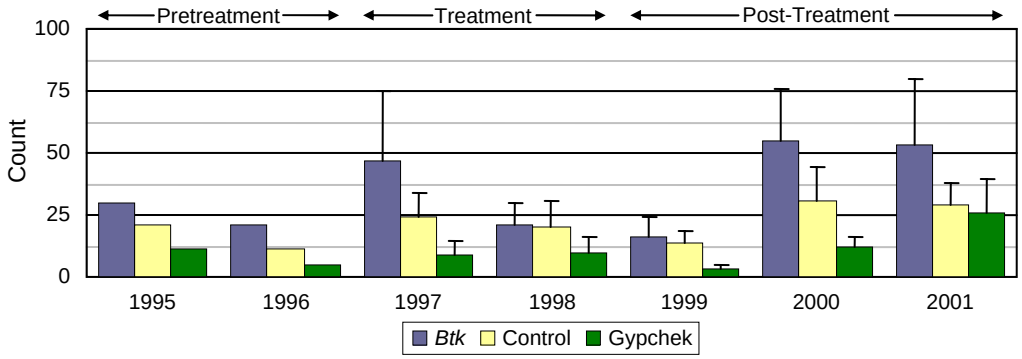

Figure 48. Total counts of *Hypoprepia miniata* (Kby.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=469. Error bars indicate one standard error.

The uneven count distribution among *Btk* plots contributes to the difficulty in interpreting *H. miniata* population fluctuations, but their low overall counts also make interpretation difficult. Another *Hypoprepia* species was the most abundant of the group we considered less sensitive to treatment timing. *H. fucosa* was also well represented on all plots. Based on our caterpillar sampling it was determined that *H. fucosa* caterpillar populations peaked a week or two later than *H. miniata*, so were less likely to be exposed to *Btk* treatments. And in fact, there were no clear declines on *Btk* plots compared to control or Gypchek plots (Figure 49). Like *H. miniata*, *H. fucosa* is a lichen and algae feeder and may be physiologically less susceptible to *Btk*.

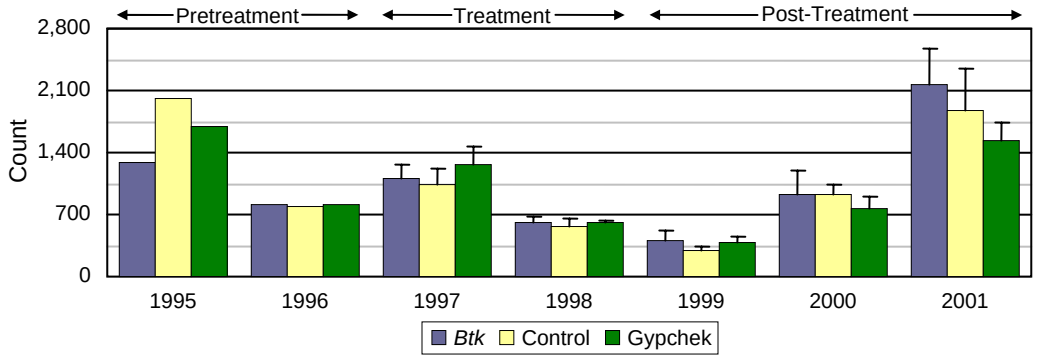

Figure 49. Total counts of *Hypoprepia fucosa* Hbn. moths, , a univoltine species considered less sensitive to treatment timing, sampled with light traps. Total count=21,857. Error bars indicate one standard error.

Other species we considered less susceptible to treatment timing have much smaller sample sizes, dropping first to *Lytrosis unitaria* (H.-S.) (Geometridae) with 2,716 individuals sampled (Figure 50). This is a species whose adults emerge the same year they are caterpillars. The relative small drop on *Btk* treatment plots and possible rebound starting in the first post-treatment year were not significant ($p<0.05$). The four next abundant species we considered less susceptible to treatment timing also had no noteworthy changes in relative counts: *Besma endropiaria* (G. & R.) (Geometridae), *Zale minerea* (Gn.) (Noctuidae), *Holomelina opella* (Grt.) (Arctiidae), *Zale unilineata* (Grt.) (Noctuidae).

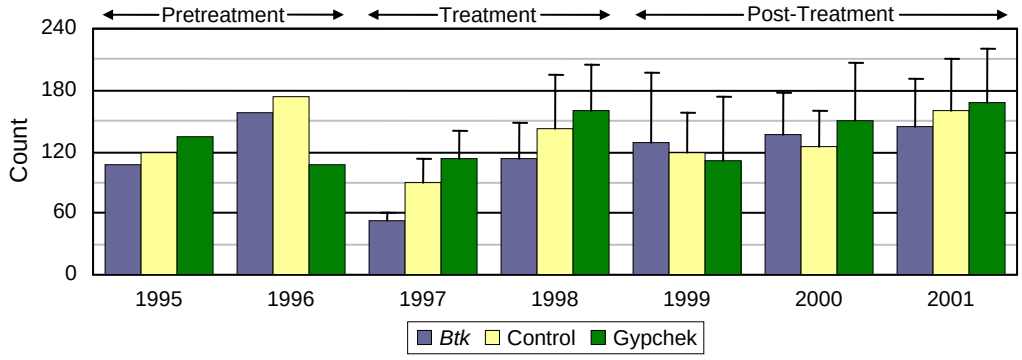

Figure 50. Total counts of *Lytrosis unitaria* (H.-S.) moths, a univoltine species considered less sensitive to treatment timing, sampled with light traps. Total count=2,716. Error bars indicate one standard error.

The two multivoltine species that were well represented on foliage, *Melanolophia canadaria* with higher counts early in the season during treatments and *Besma quercivoraria*, having higher counts after treatments were again taken as adults in large numbers. As with foliage counts, light trap counts show that *M. canadaria* had more apparent relative reductions (Figure 51) than *B. quercivoraria* (Figure 52).

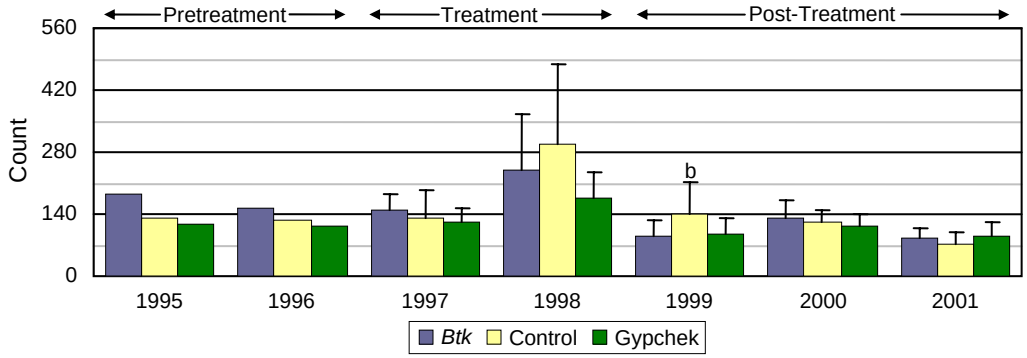

Figure 51. Total counts of *Melanolophia canadaria* (Gn.) moths, a multivoltine species considered sensitive to treatment timing, sampled with light traps. Total count=2,894. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

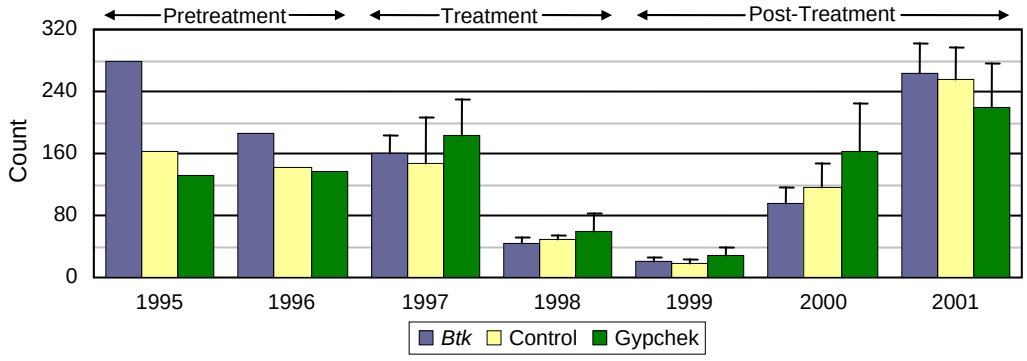

Figure 52. Total counts of *Besma quercivoraria* (Gn.) moths, a multivoltine species considered sensitive to treatment timing, sampled with light traps. Total count=2,864. Error bars indicate one standard error.

Two additional multivoltine species considered sensitive to treatment timing were collected in larger numbers, *Heterocampa guttivitta* (Wlk.) (Notodontidae) and *Hypagyrtis unipunctata* (Haw.) (Geometridae). Both species had significant declines during both treatment years (Figures 53 & 54).

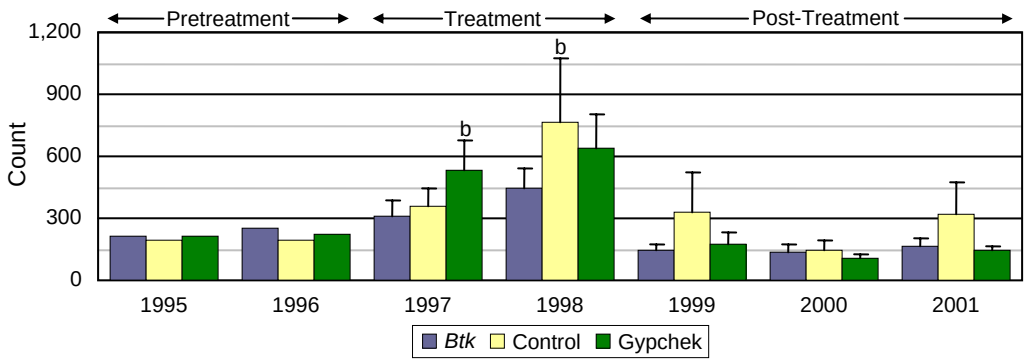

Figure 53. Total counts of *Heterocampa guttivitta* (Wlk.) moths, a multivoltine species considered less sensitive to treatment timing, sampled with light traps. Total count=6,013. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

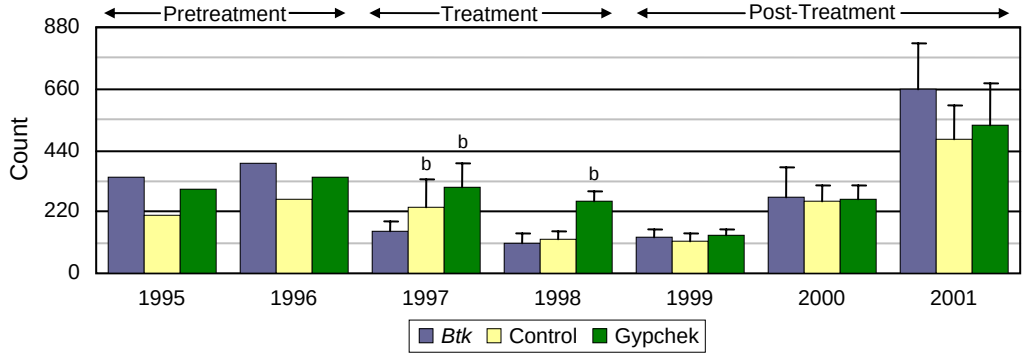

Figure 54. Total counts of *Hypagyrtis unipunctata* (Haw.) moths, a multivoltine species considered less sensitive to treatment timing, sampled with light traps. Total count=5,895. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

Pyromorpha dimidiata H. -S. (Zygaenidae) though typically grouped with the microlepidopterans, is fairly large. The showy crepuscular moth was regularly sampled with Malaise traps. Larvae have been recorded to feed on oak leaf litter, but we have not been able to confirm this through collection. It is a univoltine species with the adults most common early to mid June. The larvae may very well be present during treatments since there are significant ($p<0.05$) reductions on *Btk* plots during the second treatment year and the first and second post-treatment years (Figure 55).

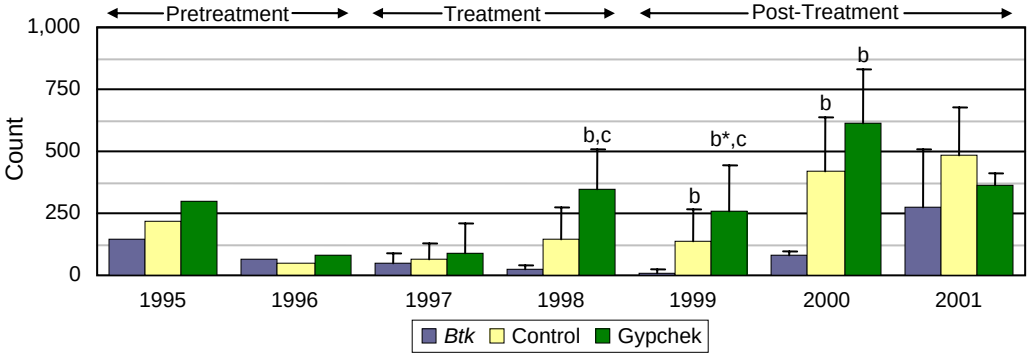

Figure 55. Total counts of *Pyromorpha dimidiata* H. -S. moths, univoltine species considered sensitive to treatment timing, sampled with Malaise traps. Total count=4,222. Lower case letters (b=*Btk*, c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. An asterisk indicates $p<0.01$. Error bars indicate one standard error.

Adults of Herminiinae (Noctuidae), many of which feed on dead leaves as caterpillars, were well represented in light trap samples. The caterpillars may serve as a food source for ground foraging birds and salamanders. Caterpillars are reported to regularly feed on oak leaf litter and may have a physiological susceptibility to *Btk* treatments. Based on lab feeding and/or literature, members

of the following genera were selected for analyses: *Bleptina* (*B. caradrinalis* Gn.), *Chytolita* (*morbidalis* (Gn.)), *Idia* (7 spp.), *Polypogon* (=*Zanclognatha*) (9 spp.), and *Phalaenophana* (*P. pyramusalis* (Wlk.). *Idia* species sampled with light traps in large numbers included *I. aemula* (Hbn.), *I. rotundalis* (Wlk.), and *I. americalis* (Gn.). For *Polypogon* species, only *P. laevigata* (Grt.) and *P. ochreipennis* (Grt.) were in large numbers. When the genera known to feed on leaf litter are analyzed as a group there may be a trend of reduction on *Btk* plots, but it is slight with small relative declines on *Btk* plots during the treatment years and the first post-treatment year (Figure 56). The relative rebound during the second post-treatment year is no better than a weak indication caterpillars may have been suppressed by *Btk*.

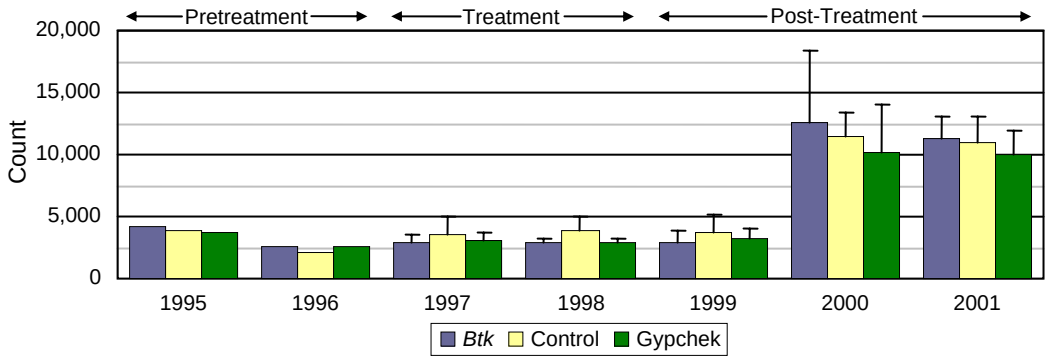

Figure 56. Total counts of light trapped Herminiinae (Noctuidae) moths. Total count=114,037. Error bars indicate one standard error.

Since spray cards were put on the leaf litter to monitor application of treatments, we know *Btk* droplets reached the forest floor. Subsequently, we conducted preliminary laboratory testing of *Idia* species that feed on dead, dry oak leaves (Kish 2004). Leaves were treated with the same formulation and with spray densities of *Btk* similar to what was seen on spray cards used on *Btk* treated plots. Mortality levels in the laboratory were very high. Possibly in the field *Idia* caterpillars feed on the lower surface of the top litter leaves and at lower levels in the litter, and they do not come into contact with *Btk*. Also, caterpillars of some species might not be present, or are mature caterpillars at the time of treatment application.

Symphyta (Sawflies)

Sawfly larvae overlap the feeding niches of caterpillars, with most species feeding exposed on foliage. By far the most abundant species feeding on tree foliage sampled during the study belong to the families Pergidae (69%) and Tenthredinidae (27%). Members of these families are typically at high numbers during treatments and peak shortly thereafter (Strazanac et al. 2003b, Braud et al. 2003). They appear not as impacted by the cold snap during the first treatment year (Figure 57) as the caterpillars. In addition, they also do not seem to have had strong trends attributable to treatment applications. The only relative change was a reduction on control plots during the second post-treatment year caused a large decline in sampled Pergidae (Figure 58). Tenthredinidae larval count fluctuations occurred, but these year to year changes did not appear related to treatment applications (Figure 59).

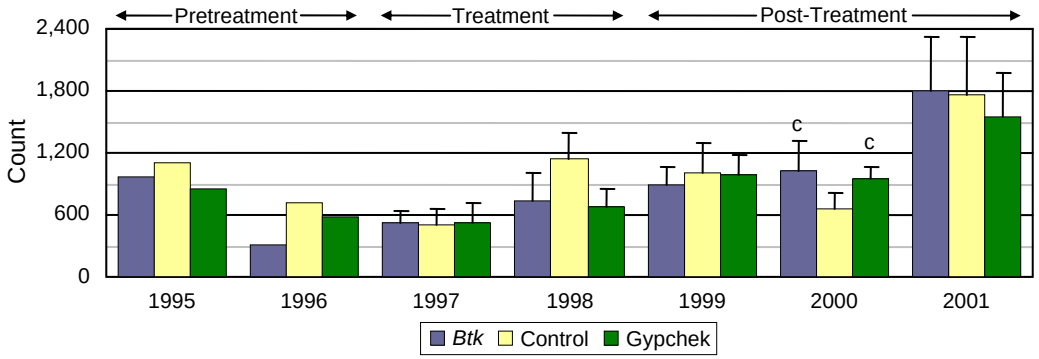

Figure 57. Total counts of Sawfly larvae (Symphyta) taken from foliage samples. Total count=19,261. Lower case letters (c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

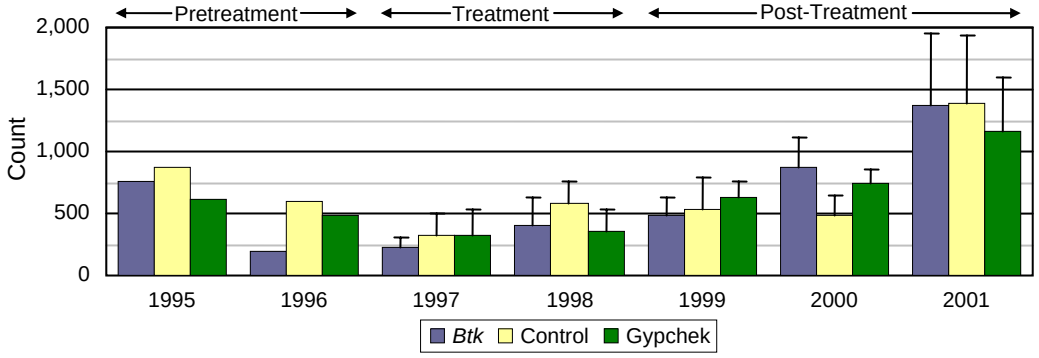

Figure 58. Total counts of Pergidae larvae (Symphyta) taken from foliage samples. Total count=13,367. Error bars indicate one standard error.

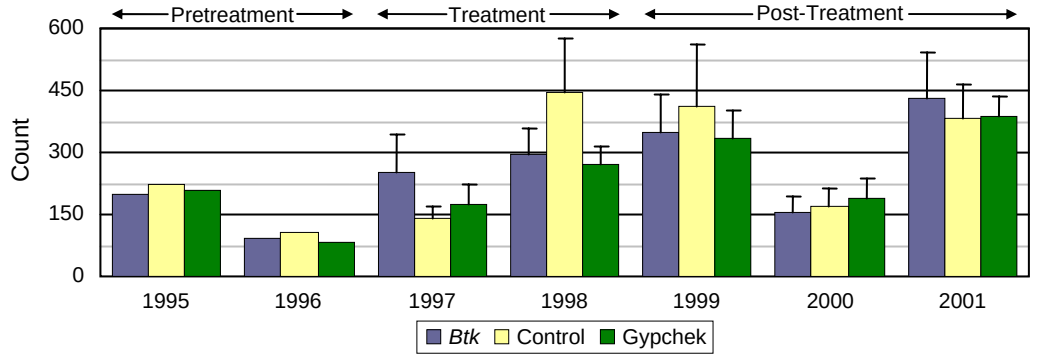

Figure 59. Total counts of Tenthredinidae larvae (Symphyta) taken from foliage samples. Total count=5,287. Error bars indicate one standard error.

For the total sawfly adults sampled with Malaise traps there were some relative reductions in *Btk* plots (Figure 60), only slightly in the first treatment year, but strong enough during the second post-treatment year to be significant ($p<0.05$). The increases and relative differences during the second post-treatment year were in large part the result of increases of *Pristiphora banksi* Marlatt

on the George Washington National Forest. It may not be defensible to say the relatively low post-treatment counts on *Btk* plots were the result of treatments. However, it should be pointed out that of the three control and three Gypchek plots on the George Washington National Forest there were large increases on five of these plots while no large increases occurred on the three *Btk* plots. When *P. banksi* is analyzed alone, there is no statistically significant support for a *Btk* effect for the post-treatment years (Figure 61), though the count reductions on *Btk* plots are clear. *P. banksi* feeds on blueberries and its relatives and is multivoltine.

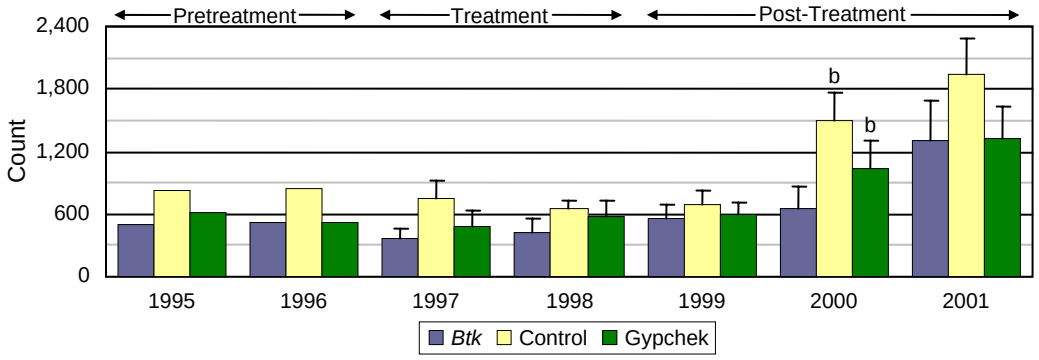

Figure 60. Total counts of adult sawflies (Symphyta) taken with Malaise traps. Total count=16,668. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

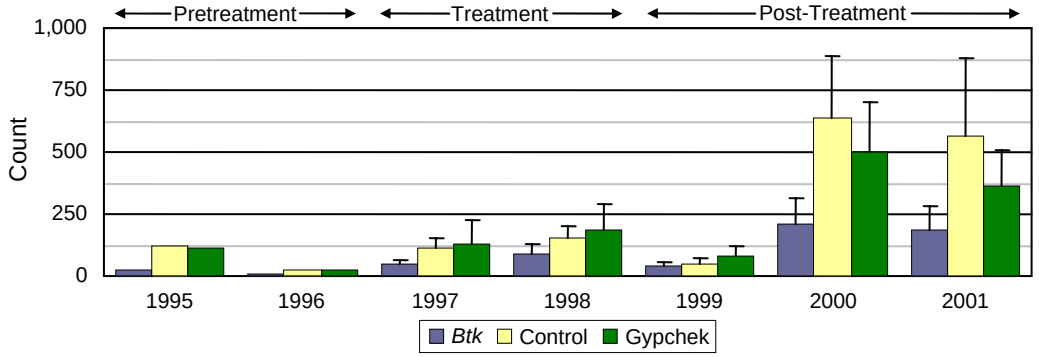

Figure 61. Total counts of adult *Pristiphora banksi* Marlatt (Tenthredinidae) taken with Malaise traps. Total count=3,692. Error bars indicate one standard error.

The most abundant species sampled as an adult was *Acordulecera dorsalis* Say. The larvae feed gregariously and exposed on oak, and a large portion of their population would be present during treatments. Counts of adults sampled with Malaise traps did not show any evidence of being impacted by *Btk* treatments (Figure 62). In a small scale laboratory assay *Acordulecera* species (probably nearly all *A. dorsalis*) demonstrated they may be susceptible to *Btk* (Braud 2001).

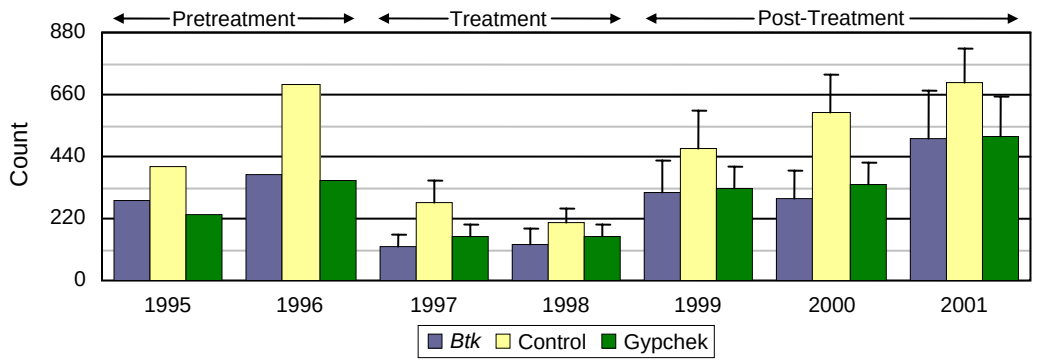

Figure 62. Total counts of adult *Acordulecera dorsalis* Say (Pergidae) taken with Malaise traps. Total count=7,441. Error bars indicate one standard error.

Parasitoids

Parasitoids play an important role moderating population fluctuations of defoliators in eastern forests (Van Driesche et al. 1996). The significant decreases in macrolepidoptera attributed to *Btk* treatment counts as previously demonstrated (Figure 27), may cause indirect impacts on their parasitoids. Members of two groups of parasitoids that attack macrolepidopteran caterpillars were sampled in sufficient numbers to analyze, the Tachinidae (Diptera) and parasitic wasps belonging to the Braconidae and Ichneumonidae.

With more than 1,300 species described from North America alone (O'Hara and Wood 2004), tachinid flies are by far the most diverse group of parasitic flies. Most species attack lepidopteran caterpillars, and the majority of these attack macrolepidopteran caterpillars (Arnaud 1978). We sampled tachinid flies with Malaise traps and reared them from caterpillars sampled from foliage (Strazanac et al. 2001). When the species that are known to attack macrolepidoptera are pooled (Figure 63), there is a strong relative decline in the second treatment year on *Btk* plots compared to control and Gypchek plots. As with the decline in early spring macrolepidopteran caterpillars that seems attributable to a cold snap in 1997, tachinid fly counts declined as well. The significant decline in 2001 *Btk* counts, however, did not occur for either of the two most abundant tachinid genera, *Tachinomyia* and *Phorocera* (total count=354), suggesting it to be an anomaly (Figure 64).

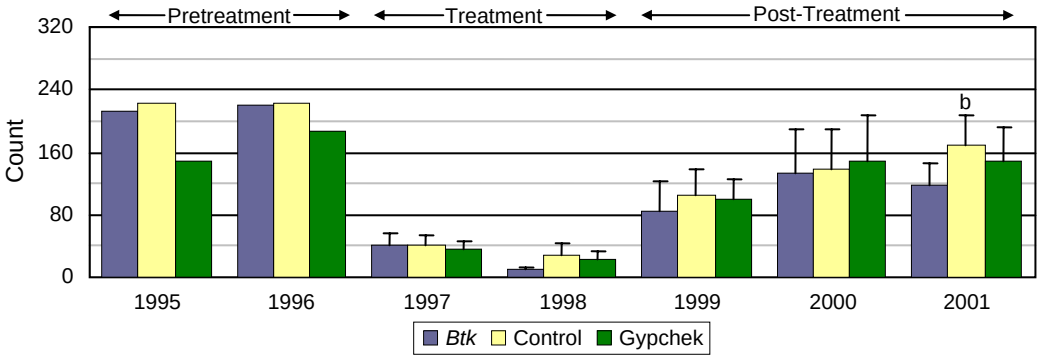

Figure 63. Total counts of Tachinidae species known to attack macrolepidoptera taken with Malaise traps. Total count=2,540. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with p<0.05.

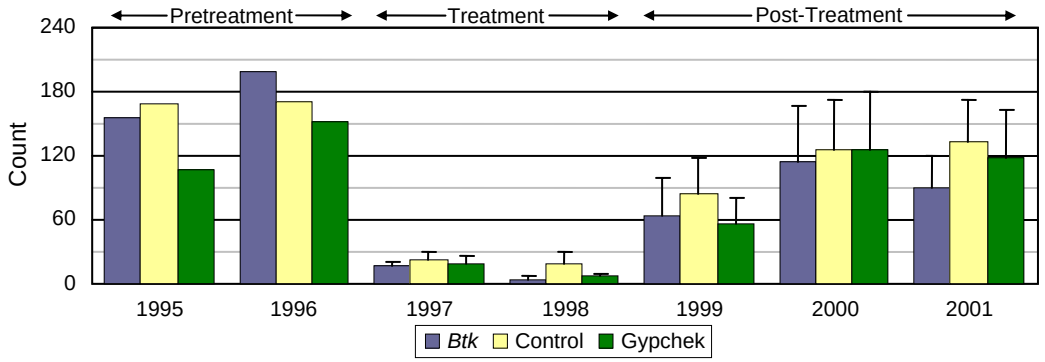

Figure 64. Total counts of *Tachinomyia* species known to attack macrolepidoptera taken with Malaise traps. Total count=1,951. Error bars indicate one standard error.

Members of *Tachinomyia* and *Phorocera* are univoltine (based on rearing results) and after emerging from their host, pupate in the soil and overwinter as puparia, eclosing as adults the following spring. As might be expected, declines were found on *Btk* plots in the second treatment year in both of these genera. Members of the *Tachinomyia* had the highest counts of any group that attack macrolepidopteran caterpillars. *Tachinomyia variata* Curran was reared from foliage caterpillars more than any other member of that genus. Declines for this species were significant for the second treatment year and the first post-treatment year (Figure 65). The low counts of *Phorocera aequalis* (Reinhard) (total count=347) may make the results no more than suggestive, but counts dropping to zero on *Btk* plots the second treatment year seems noteworthy (Figure 66).

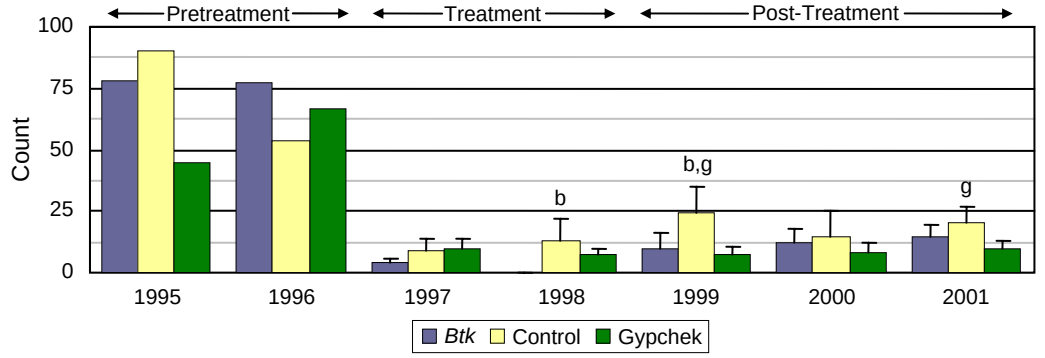

Figure 65. Total counts of *Tachinomyia variata* Curran taken with Malaise traps. Total count=575. Lower case letters (b=*Btk*, g=Gypchek) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with p<0.05.

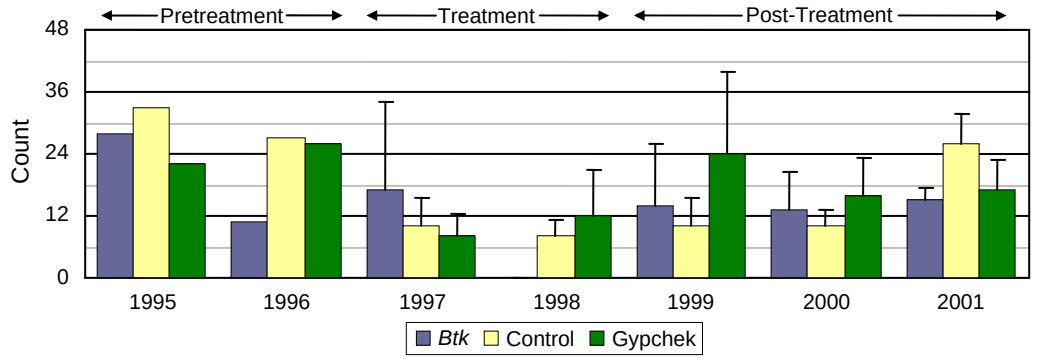

Figure 66. Total counts of *Phorocera aequalis* (Reinhard) taken with Malaise traps. Total count=347. Error bars indicate one standard error.

Within the highly diverse Ichneumonoidea, only the Ichneumoninae (Ichneumonidae) (Wahl 1993) and Microgastrinae (Braconidae) (Whitfield 1997) are made up of species that are known to only attack caterpillars. As with the tachinid flies, Ichneumonoidea were sampled with Malaise traps and reared from foliage caterpillars (Petrice et al. 2004). These two groups appear not to have been similarly affected by the 1997 spring cold snap, with Ichneumoninae increasing and Microgastrinae decreasing in sample counts. If an indirect impact of *Btk* occurred through the lowering of caterpillar populations, it was weak based on counts. The Ichneumoninae counts did decrease the second treatment year (Figure 67), but only compared with its counts relative to control and Gypchek the first treatment year. The Microgastrinae after a decrease across all plots the first treatment year (Figure 68), rebound less on *Btk* plots the second treatment year. They then decrease significantly the first post-treatment year. Whereas, the tachinid flies had similar phenologies, within the Ichneumoninae and Microgastrinae phenologies are more varied and may influence results differently when species are grouped.

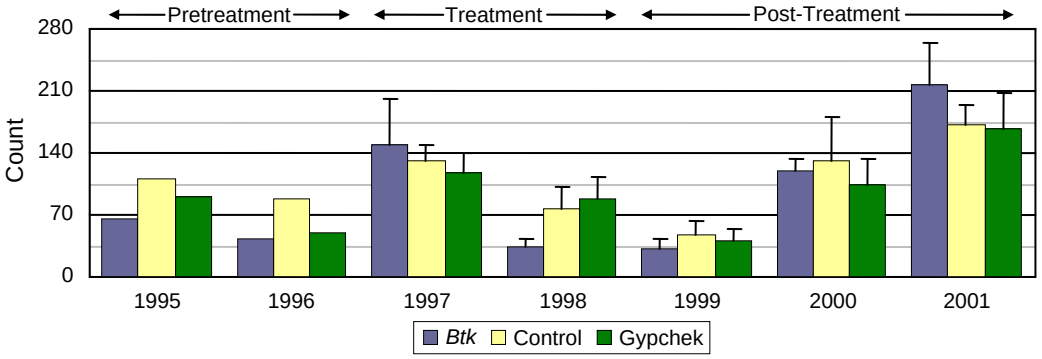

Figure 67. Total counts of Ichneumoninae taken with Malaise traps mid-season. Total count=2,071. Error bars indicate one standard error.

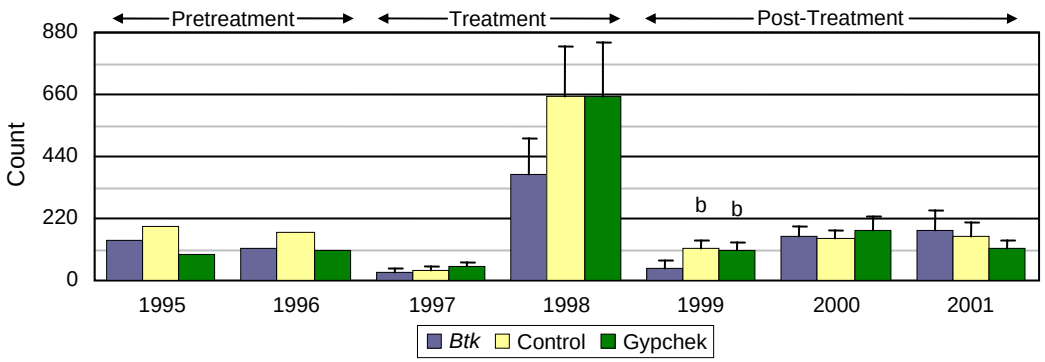

Figure 68. Total counts of Microgastrinae taken with Malaise traps mid-season. Total count=3,796. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

Predators

Carabid beetles and spiders (Araneae) were sampled in high numbers from under canvas bands and in pitfall traps. Spiders were also sampled from foliage. The predatory stinkbugs (Pentatomidae) were sampled from foliage, under canvas bands, and with Malaise traps. As with the parasitoids, an impact from *Btk* treatments would likely only be indirect through the loss of *Btk* susceptible caterpillars. Whereas parasitoids have some level of host specificity, predatory arthropods are generally opportunistic. Obvious exceptions within our sampling are some species of carabid beetles and the predatory stinkbugs that prefer caterpillars.

Spiders' general lack of prey specificity for caterpillars may be indicated by the lack of any trend in any species that might implicate having *Btk* treatment impacts. When analyses were made of various taxonomic level groupings (i.e., genus, family, subfamily) or all species grouped, no indication of indirect *Btk* impact could be detected. In an attempt to test if grouping species by habitat preference and predation method could pull together species that as a group would indicate *Btk* indirect impacts, again no impacts were found. Foliage, for example, from which caterpillars and spiders were sampled, did not indicate any distinct trends in spider decline when caterpillars were reduced in total counts (Figure 69). No indication of impact was seen after separating species that capture prey with webs (Figure 70) versus ambushing/hunting behavior (Figure 71).

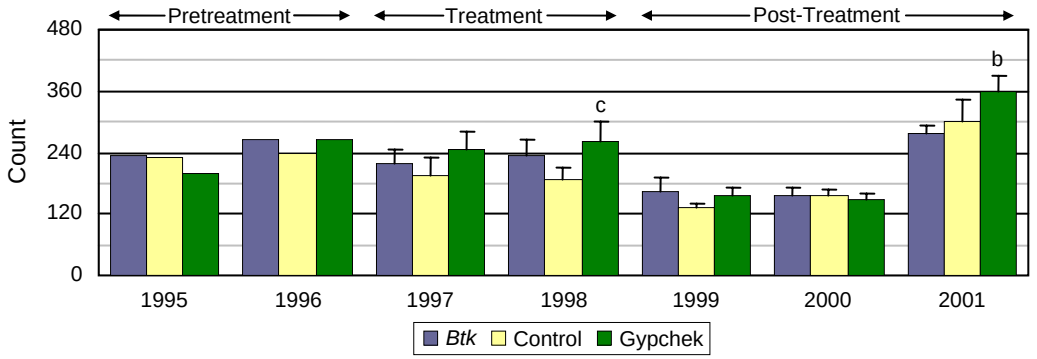

Figure 69. Total counts of adult spiders sampled from foliage. Total count=4,624. Lower case letters (b=*Btk*, c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

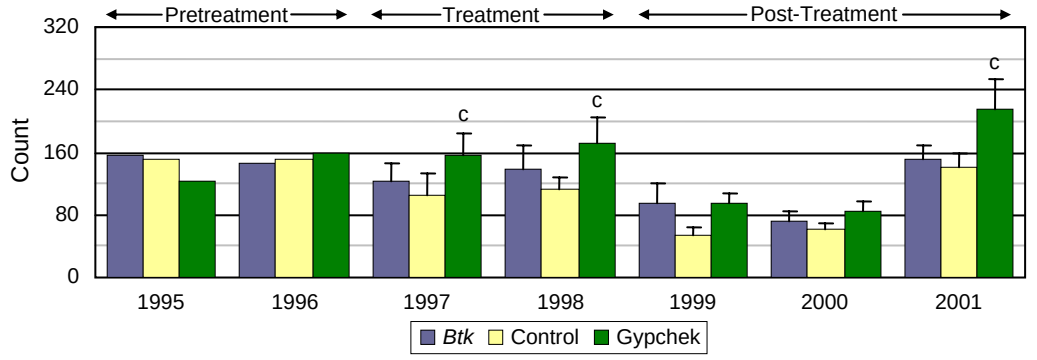

Figure 70. Total counts of adult spiders that build webs to capture prey sampled from foliage. Total count=2,658. Lower case letters (c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

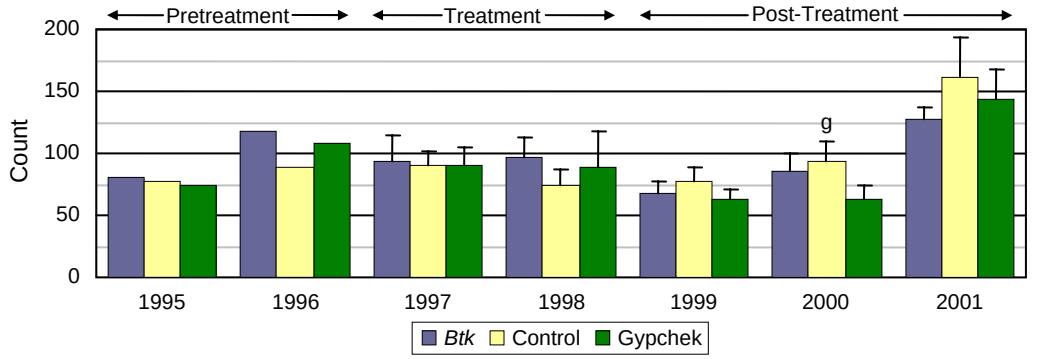

Figure 71. Total counts of adult spiders that hunt or ambush prey sampled from foliage. Total count=1,966. Lower case letters (g=Gypchek) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

Carabid beetle counts when grouped only by sampling method include many species with no known prey specificity (based on Larochelle and Larivière 2003). In these groupings, no indirect impact of *Btk* was indicated (Figures 72 & 73). A number of species sampled have been reported to prey specifically on caterpillars, from under canvas bands, *Cymindis limbatus* Dejean, *Cymindis platicollis* (Say), and *Platynus decentis* (Say), and in pitfall traps, *Pterostichus* species. Also from pitfall traps, *Carabus goryi* Dejean, sampled in large numbers, is reported to feed prey on caterpillars and caterpillar-like larvae. Of these, only one species appeared to have any indication of impact by the removal of caterpillar prey from *Btk* plots by treatments, *Pterostichus tristus* (Dejean) (Figure 74). Relative decreases on *Btk* plots compared to control and Gypchek plots were not significant for any single year, but were when treatment and post-treatment years were combined. It should be noted that the last post-treatment year accounts for most of the overall decrease on *Btk* plots.

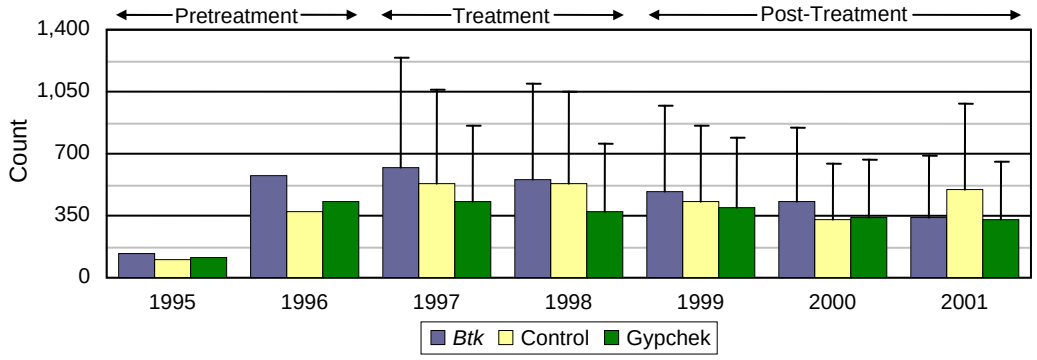

Figure 72. Total counts of Carabidae sampled from under canvas bands. Total count=8,317. Error bars indicate one standard error.

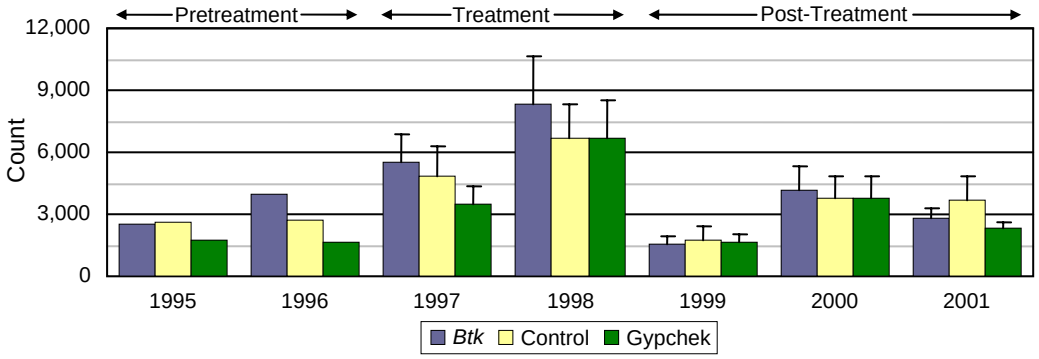

Figure 73. Total counts of Carabidae sampled with pitfall traps. Total count=76,018. Error bars indicate one standard error.

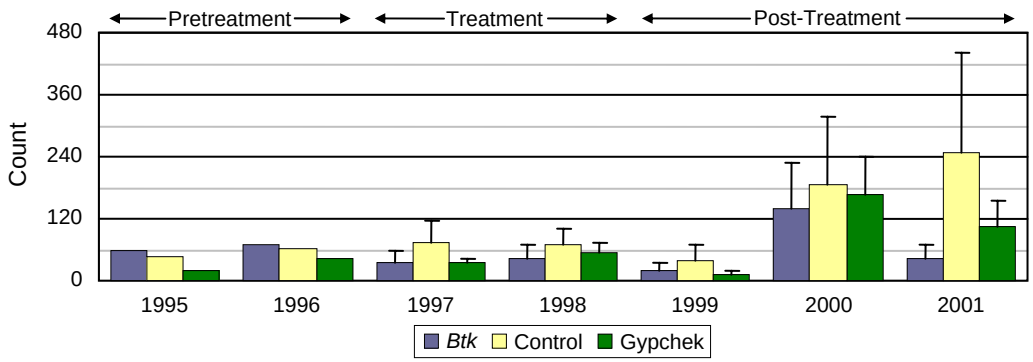

Figure 74. Total counts of *Pterostichus tristus* (Dejean) sampled with pitfall traps. Total count=1,558. Error bars indicate one standard error.

Most predatory stinkbugs feed on caterpillars, but will take other slow moving soft bodied insects, such as sawfly larvae. Total counts sampled from foliage and under canvas bands were similar, 662 and 612 respectively. Canvas band counts were not well distributed across years for analyses, with three-quarters of the total count collected the third post-treatment year, and only 5 specimens the first treatment year. Foliage counts were better distributed across years (Figure 75). Stinkbugs typically overwinter as adults, less often mature nymphs, so if a reduction of caterpillars impacts their adults counts, it would be more likely indicated during the second year of treatments. This is the case with *Btk* plots having relative declines the second treatment year, though not significant. Other fluctuations, most notably the rebound on *Btk* plots the first post-treatment year when caterpillar counts were still low, may weaken any indication of a *Btk* impact.

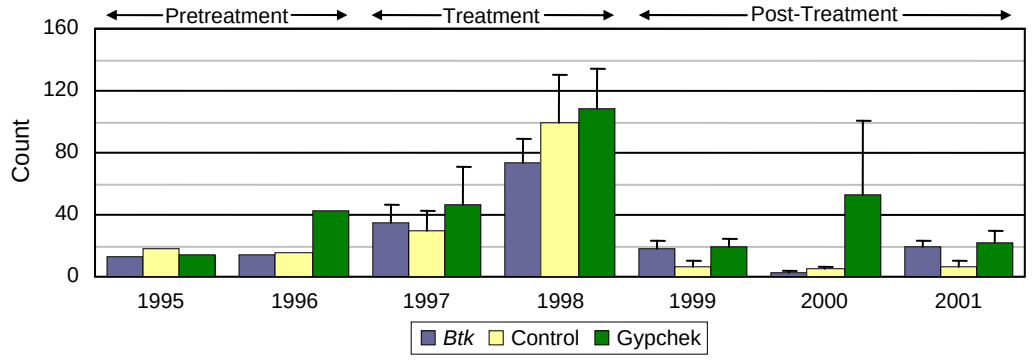

Figure 75. Total counts of adult predatory stink bugs (Pentatomidae) sampled from foliage. Total count=662. Error bars indicate one standard error.

Counts from Malaise samples, though quite low, do indicate a significant *Btk* treatment impact during the second treatment year as might be expected (Figure 76). In the following post-treatment year, *Btk* plot counts continue to be low, as might be expected, before relative counts return to pretreatment year levels.

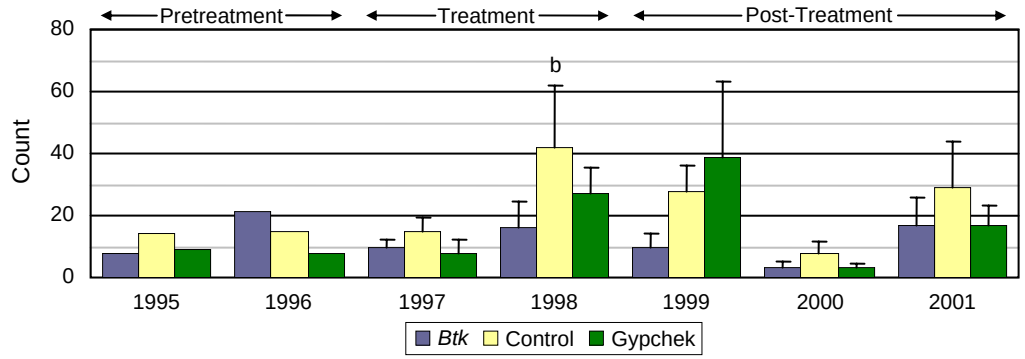

Figure 76. Total counts of adult predatory stink bugs (Pentatomidae) sampled with Malaise traps. Total count=347. Lower case letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

Pollinators

Pollinators were included for study because of the original intent to include a defoliation treatment caused by gypsy moth. Though defoliation started at low levels in the baseline years, supporting this plan, *Entomophaga maimaiga*, soon became established and very thoroughly removed gypsy moth from the plots. Low gypsy moth numbers continued throughout the study. The most important pollinators of flowering plants, the bees (Apoidea) and less efficient pollinators, the hover flies (Syrphidae) continued to be tallied in case major defoliation did occur. Although not statistically significant, the bee counts did decline on *Btk* plots relative to control plots during the treatment years, then rebounded on the first post-treatment year (Figure 77). The hover fly counts were more as expected, with pretreatment and treatment years having similar relative counts (Figure 78). The apparent rebound in the bee counts repeated in the first post-treatment year is very similar to what occurred with the hover flies and may indicate the *Btk* plot count increases were not related to treatments.

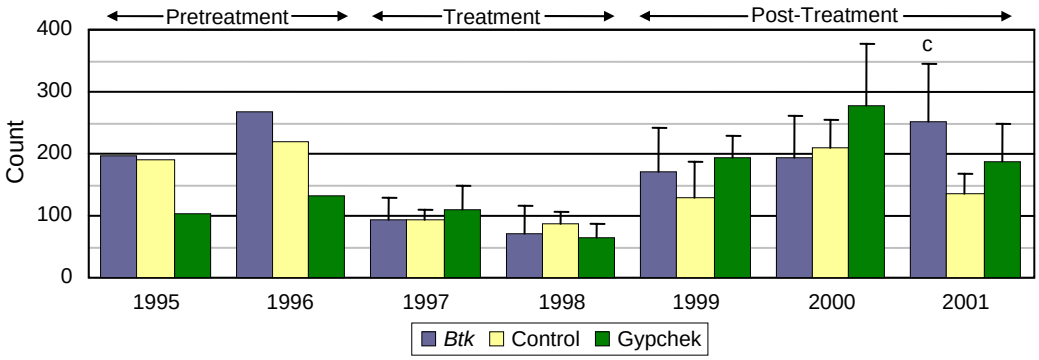

Figure 77. Total counts of bees (Apoidea) sampled with Malaise traps. Total count=2,800. Lower case letters (c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

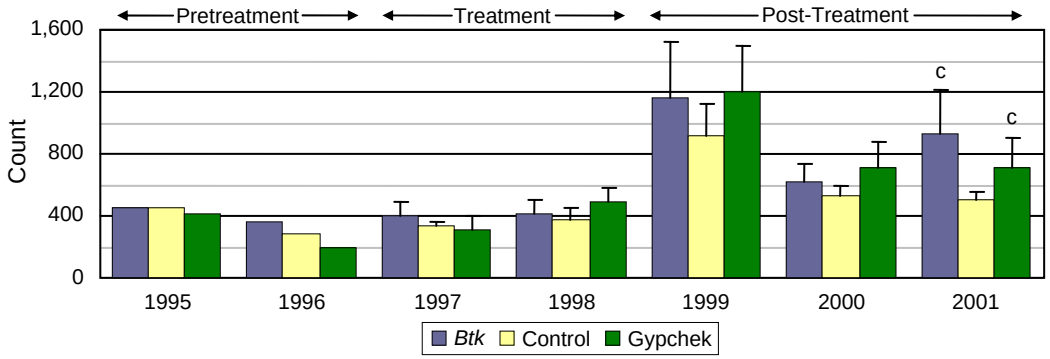

Figure 78. Total counts of hover flies (Syrphidae) sampled with Malaise traps. Total count=7,752. Lower case letters (c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$. Error bars indicate one standard error.

Detritivores and Omnivores

Other groups tallied for possible defoliation treatment effects included various detritivores and omnivores sampled with pitfall traps. The groups with the highest counts sampled included the ants (Formicidae), crickets and grasshoppers (Orthoptera), and the harvestmen/daddy-long-legs (Opiliones). Comparing the first treatment year ant counts with pretreatment years Wang (2000) determined that there was no *Btk* impact, though there was an increase of ants on *Btk* plots (Figure 79). Ants continued to be tallied throughout the study with no trend of *Btk* impacts indicated.

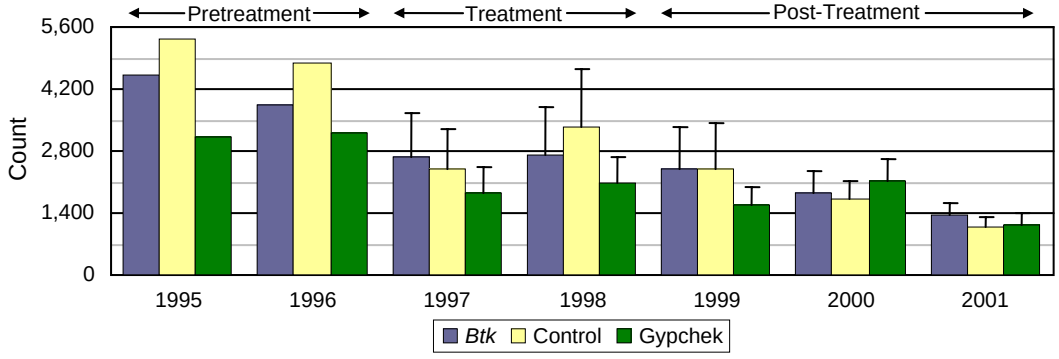

Figure 79. Total counts of ants (Formicidae) sampled with pitfall traps. Total count=55,462. Error bars indicate one standard error.

The camel crickets (Gryllacrididae) made up more than 95% of orthopteran insects sampled with pitfall traps. No significant difference in counts on the *Btk* plots compared to the control or Gypchek plots occurred (Figure 80). The significant decline on Gypchek plots compared to control plots is considered an anomaly. The harvestmen (Opiliones) are opportunistic omnivores, including taking live prey. There are relative weak declines on *Btk* plots compared to the control plots during the treatment years, then an apparent rebound the first post-treatment year; none are statistically significant (Figure 81). The same is true comparing control and Gypchek plots.

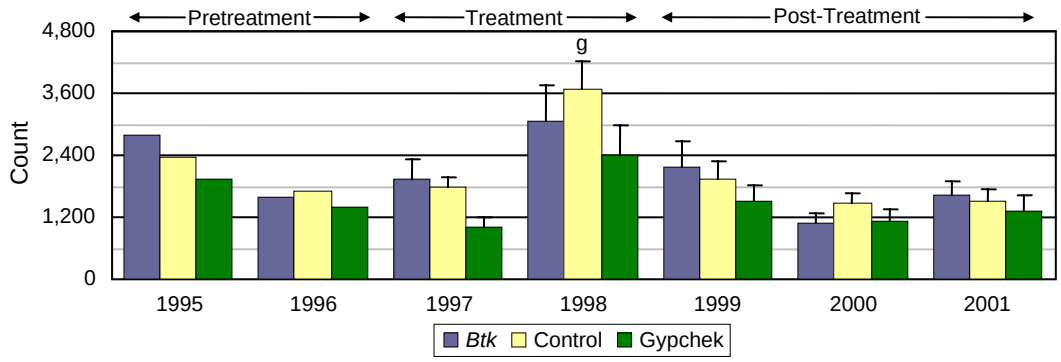

Figure 80. Total counts of crickets and grasshopper (Orthoptera) sampled with pitfall traps. Total count=39,409. Lower case letters (g=Gypchek) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year with $p<0.05$.

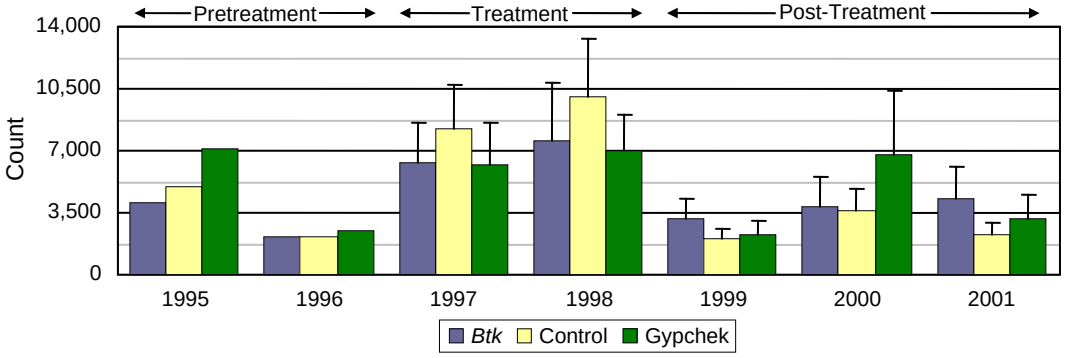

Figure 81. Total counts of harvestmen (Opiliones) sampled with pitfall traps. Total count=99,803. Error bars indicate one standard error.

Gypsy Moth Egg Mass Survey

Surveys of gypsy moth egg masses were conducted in late winter and early spring along transects on subplots by a standard method (Kolodny-Hirsch 1986). During each of the 7 years of sampling, the survey was conducted along the central four transects (A, B, C, D) starting at the beginning of each, then at every 100 meters for a total of 28 points per subplot (Figure 3). At the survey points, counts were made in a 0.01 ha (1/40 acre) area. Each year the counts were averaged for each subplot and scaled to egg/masses per hectare (Table 4). Counts were made using binoculars by the same experienced individual for all years.

Gypsy moth began to enter all of the study plots in 1995, and produced relatively high egg mass counts on the northern George Washington National Forest plots and two widely spaced plots on the Monongahela National Forest. Counts continued at similar levels on both forests in 1996, but dropped to a mean of 0 to 12 egg masses per hectare on all plots in 1997 due to the spread of *E. maimaiga* across the study region. Small increases in counts occurred on some MNF plots by the end of the study.

Table 4. Mean gypsy moth egg mass counts taken along transects on study plots.

George Washington N. F.							
Plot	1995	1996	1997	1998	1999	2000	2001
1	916	464	0	0	0	0	0
2	140	116	4	0	0	0	0
3	92	64	0	0	0	0	0
4	28	44	0	0	0	0	0
5	104	104	0	0	0	0	0
6	96	80	4	0	0	0	0
7	24	4	0	0	0	0	0
8	190	72	0	0	12	0	0
9	20	24	0	0	0	0	0

Monongahela N. F.							
Plot	1995	1996	1997	1998	1999	2000	2001
10	64	8	0	0	0	4	0
11	384	708	12	0	0	0	0
12	48	4	0	0	0	0	0
13	84	84	8	4	0	0	0
14	8	4	0	4	4	12	0
15	330	472	8	0	0	0	0
16	4	0	0	4	12	12	84
17	4	4	4	0	0	0	8
18	4	0	4	0	0	4	0

DISCUSSION

Previous studies by Miller (1990), Wagner et al. (1996), and Sample et al. (1996) have shown the negative effect of *Btk* applications for gypsy moth control on nontarget Lepidoptera. The design of this study was to take a longer term ecological approach to understanding the effect of intense gypsy moth control using *Btk* and Gypchek on nontarget organisms. This should broaden our understanding of the direct and indirect impacts of *Btk* on nontarget organisms and ultimately on forest ecosystem function. Table 5 summarizes our study approach.

Table 5. Study design elements and importance.

Study Design Element	Summary of Importance
1. Two years of pretreatment data.	Establish a baseline for comparison of treatment and post-treatment data.
2. Two consecutive years of treatment at the highest allowable dosages.	Apply current scenario to suppress major outbreaks or new isolated infestations in otherwise gypsy moth free regions.
3. Three years of post-treatment sampling.	Monitor rebounds in *Btk* or Gypchek sensitive populations that might occur and some portion of the recovery; impacts on long term population dynamics.
4. Large study plots.	Reduce influence of migration from outside of treatment plots on sampling results and minimize impacts of localized weather conditions.
5. Five sampling methods over a wide geographic area.	Monitor a large number of species and additional environments.
6. Inclusion of natural enemies and competitors	Monitor population release of competitors of treatment sensitive taxa, and the recovery of natural

	of the treatment sensitive groups.	enemies; impacts on feeding guilds and defoliator natural controls.
7.	Survey vegetation and physical attributes, and monitor weather.	Contribute to an ecosystems approach.
8.	Longer yearly sampling periods.	Monitor adult stages of univoltine species, and population dynamics of multivoltine treatment sensitive species, their competitors and their natural enemies.

Statistical analysis considerations of the field and laboratory studies were also met by the study design. The inclusion of two years of pretreatment data did more than allow us to establish a baseline that could be compared against year to year changes on the study plots. By using counts from the pretreatment years as a covariant in our analysis, we also compared relative plot counts grouped by treatments with the baseline plots grouped in the same way. Baseline data gives an idea of normal relative counts among plots; blocking plots on vegetation minimizes some inherent differences among plots.

Since the plots were spread over two areas within the Monongahela and George Washington National Forest, the impact of localized weather conditions was minimized. Two wide spread weather events were felt across all plots, a cold snap in the early spring of the first treatment year and a summer long drought the first post-treatment year. These had obvious impacts on total sampling counts of abundance. Even with these great reductions on non-treatment plots, significant *Btk* direct and indirect treatment effects were seen.

The caterpillars that we would expect to be sensitive to treatment timing, when pooled, did have great relative declines during the two treatment years on *Btk* plots compared to control and Gypchek plots. This reaffirms the negative impact *Btk* has on spring caterpillar populations. The specificity of Gypchek to gypsy moths was also reaffirmed. Moths were sampled with light traps to increase sample size and provide additional species for analysis. Knowing most moths are strong fliers, but not how far their flights range, undermines our ability to put as much weight on these results as sampling caterpillars. Pooling just the species we consider sensitive to treatment timing as caterpillars, indicates significant decreases in moth counts on *Btk* plots. The significant decreases were only seen the second treatment year. Whereas the foliage caterpillars had a significant rebound on *Btk* plots beyond “normal” baseline counts the second post-treatment year, the moth rebound was not statistically significant and only brought the treatments’ relative counts back to baseline normal.

The ability of a population to recover from decline in size relates in part to the period of time between generations. When only considering the number of generations in a year, species with multiple generations (multivoltine) should recover faster than species with just one generation (univoltine). This is seen in the results when univoltine and multivoltine species are analyzed separately, with univoltine species having larger and longer term decreases on *Btk* plots.

We focused on macrolepidopterans because of their importance as a food source for other arthropods and vertebrates. Microlepidopterans also serve as food sources and were also impacted by treatments. The two most common families encountered as caterpillars on foliage were the Tortricidae and Gelechiidae. Both of these groups had relative decreased counts on *Btk* plots during treatment years, but only for the tortricids were they significant. Recovery for both families on *Btk* plots occurred the second year of post-treatment. Another microlepidopteran, *Pyromorpha dimdiata* (Zygaenidae), sampled as adults with Malaise traps, had significant decreases in counts on *Btk* plots. Little is known of this species' life history, illustrating that we do not know what lepidopterans may be directly impacted. There was no indication of Gypchek impacting the microlepidoptera.

One group difficult to place with the nontargets that may be directly or indirectly impacted by *Btk* are the sawflies (Symphyta). Species that feed on the same host plants and at the same time as lepidopterans could be released from competition when caterpillar populations are reduced thus, an indirect impact. There is laboratory evidence that *Btk* is toxic to larvae (Smirnoff and Berlinguet 1966, Gorske et al. 1976, Braud 2001), a direct impact. With the data we gathered, there was no indication of an effect on sawflies from *Btk* treatments. Our limited knowledge of the immature stages will have to be overcome to address *Btk* impacts in this group, including larval species identification, host plant preferences, and feeding behavior.

Negative indirect effects of *Btk* were identified in parasitoid communities that attack Lepidoptera. Trends of decline during the treatment years occurred in parasitic flies (Tachinidae) and wasps (Ichneumonidae: Ichneumoninae, Braconidae: Microgastrinae) that specialize on Lepidoptera. Significant decreases as the result of caterpillars being reduced on *Btk* plots were found in *Tachinomyia variata* Curran (Tachinidae) and the Microgastrinae (Braconidae). These were sampled as adults, many of which are univoltine, overwintering as immature larvae or pupae (e.g., *T. variata*). Therefore, the significant decreases in adults began the second treatment year and included the first post-treatment year.

Predators are typically more generalized than parasitoids. When the caterpillar counts decreased on *Btk* plots, generalist predators such as spiders (Araneae) and most carabid beetles were not significantly impacted. In fact, there was no indication these were impacted, positively or negatively. We sampled a number of carabid beetles reported to selectively prey on caterpillars including species of *Cymindis*, *Platynus*, *Carabus* (and other caterpillar-like prey), and *Pterostichus*. There were weak trends of decreases on *Btk* plots for the caterpillar specialists. *Pterostichus tristus* (Dejean) was the only species in which significant declines occurred, and only when treatment and post-treatment years were combined.

The predatory stinkbugs (Pentatomidae) sampled specialize on caterpillar or caterpillar-like larvae. Counts on *Btk* plots from foliage and Malaise trap samples indicated a trend of decline after caterpillar counts were reduced, but only statistically significant in the Malaise traps for one treatment year and when treatment and post-treatment years were combined. Recovery of predatory stinkbugs based on the Malaise trap samples occurred the second treatment year, when caterpillars recovered as well.

When this study began, gypsy moth defoliation was expected to be a treatment, but the arrival of the highly pathogenic fungus, *Entomophaga maimaiga* kept gypsy moth numbers low. We continued surveying some organisms in case defoliation did occur, including ants, orthopteroid insects and harvestman (Opiliones). These groups could serve as alternate food sources for generalist predators, but no trend of an indirect effect was found when caterpillars were removed as a food source.

Btk is not highly specific to gypsy moth as is Gypchek, but unlike diflubenzuron (Butler et al. 1997a), it is highly specific to foliage feeding Lepidoptera. Also, unlike tebufenozide, it does not have season-long nontarget caterpillar impact (Butler et al. 1997b). *Btk* is a naturally occurring pathogen, that when applied aerially in current formulations remain toxic on the foliage for a short period of time (Reardon et al. 1994). It appears not to increase the long term pathogen load in the leaf litter, since we saw no impact on litter feeding caterpillars (i.e. Herminiinae). When applied to control gypsy moth, *Btk* negatively impacts many other spring lepidopterans. Some natural enemies specific to the impacted spring lepidopterans are also negatively impacted. If the subsequent reduction of natural enemies allows a release of potential pest defoliators, our results do not strongly indicate this.

One element requested in the original call for proposals for this research was that the study design include large treatment plots to limit migration from untreated areas that influence sample composition. The 500 acre (200 ha) treatment plots were relatively large compared to other studies on *Btk* impact, but possibly not large enough for sampled arthropods to have originated only on the treatment plots. The significant reduction of treatment timing sensitive macrolepidopteran nontarget species as caterpillars was not nearly equally reflected in reduction in their adult stage as indicated by light trap samples. This may also be true for the specialized parasitoids which had only limited decreases when their hosts were mostly removed. Macrolepidopterans as adults are usually strong fliers, as are many of their specialized natural enemies (or easily wind blown). The positive side of these results is when sensitive species and their specialized natural enemies within a *Btk* treatment area are also present in adjacent areas, migration can quickly re-establish their populations and host/prey relationships. Many other factors not addressed here must be considered with regard to migration compensation for *Btk* impacts, including suitable habitat corridors between treated and untreated areas, preserving genetic variability of small populations, and maintaining pathogen loads to name a few.

This study broadens our knowledge of how forest ecosystems are impacted by *Btk* at high dosages for two consecutive years. The compounded impact of record low temperatures the first treatment year that reduced spring caterpillars, which then received *Btk* treatments, illustrates that populations of these primary consumers can be quite resilient. Interpretation of the results here, like any similar field study, must take into account migration from outside of study plots altering samples sizes. The practice of applying Gypchek in areas of rare species or isolated forests should be continued. Wider use of this product should be promoted because of its specificity. During this study, the influx of *Entomophaga maimaiga* quickly and thoroughly removed gypsy moth from the study plots, but also eliminated the possibility of having defoliation as a treatment. The relative specificity, pathogenicity, and longterm high spore load remaining in soil after the gypsy moth population collapsed on the study plots indicates how very influential this pathogen has become in gypsy moth population dynamics. We do not know yet if

the ultimate range of gypsy moth will be suitable for *E. maimaiga* survival. At this time, gypsy moth management by having *E. maimaiga* established in vulnerable forests and occasionally using spot applications of Gypchek would be, by far, the most environmentally friendly combination. *Btk* as a treatment is also clearly a good option, and suitable in place of Gypchek in ecosystems when applied to limited areas of homogenous forests.

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CHAPTER 5. *ENTOMOPHAGA MAIMAIGA* STUDIES

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INTRODUCTION

The Asian fungus, *Entomophaga maimaiga* (Humber, Shimazu & Soper), was first found to be infecting North American gypsy moth caterpillars in several northeastern states in 1989 (Andreadis and Weseloh 1990, Hajek et al. 1990). As *E. maimaiga*, recognized as a highly virulent pathogen, was spreading rapidly in this country and affecting population dynamics of gypsy moth, concern was expressed as to its host specificity (Reardon and Hajek 1993) and several studies were conducted.

This fungus is now a part of the natural history of forests within contiguous gypsy moth-infested states of eastern U. S. and the Great Lakes region. It has dramatically influenced gypsy moth populations and will certainly have an influence on populations of gypsy moth natural enemies and, potentially, on populations of some nontarget Lepidoptera. When *E. maimaiga* moved into our study plots in 1995 and 1996, we were provided an opportunity to collect data to help answer some outstanding questions.

See Chapter 1 for an expanded discussion of the biology and importance of *E. maimaiga*.

METHODS

Resting Spore Quantification

Soil samples were collected yearly from each plot for quantification of *Entomophaga maimaiga* resting spores, to determine the abundance of the environmental reservoir of this pathogen. When taking soil samples, only the organic layer of soil was collected from within 10 cm of the bases of co-dominant oaks. For each plot, at three locations within the plot, five trees were chosen and soil was collected from 60°, 180°, and 300° around the bases of each of the trees to total approximately 20 g of soil per tree and 100 g per location within the plot and therefore 300 g total per plot. Soil samples were stored at 4° C until quantification. For each site, equal amounts of soil from the three subplots were thoroughly mixed. Resting spores in soil were quantified using standard techniques (Hajek & Wheeler 1994). For each plot, three 5 g samples were randomly selected for wet sieving through a 65 ìm sieve and collection on a 20 ìm sieve. A discontinuous density gradient made with Percoll™, a colloidal PVP-coated silica, was used to fractionate the samples and resting spores were then visually counted at 50X magnification. During 1997, 1998, and 1999, soil samples were collected in late fall/early winter (October to December) while for each of the 5 years from 1997 to 2001, soil samples were collected in early spring (March to May).

Sampling Macrolepidopteran Larvae for *E. maimaiga* Infection

As part of sampling efforts, each year a subset of the larvae that were collected from foliage and under bands was sent to A. Hajek's laboratory at Cornell University to detect infection by *E. maimaiga*. Emphasis was placed on larvae of gypsy moth and native lymantriids (Figure 82).

Larvae were individually provided with the species of foliage on which they had been collected within 96.1 ml plastic cups. Over 14 days after collection, larvae were provided with fresh foliage as necessary at 20 to 25° C and 14:10 (L:D) and were monitored daily for death. To detect infection by *E. maimaiga*, cadavers were placed on 1.5% water agar at 20° C and over the 3 days following death were observed daily for external outgrowth of *E. maimaiga* conidiophores. Cadavers remained at 20° C for 7 more days to allow for maturation of resting spores that could occur. Cadavers were then stored at 4° C and were subsequently dissected and body contents were examined microscopically to detect *E. maimaiga* resting spores.

Figure 82. *Orgyia leucostigma* (J. E. Smith) is a native lymantrid caterpillar.

Conidial production by *E. maimaiga*

We conducted field bioassays to evaluate the extent to which *E. maimaiga* could be infecting native lymantriid larvae in early spring (Hajek et al. 2004). The bioassays were conducted on Plot 1 (George Washington National Forest) during early spring 1997. Early fourth instar gypsy moth larvae (obtained from USDA, APHIS) were placed in cages made with 20 x 20 mesh aluminum window screening and containing a cube of artificial diet. Twenty larvae were placed in each cage and one cage was placed at the base of each of three co-dominant oaks. The soil beneath the cages and around the bases of each tree was watered once each week with 3.8 liters to promote resting spore activity in case it did not rain. After the first two exposure periods, cages were placed at the bases of three more trees beneath which the soil was not watered.

Since resting spores actively eject infective germ conidia, we evaluated whether larvae above the ground became infected by germ conidia during early spring when conidia from cadavers would not yet be present. Using the same cage design, we contained gypsy moth larvae on hardware cloth platforms within 10 cm of the trunks of three watered oaks at 2, 5, 10, and 50 cm above the ground as well as at 2 m height, hanging from a nearby branch. For each exposure period, cages remained in the field for 48 h, after which larvae were removed from cages and provided with artificial diet in groups of 10 in plastic cups with paper lids at room temperature. Larvae were monitored daily for 10 days to detect mortality, and dead larvae were treated as described above to detect *E. maimaiga* infections. Between 4 April and 8 May, groups of larvae had 15 exposure

periods at watered trees and 13 exposures at nonwatered trees. Electronic weather recording equipment quantified leaf wetness and soil temperature and moisture during the exposure periods (Hajek et al. 2004).

The potential for production of airborne conidia was evaluated throughout the season by quantifying production of conidia versus resting spores from cadavers of field collected gypsy moth larvae dying from *E. maimaiga* infections. This was only possible in 2000 and 2001 because no infections were found in larvae from the study areas from 1997 to 1999. Sampling began 7 to 9 May and continued weekly while larvae were present in the field. All cadavers were treated as described above and instars were recorded as well as whether conidia only, resting spores only, or both spore types were produced in or on cadavers.

Analysis

Statistical analysis could not be used to evaluate levels of infection among native lymantriid larvae because densities were too low. Season-long infection rates for gypsy moth larvae were calculated as in Hajek et al. (1990). Proportions of survival for each week were multiplied by each other to estimate the proportion of larvae surviving to pupation that year, after which proportion *E. maimaiga* infection was estimated as 1 - (proportion survival).

To compare *E. maimaiga* resting spore density in winter versus spring, soil samples and changes in resting spore density across years, Poisson regression models adjusted for overdispersion of data were used (SAS Institute 1999). Post hoc comparisons among years within states used least square means with Bonferroni corrections.

RESULTS

Entomophaga maimaiga infections among lymantriids

After the initial collapse of building gypsy moth populations in 1995 and 1996 due to *E. maimaiga* infection, gypsy moth populations remained extremely low considering that this is a species that can characteristically increase to >1,000 egg masses/ha during outbreaks. Egg mass densities in the George Washington (GWNF) and some northern Monongahela (MNF) National Forests plots averaged from 267 to 697/ha, in 1995 and 1996, but populations crashed during the 1996 field season (Table 4). From 1997 to 2001, maximum densities of gypsy moth in GWNF averaged 3 ± 3 egg masses/ha and in MNF averaged 25 ± 23 egg masses/ha. At such low densities, larval densities rather than egg mass densities are more indicative of changes in population level. Counts of gypsy moth larvae from canvas bands and foliage pruning demonstrated a trend of increase during 2000 and 2001, especially in MNF.

Populations of native lymantriids found in plots were also sparse, with only 16 and 21 total larvae per year collected during 1997 and 1998, respectively, increasing to 49 larvae collected during 1999. While gypsy moth populations increased steadily in 2000 and 2001, native lymantriids did not increase significantly, with 15 and 50 total larvae collected, respectively. Throughout the five years of the fungus study, gypsy moth larvae were predominately first instars with a few second instars when sampling began 5 to 12 May each year, while during this

same sampling week, some of the native lymantriids, particularly *Dasychira* spp., were predominantly instars 4 to 6, having overwintered as partially mature larvae.

No gypsy moth larvae collected in 1997 to 1999 were infected by *E. maimaiga*. During these years, rainfall during the period of larval sampling ranged from a low of 23.1 cm total in 1999 to 44.5 cm (1997) and 54.0 cm (1998). Total rainfall throughout the larval collection periods in 2000 to 2001 was more similar to 1998 with 53.1 (2000) and 56.8 (2001) (Figure 11). In 2000, the first infected gypsy moth larvae were collected during the early fifth instar (4 to 5 June) and infections were thereafter found weekly. During 2000, all gypsy moth infections were found in MNF, where gypsy moth populations were more abundant. A total of 3 larvae were infected out of 15 native lymantriids collected in 2000. Two of these infected larvae were collected in two plots in MNF (19 to 20 June; 85.2% infection among gypsy moth larvae collected that week in MNF) and one was collected from GWNF (June 13) where no infected gypsy moth larvae were collected from 1997 to 2000.

In 2001, as gypsy moth populations became more abundant, *E. maimaiga* infections among gypsy moth larvae became abundant on both national forests. Only 4 of the 50 native lymantriids collected became infected. In 2001, infected native lymantriids were all collected as later instars on the same sample date (June 4) at three different plots in GWNF (32% infection among gypsy moth larvae collected that week in GWNF).

Of the 7 species of native lymantriids collected during this study, infections only occurred in three species, *Dasychira obliquata* (G. & R.), *Dasychira vagans* (B. & McD.), and *Orgyia leucostigma* (J. E. Smith). The species with most infection was *D. obliquata*. Surprisingly, a species previously found infected with *E. maimaiga*, *Dasychira basiflava* (Pack.), was never found infected throughout this 5-year study, although it was the most abundant of the native lymantriids collected. The seven individuals of native lymantriids that were infected all were 4 to 6 instar. Six of the 7 were collected under canvas bands, so they had wandered from the foliage. Of the total 151 native lymantriids collected, 86.0% were found under bands rather than on foliage.

Resting spore persistence

A significant difference was found between resting spore densities in December 1996 and April 1997 ($t = -2.58$; $p < 0.0157$) but, contrary to our hypotheses, the densities of resting spores across plots in spring (6359.1 ± 1788.2) were greater than the densities in winter (4154.9 ± 1067.8). These data included counts from MNF plot 15 that were greater than 8 times higher in spring than fall. Spring counts from plot 15 were also much higher than counts from other plots where winter and spring counts were more similar. When we removed counts from plot 15, as an outlier, resting spore densities among other plots did not differ between winter and spring ($t = 0.61$; $p > 0.5464$). GWNF counts were greater than MNF counts ($t = 3.48$; $p < 0.0064$) because gypsy moth populations had been present longer in GWNF and had been higher before crashing, so that more resting spores would potentially have been produced (Hajek et al. 2004).

Comparing spring resting spore densities across all years, overall results were similar with or without the high counts from plot 15 in April 1997. Patterns of densities differed for sites in

GWNF versus MNF (Figures 83 & 84). In GWNF, resting spore densities declined after 1998 while, in MNF, resting spore titers showed a trend of decrease from 1997 through 2000 but then increased again in 2001.

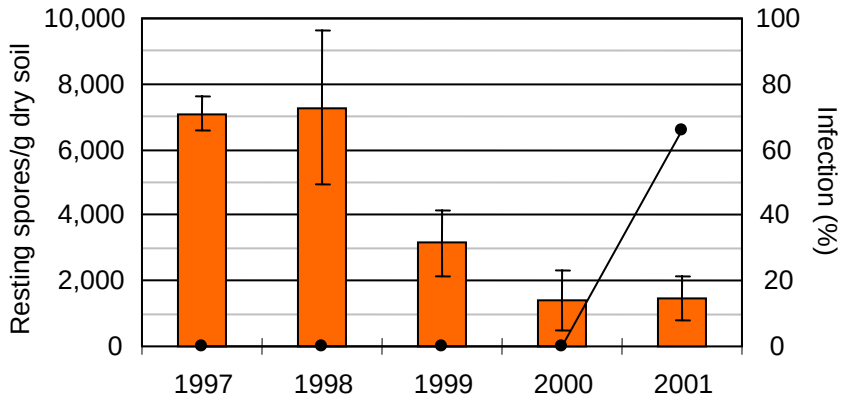

Figure 83. Resting spores/g dry soil (±SE) during early spring associated with total yearly percent infection among gypsy moth larvae collected throughout the field season from 1997 to 2001 on all nine George Washington National Forest plots.

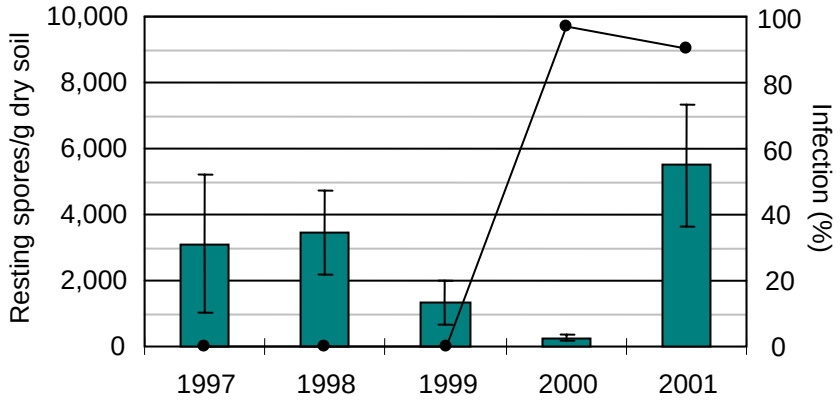

Figure 84. Resting spores/g dry soil (±SE) during early spring associated with total yearly percent infection among gypsy moth larvae collected throughout the field season from 1997 to 2001 on all nine Monongahela National Forest plots.

Conidial production by *E. maimaiga*

Bioassays conducted in 1997 confirmed that the resting spores at bases of trees in plot 1 were *E. maimaiga*. The very first infections seen were few, only occurring among larvae caged on the ground under watered trees from 4 to 6 April. For cages on watered soil, infections started in earnest beginning 25 April, a time when soil moisture was high. Under trees where the soil was not watered, infections were only seen during four exposure periods, between 25 April and 4 May. High variability in infection was seen among trees, with no infections at all occurring among larvae caged under one unwatered tree.

Larvae caged above the soil that could only be infected by airborne conidia were very seldom infected. One larva out of 60 was infected at 2 cm height during three intervals (4 to 6 April, 25 to 27 April, and 6 to 8 May), one larva was infected at 10 cm height (2 to 4 May), and none of the larvae caged at 5 or 50 cm and 2 m height were infected throughout the study.

Collections of gypsy moth larvae during the field season demonstrated that early in the season, conidia were exclusively produced and resting spore production only increased late in the season. Fourth instars were predominately collected in weeks 4 to 5 in both 2000 and 2001 and fourth instar cadavers never contained resting spores. Resting spore production only began when fifth and later instars were present although cadavers that produced conidia as well as resting spores were found until the end of the season. *E. maimaiga*-infected native lymantriids were only collected weeks 6 and 7 in 2000 when gypsy moths were about fifth instar and week 5 in 2001 when instars 4 to 5 predominated.

DISCUSSION

Native lymantriids were at low densities throughout this study and only became infected by *E. maimaiga* during years when gypsy moth populations were more abundant and gypsy moth larvae were infected (2000, 2001). The highest percent infection among native lymantriids was found among *D. obliquata* (21.7%) although this was not the most abundant native lymantriid species collected. Among all native lymantriid species collected during this study, infection was never greater than 50% for any one year and, in fact, only approached this level for one species out of seven, during one year out of five.

Results of this study agree with other studies (Hajek and Eastburn 2001) that unless resting spores are added, titers generally decrease in density to some extent each year because some resting spores germinate whether gypsy moth larvae are present or not, although many persist as a soil reservoir. Indeed, between 1997 and 1998, resting spore titers declined when gypsy moth larvae were scarce. Field bioassays conducted during 1997 demonstrated the importance of soil moisture for resting spores to germinate. Because rainfall was frequent in 1997 and 1998 and was similar to that of 2000 and 2001, we suggest that the reasons we did not find infected gypsy moth or native lymantriid larvae from 1997 to 1999 was not due to moisture limiting resting spore germination. The present study documented for the first time a rapid increase in resting spore titer after *E. maimaiga* infections were abundant during one season (2000). This increase was seen in the MNF plots in 2001 and occurred although gypsy moth density in the plots was very low (9 ± 4 egg masses/ha in 2000).

For *E. maimaiga* to begin infection cycles each season, resting spores in the soil germinate to actively eject infective germ conidia that can cause “primary infection.” Although germ conidia are actively ejected, no one previously studied to what extent they become airborne. Results from our 1997 early season studies provide little indication that germ conidia infect many larvae above the ground level. After gypsy moth larvae become infected and die, then infective conidia actively ejected from larval cadavers cause “secondary infections” and these conidia definitely become airborne (Hajek et al. 1999). Infections among larvae while they are in the tree and shrub canopies are most likely due to secondary infections. It is these secondary infections that are

responsible for the exponential increases in infection characteristic of epizootics (Hajek el al. 1993).

Lymantriid larvae other than gypsy moth are not known to commonly rest in the leaf litter. Therefore, native lymantriids would have much less risk of infection from the bank of resting spores in the soil compared with gypsy moth larvae. We hypothesize that it is more likely that native lymantriids become infected from conidia produced from cadavers while they are in the understory or tree canopy. During this study, cadavers of gypsy moth larvae predominantly produced conidia through much of the season, until late instars were present, at which time many late instar cadavers produced resting spores. We hypothesize that *E. maimaiga* infections were not found from 1997 to 1999 among native lymantriids because gypsy moth densities were too low to produce abundant conidia for secondary infections. In addition, because high levels of *E. maimaiga* infection in gypsy moth populations often occur late in the season (Hajek 1999) and many of the native lymantriids are earlier than gypsy moth and would have pupated by the time gypsy moth are late instars, we hypothesize that relative seasonality of these species would result in native lymantriids largely escaping periods when airborne conidia of *E. maimaiga* might be abundant.

While we could not confirm that each of the infections in native lymantriids found during this study were caused by *E. maimaiga* [and not the native *E. aulicae* (Reichardt in Bail) Humber, a morphlogically identical species of fungus (Hajek et al. 2004)], we feel confident in making that assumption. Supporting evidence includes the following: *E. maimaiga* has previously been shown to infect native lymantriids in the field; the 1996 gypsy moth populations in the GWNF and MNF crashed and resting spores in soil were subsequently abundant and high percentages of gypsy moth larvae caged over soil became infected by *E. maimaiga* in 1997; and abundant infections were found among gypsy moth larvae collected at the same times and locations as native lymantriids in 2000 and 2001.

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CHAPTER 6. BIRD STUDIES

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INTRODUCTION

Effects of Pesticide Options on Bird Populations

Because applications of *Btk* reduce the abundance of nontarget (i.e., non-gypsy moth) Lepidoptera larvae in both the year of spray and the year post spray (Miller 1990, Rodenhouse and Holmes 1992, Lih et al. 1995), many bird populations that depend on Lepidoptera larvae for survival and provisioning of young (MacArthur 1959, Robinson and Holmes 1982, Martin 1987, Holmes and Schultz 1988) may be negatively affected.

The importance of Lepidoptera larvae in the breeding season diets of temperate forest insectivorous birds has been demonstrated for both adults (Holmes et al. 1986, Cooper et al. 1990, Sample et al. 1993) and young (Biermann and Sealy 1982, Goodbred and Holmes 1996, Brodmann and Reyer 1999, Naef-Daenzer and Keller 1999). Greenberg (1995) hypothesized that Lepidoptera larvae may serve as the "breeding currency" by which the reproductive ecology of migratory insectivores is moderated. It has also been demonstrated that predation by birds can significantly reduce the abundance of caterpillars (Holmes et al. 1979b) and that this predation may increase the vigor of the plant substrates (Marquis and Whelan 1994), illustrating a tight ecological link between these taxa (Robinson and Holmes 1982). Furthermore, the annual productivity of at least one forest bird species, the Black-throated Blue Warbler (*Dendroica caerulescens*) (Holmes et al. 1992, Rodenhouse and Holmes 1992), is higher in years when caterpillars are unusually abundant (i.e., an outbreak year). This has led Holmes et al. (1986) to conclude that birds breeding in temperate deciduous forests can be food limited during non-outbreak years. Martin (1987) and Boutin (1990) also concluded that food can limit the reproductive success and survival of passerine birds breeding in eastern temperate forests. Variation in food abundance (primarily Lepidoptera larvae), both natural and experimentally induced by pesticides, has resulted in changes in the nutritional status (Whitmore et al. 1993), nest provisioning rate (Rodenhouse and Holmes 1992, Nagy and Smith 1997), frequency of second broods (Rodenhouse and Holmes 1992), time of breeding (Kelly and VanHorne 1997), and overall reproductive success (Holmes et al. 1979a, Holmes et al. 1992, Rodenhouse and Holmes 1992) of several bird species.

METHODS

Study Methods on Avian Populations and Productivity

Given the current management preference for *Btk* and the extent to which this insecticide is applied to forested systems (1.5 million hectares were sprayed with *Btk* to control gypsy moths from 1990 to 1998), evaluating the potential impacts on nontarget organisms, such as birds, is imperative. The objective of this portion of the study was to investigate the indirect (i.e., non-

toxological, food chain related) effect of *Btk*, applied in a typical gypsy moth management scenario, on avian abundance and breeding ecology of forest breeding songbirds.

Our original research hypotheses were that (1) the reduction of nontarget Lepidoptera larvae would negatively affect the reproductive ecology of those species whose diet is largely composed of Lepidoptera larvae, while (2) changes in the forest structure due to defoliation would alter abundance and species composition. To examine the possible effects of defoliation and food reduction on avian populations we monitored both abundance and reproductive output, through point counts and nest monitoring, respectively. Point counts were conducted to monitor possible annual changes in avian density and species richness. For reproductive output, we specifically examined whether four focal species had smaller clutches, decreased hatching success, increased nestling mortality, fewer young per successful nest, and an overall decrease in nest success, due to the *Btk* applications. Additionally provisioning rates and nestling weights were monitored for one species to determine if food reductions altered adult activity and nestling growth.

This study utilized a blocked design with the treatments (aerial application of *Btk* and Gypchek) and the control randomly allocated to one of three plots within each of three blocks (Table 1). The Gypchek applications were originally intended to serve as experimental controls to prevent defoliation on study plots that would have otherwise been defoliated during an anticipated gypsy moth outbreak. However, defoliation did not occur during the course of this study due to the unforeseen presence of the fungus *Entomophaga maimaiga* that extirpated the gypsy moth from the region (Webb et al. 1999). Because of the host specificity of Gypchek (only gypsy moth larvae are affected; Lewis and Podgwaite 1981, Podgewaite et al. 1992), these plots were subsequently grouped with the control plots and both are referred to as the non-*Bacillus* (NB) plots hereafter. Thus, each of the three blocks contained one *Btk* treated and two untreated (NB) plots. Blocks were separated by approximately 12 km and plots within a block were separated by less than 3 km (Figure 4).

All avian research was conducted on 30 ha (600 m x 500 m) subplots that were randomly located completely within each of the 18 plots. The perimeter boundary and four 600 m long fixed transects, situated 100 m apart, were marked with colored flagging every 25 m in each of the subplots (Figure 3). The use of subplots facilitated data collection, insured that treated plots were in fact treated, and prevented edge effects such as birds having territories that were only partially in treated areas.

Avian Abundance

Field Methods

Point counts were conducted on all subplots from 1996 to 2001 following the methods of Ralph et al. (1993). Two, two-week sampling periods were conducted each year in early spring while male birds were advertising territories (i.e., singing). Each subplot had twelve sample points located along alternating grid lines (AA, B, D; except for plot 16 which followed A, C, DD) spaced at 200 m intervals (grid points 0, 200, 400, 600 m along each grid line surveyed; Figure 3). Counts began after dawn and were completed by 10:00 am to coincide with peak morning singing activity. After a 3 minute acclimation period, all birds detected during a 10

minute period were recorded and placed in 1 of 3 distance categories; <25 m, 25 to 50 m, or >50 m from the observer. Counts were not performed in rain or during high wind. For each avian detection, the species, sex (if known), and method of detection (vocalization versus visual) were recorded. To aid in determining the number of individuals of a particular species at a point, an effort was made to keep track of conspecifics by noting countersinging and calling.

Analysis

A randomized complete block design was employed, with six blocks (groups of three plots at each national forest) and the three treatments applied to one plot in each block. The purpose of blocking was to explain extraneous sources of variation (due to study plot location) not directly of interest but recognized as being potentially important in explaining the response variables. An additional way to block in this experiment was to use the national forest as a block (i.e., two blocks reflecting national forest instead of six blocks representing groups of study plots). The data were analyzed using a repeated measures analysis of variance on both blocking designs, to assess the treatment effects on species richness and abundance.

Species Richness

For each year of the study, the total number of species detected on each plot during the point counts (all birds heard or seen regardless of distance) was tallied. Repeated measures ANOVA (von Ende 1993) was then used to determine variations due to treatment over the six years sampled.

Abundance

All birds detected within the 50 m distance category were totaled by species for each survey, and then the two surveys were averaged to yield the number of birds detected per point. These averages were then summed for a yearly plot total for each species. Those plots with no recordings of a species were excluded from the particular species' analysis. Repeated measures ANOVA was then used to determine variations in abundance due to treatment over the six years sampled.

Reproductive Success

Study Species

Four Neotropical migrant songbird species were chosen as focal species to examine the indirect effects of *Btk* applications on songbird populations and reproductive output (Figure 85). All were chosen primarily based on food habits. Each is largely insectivorous during the breeding season, and three of the four are believed to be particularly susceptible to indirect effects of insecticide application because of their reliance on caterpillars as a food-source (Robinson and Holmes 1982, Cooper 1988). We selected two vireo species, one thrush species, and one warbler species that nest, respectively, in the canopy, subcanopy and on the ground, to represent the diversity of bird species present on the study plots. Furthermore, the density of

these four species was sufficiently high at both national forests to collect adequate reproductive data for statistical analyses.

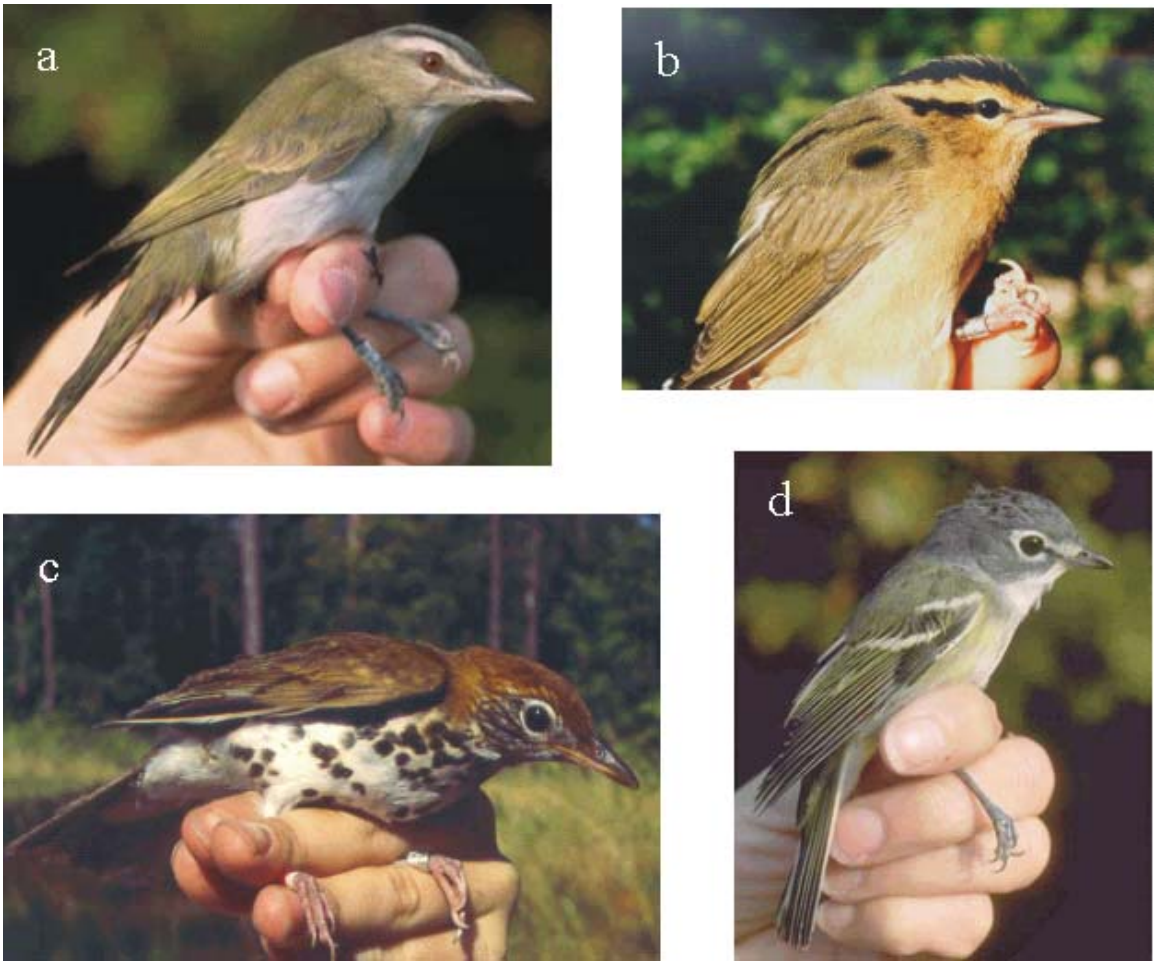

Figure 85. Reproductive output of four focal species, (a) Red-eyed Vireo (photo: J. DeCecco), (b) Worm-eating Warbler (photo: A. Williams), (c) Wood Thrush (photo: L. Powell), and (d) Blue-headed Vireo (photo: J. DeCecco), was monitored on the subplots in Virginia and West Virginia from 1995 to 1999.

Table 6. Selected life history characteristics for the Red-eyed Vireo, Blue-headed Vireo, Wood Thrush, and Worm-eating Warbler from the Monongahela National Forest in West Virginia and the George Washington National Forest in Virginia during 1995 to 1998. Parameters (except breeding season length) are expressed as 0 ± SD. Table reprinted from DeCecco et al. (2000).

Parameter	Red-eyed Vireo	Blue-headed Vireo	Wood Thrush	Worm-eating Warbler
Incubation stage length (days)	14.0 ± 0.9	14.5 ± 1.0	13.0 ± 1.4	12.0 ± 2.6
Nestling stage length (days)	11.5 ± 1.4	11.9 ± 1.0	12.0 ± 1.6	8.6 ± 1.9
Breeding season length: median days (range)	38 (28-43)	73 (48-92)	60 (47-66)	47 (35-54)
Clutch size: 0 cowbird eggs	3.2 ± 0.6	3.8 ± 0.4	3.6 ± 0.6	4.5 ± 0.9
Clutch size: ≥ 1 cowbird eggs	2.2 ± 1.1	2.8 ± 1.8	3.1 ± 0.8	3.8 ± 0.9

Nest Searching

Nest searching was conducted on all 18 study plots from 25 April to 1 August 1995 to 1999, although the most intensive searching was done in May and June. Nests were found at all stages of the nesting cycle and were monitored every three days until they either failed or fledged young according to BBIRD (Breeding Bird Inventory and Research Database) protocols (Martin et al. 1997). Nests were found through a variety of techniques covered in Martin et al. (1997) including systematic search, parental behavior (e.g., witnessed the adult taking nesting material or food to nest), adult flushed from nest, sound of begging young, based on location of nest from a previous year, and luck. Once a nest was located, it was flagged and detailed directions to the nest were recorded from a known transect point. Specific details about the nest substrate and location (i.e., species, DBH, height of tree, distance and cardinal direction from bole of tree for tree nests; distance, direction, and substrate for ground nests) were also recorded to facilitate relocation and reduce time spent at the nest on future checks. Because predators can learn to associate flagging with nests, flags were placed as far away as possible (usually 5 to 10 m), with nests still visible from this flag.

Nest Monitoring

In order to minimize possible negative effects associated with increased human presence around nests the following precautions were observed when checking nests:

1) Observers moved to nest sites using different paths both to and from the nest site to minimize the risk of predators locating nest by following “trails” to the nest.

2) Observers avoided checking nests while potential avian predators (e.g., crows, blue jays) or mammalian predators were within visual contact.

3) Care was taken to minimize the amount of time spent checking nest contents.

4) If there was a heavy rain during a nest check the adult was not flushed to check nest, instead the nest was checked the next day.

Data collected at each nest check included clutch size, number of eggs hatched, number of nestlings, number of Brown-headed Cowbird (*Molothrus ater*; hereafter cowbird) eggs and nestlings, adult activity, and the fate of the nest. Detailed descriptions of nestlings were also recorded to help determine nestling ages of newly found nests. The date the first egg of the nest attempt was laid (first egg date), the hatch date, and the fledging date were also recorded. Those nests not found during the building stage, were back-dated from known periods in the nesting cycle. When back-dating, nesting stage lengths and clutch size were based on the respective means of the species (Table 6). If on a nest check, the nest was no longer active, details on nest condition (e.g., lining pulled out, side of nest flattened, no sign of disturbance), adult activity (e.g., adults chipping, adults in area, no sign of adults), signs around nest (e.g., fecal matter, egg shells or dead nestlings in or below nest), or visual/audible conformation of fledglings were recorded in order to help determine final nest fate. A mirror pole was used to see inside nests up to 8 m in height. If a nest was too high to check with the mirror-pole, the observer watched the nest for 60 minutes or until activity was seen at the nest, whichever was shorter. After two consecutive visits of 60 minutes without observing activity, the nest was considered failed. The inability to access these high nests lead to some unknown outcomes if fledging or failure could not be determined. For those nests that could be directly observed, if one nestling of a brood disappeared between visits to the nest, it was assumed that it died and was removed by an adult bird.

The ground level nests of Worm-eating Warblers were fully accessible and provided an opportunity to examine the effects of the *Btk* application on provisioning rates and nestling weights. In 1998, the field crew videotaped nests containing five-day old young (although every attempt was made to film on day five, occasionally the exact age of the nestling was not known and therefore in a few cases young may have been either four or six days old). The age of the young was determined using hatching date and nestling development. Technicians placed and camouflaged a 8 mm Sony® Handycam® video camera within 2 to 7 m of the nest. The camera was positioned to record the arrival and departure of both parents, the type of food brought, and any other behaviors in a 0.25 m radius of the nest. The camera recorded color video continuously for 3 to 4 hours from 5:30 to 11:00 am and was set to stamp the time and date on all recordings made. Later the videos were reviewed using a Sony® (EVS7000 NTSC) 8 mm video cassette player/recorder. Additionally, when nestlings were five to six days old and after they had been video taped, they were all weighed, measured, and banded. Due to high concentrations of Worm-eating Warblers on the George Washington National Forest, the effort was concentrated on these sites.

Reproductive Success

Survival probabilities for each stage in the nesting cycle were estimated using the Mayfield method (Mayfield 1975). Only nests that contained at least one host egg were used for Mayfield estimates; abandoned nests or those that failed during the building phase were not used. In particular, many Blue-headed Vireos were observed building partial nests that were subsequently never used; James (1978), gives detailed descriptions of the use of these partial nests for display areas during courtship. Similarly, a nest was considered successful if one host young fledged. A nest was considered as failed at the time of host failure, even if the cowbird young survived in the nest until fledging. Survival probabilities at each stage of the nesting cycle (egg-laying, incubation, and nestling) were calculated separately and weighted by the number of days in the respective stage for each species. These separate probabilities were then multiplied together for an overall probability of nest success, and standard errors were calculated following Hensler (1985). All nests in which an outcome could be reliably assessed were used in the analysis and differences were tested using the program CONTRAST (Hines and Sauer 1989). The average number of fledglings per successful nest included nests parasitized by cowbirds as long as at least one host young fledged. The height of many Red-eyed Vireo nests exceeded 8 m (DeCecco et al. 2000) which did not allow the exact determination of nest contents (e.g., number of eggs/young, presence of cowbird eggs/young, disappearance of young, etc.), although the activity status of a these nests was determined by behavioral cues. Many analyses required that the exact nest contents be seen. Therefore, not all nests found could be used in all analyses.

Reproductive Variables

To further examine the effects of food reduction, we examined a number of other reproductive variables between *Btk* and NB plots. Clutch size (average number of eggs laid) was calculated for all nests on *Btk* and NB plots to determine if reductions in clutch size might reflect the reduced availability of food resources as perceived by laying females. Because incubating females might have trouble meeting their energetic demands because of reduced food availability, the time they spend off the nest might increase which in turn might reduce the efficiency of their incubation and reduce hatching success of the eggs. Therefore, hatching success (number of young that hatched divided by the number of eggs laid) was also estimated. Finally, if adult birds have trouble finding adequate food to provision young because of reduced prey levels, then loss of either the entire or part of the brood may occur due to nestling starvation. Although it is difficult to speculate on the exact cause of failure for any nest, we did note those nests found on both *Btk* and NB plots in which we found dead individual(s) nestling(s). The percentage of young that fledged from nests in which the exact clutch size was known (found before hatching) was determined to assess if a lower ratio of young may fledge from nests on *Btk* plots (due to starvation or other reasons). However, because all nests were not found before hatching, calculation of a measure of fledging success (mean number of young fledged per nest) between *Btk* and NB was done using nests found in any stage but in which the exact number of fledglings was known. For estimates of hatching success rates, clutch size, and egg/fledging ratio, program CONTRAST was used to test for differences between treatments (Sauer and Williams 1989).

Provisioning Rates

The total numbers of three prey types, Lepidoptera larvae, other prey, and unknown prey were calculated for each Worm-eating Warbler nest. Any food item with visible appendages was scored as other prey and prey types scored as unknown prey showed no detail as to shape or form (particularly on smaller prey items). We recognized that parents often removed wings and legs from prey prior to returning to the nest so we paused the video tape at each feeding to allow for adequate scrutiny of each prey item. The total number of all food items and the total number of provisioning trips made by both parents were tallied for each nest. These totals were then divided by the number of young in the nest and the number of hours of observation to obtain a provisioning rate/young/hour for each nest. We estimated the effect size, or the magnitude of difference between *Btk* and NB plots for provisioning data, and constructed a 95% confidence interval around this estimate (Dowdy and Wearden 1991). These estimates indicate if the size and direction of the effect were in the predicted direction, with the confidence intervals giving a measure of uncertainty that is similar to calculating power (Steidl et al. 1997, Gerard et al. 1998).

For nests with adults that were color-banded we calculated the proportion of time each parent was out of view of the camera (away from the nest). We averaged the percentage of time that each individual bird was out of view of the nest by sex and treatment, arcsine square root transformed the mean percentages, then compared them using a 2-way factorial design (SAS Institute 1990). Differences between the treatments and sexes were assessed using Tukey's Studentized Range (HSD) Test ($\alpha = 0.05$).

Nestling Weights

The total weight of all nestlings was obtained for each nest and then was divided by the number of nestlings present to obtain an average nestling weight per nest. We also wanted to examine if the variability within nests on *Btk* plots may be greater than that of NB plots. A lack of preferred food could cause the adults to preferentially feed certain young over others, causing a greater discrepancy between the smallest and largest individual in a brood (Rodenhouse and Holmes 1992). Variation of nestling weight between treatments was compared using effect size and 95% confidence intervals.

Variability Due to Clutch Sizes

Because Worm-eating Warblers lay a variety of clutch sizes during the season (either due to failure of successive nesting attempts or other factors), we also wanted to examine several of the variables on a per clutch or per young basis to determine if the effects of the treatment may disproportionately affect either larger or smaller clutches (or broods). Nesting success, provisioning rates of both Lepidoptera larvae and total prey as well as nestling weights were examined according to the number of either eggs or young that were in the nest. Although they may lay as few as one egg or as many as six, due to small sample sizes, only nests with 3 to 5 eggs or young could be evaluated for most analyses.

RESULTS

Avian Abundance

Point Counts

Point counts are the primary way that we assessed the abundance of most bird species on our plots. Although not believed to be as meaningful as demographic parameters such as productivity and survivorship, they are much easier to obtain and therefore serve as our primary means of assessing trends of individual species and communities relative to *Btk* application.

Species Richness

Our results show that regardless of blocking method, local block or national forest area, block had a significant effect on species richness (Figure 86). This result was consistent with our findings that the Monongahela National Forest (MNF) plots were more diverse in vegetation composition and therefore contained more bird species. Species such as Hermit Thrush (*Catharus guttatus*), Least Flycatcher (*Empidonax minimus*), Northern Parula (*Parula americana*), American Redstart (*Setophaga ruticilla*), and Dark-eyed Junco (other scientific names listed in Table 7) rarely, if ever, occurred on the George Washington National Forest (GWNF) while they were commonly found on the MNF. There was no significant effect of treatment on species richness (Figure 87), although we noted a slight decrease in species richness on the *Btk*-treated plots in both years of *Btk* application (1997 and 1998).

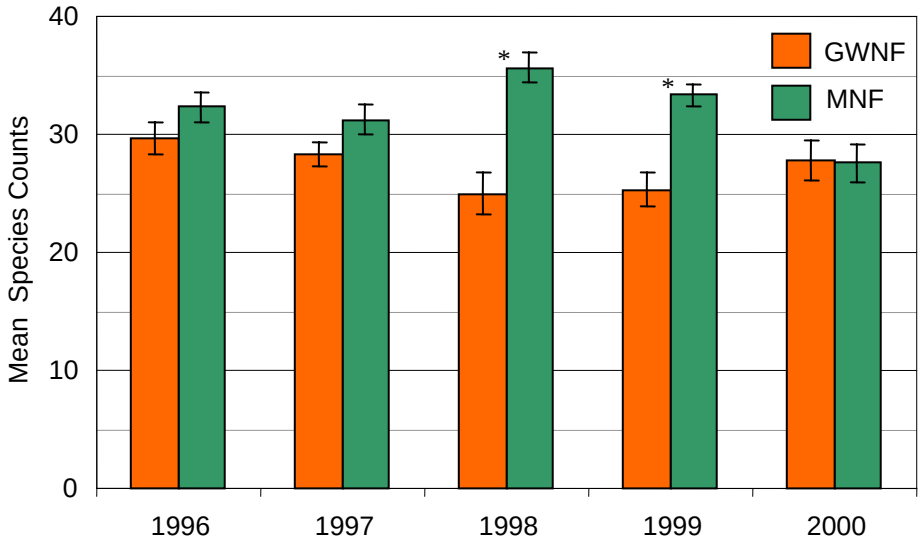

Figure 86. Species richness means for all plots (n=9) in the George Washington (GWNF) and Monongahela (MNF) National Forests from 1996 to 2000. Error bars indicate one standard error. Asterisks indicates significant difference between forests ($p<0.05$).

Table 7. Mean (± SE) number of birds found per plot (for each treatment; *Btk* and NB) for the most common species detected on point counts on the George Washington and the Monongahela National Forests during 1996 to 2001.

		1996		1997		1998		1999		2000		2001		p Value[1]				
Species	Trt	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	F	T	Trt*T	F*T	Trt*T*F
Acadian Flycatcher	*Btk*	1.88	0.96	1.92	0.51	1.63	0.70	0.92	0.69	1.29	0.47	1.83	0.86	0.51	0.66	0.17	0.45	0.35
Empidonax virescens	NB	1.08	0.63	0.40	0.34	0.92	0.47	1.12	0.46	0.83	0.31	0.90	0.57					
Black-and-white Warbler	*Btk*	1.00	0.65	2.33	0.59	1.42	0.71	1.83	0.48	0.92	0.59	1.17	0.33	0.01	0.46	0.10	0.60	0.09
Mniotilta varia	NB	2.33	0.46	1.83	0.41	1.58	0.50	1.42	0.34	2.00	0.42	1.46	0.23					
Black-capped Chickadee	*Btk*	1.58	0.53	0.83	0.44	1.08	0.54	1.08	0.43	0.75	0.26	0.83	0.35	0.57	0.04	0.74	0.19	0.91
Poecile atricapilla	NB	2.08	0.37	1.79	0.31	1.21	0.38	1.42	0.30	0.96	0.19	0.79	0.25					
Black-throated Green Warbler	*Btk*	3.042	0.56	1.5	0.95	2.208	0.78	1.542	0.71	1.917	0.37	1.375	0.70	0.00	0.19	0.40	0.21	0.05
Dendroica virens	NB	1.75	0.37	1.558	0.63	2.533	0.52	2.092	0.47	2.625	0.25	1.492	0.46					
Blue Jay	*Btk*	1.42	0.48	0.75	0.45	0.08	0.28	0.33	0.27	0.50	0.23	1.00	0.31	0.23	0.04	0.39	0.74	0.41
Cyanocitta cristata	NB	1.17	0.34	0.96	0.32	0.20	0.42	0.92	0.19	0.50	0.16	0.29	0.22					
Blue-gray Gnatcatcher	*Btk*	1.75	0.63	1.00	0.51	0.54	0.24	0.46	0.20	0.67	0.34	0.75	0.38	0.12	0.01	0.95	0.63	0.92
Polioptila caerulea	NB	1.12	0.42	0.76	0.34	0.39	0.16	0.36	0.13	0.49	0.22	0.67	0.25					
Blue-headed Vireo	*Btk*	1.5	0.62	1.583	0.61	2.667	0.78	1.917	0.64	1.417	0.38	1.083	0.42	0.81	0.08	0.91	0.60	0.39
Vireo solitarius	NB	1.292	0.44	1.667	0.43	2.333	0.55	2.375	0.45	1.958	0.27	1.417	0.30					
Brown-headed Cowbird	*Btk*	1.92	0.69	1.42	0.50	0.92	0.57	0.75	0.40	0.58	0.32	1.25	0.40	0.17	0.03	0.78	0.02	0.49
Molothrus ater	NB	1.92	0.49	1.92	0.35	1.42	0.40	1.17	0.28	1.04	0.22	0.83	0.28					
Cedar Waxwing	*Btk*	1.25	0.35	0.50	0.32	0.00	0.13	0.00	0.24	0.00	0.20	0.00	0.35	0.03	0.03	0.10	0.22	0.45
Bombycilla cedrorum	NB	0.31	0.17	0.38	0.16	0.06	0.06	0.31	0.12	0.25	0.10	0.25	0.18					
Dark-eyed Junco[2]	*Btk*	0.75	1.43	0.25	0.68	0	1.04	0	1.30	0.25	1.16	0	1.01	-	0.98	0.93	-	-
Junco hyemalis	NB	1.667	1.17	1.167	0.56	2	0.85	2.167	1.06	2	0.95	1.667	0.83					
Downy Woodpecker	*Btk*	0.58	0.34	0.08	0.13	0.00	0.05	0.25	0.10	0.33	0.14	0.58	0.18	0.08	0.01	0.88	0.02	0.48
Picoides pubescens	NB	0.44	0.27	0.17	0.10	0.06	0.04	0.08	0.08	0.08	0.11	0.44	0.14					
Eastern Towhee	*Btk*	0.75	0.81	0.63	1.05	0.13	0.52	0.13	0.52	0.13	0.13	0.00	0.35	0.83	0.16	0.96	0.86	0.94
Pipilo erythrophthalmus	NB	1.08	0.52	0.98	0.68	0.88	0.34	0.50	0.33	0.15	0.08	0.35	0.23					
Eastern Tufted Titmouse	*Btk*	3.167	0.62	0.833	0.36	1.167	0.58	0.833	0.26	0.75	0.28	0.417	0.30	0.38	<0.0001	0.40	0.52	0.00
Baeolophus bicolor	NB	2.417	0.44	1.417	0.25	1.542	0.41	0.917	0.19	0.708	0.20	1	0.21					
Eastern Wood Pewee	*Btk*	2.58	0.74	0.75	0.52	1.92	0.78	0.67	0.26	1.42	0.48	0.58	0.31	0.61	0.00	0.30	0.26	0.92
Contopus virens	NB	1.29	0.52	1.17	0.37	1.38	0.52	0.58	0.18	0.75	0.34	0.50	0.22					

Table 7. Continued.

		1996		1997		1998		1999		2000		2001		p Value[1]				
Species	Trt	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	F	T	Trt*T	F*T	Trt*T*F
Great-crested Flycatcher	*Btk*	1.833	0.55	0.417	0.36	0.833	0.20	0.5	0.22	0.583	0.29	0.25	0.20	0.78	<0.0001	0.46	0.01	0.30
Myiarchus crinitus	NB	1.558	0.41	1.067	0.27	0.433	0.14	0.3	0.16	0.533	0.22	0.342	0.15					
Hairy Woodpecker	*Btk*	0.667	0.23	0.25	0.23	0.167	0.15	0.333	0.18	0.167	0.11	0.333	0.17	0.77	0.18	0.21	0.63	0.87
Picoides villosus	NB	0.408	0.17	0.767	0.17	0.408	0.11	0.35	0.14	0.225	0.08	0.667	0.12					
Indigo Bunting	*Btk*	1.708	1.00	0.708	0.81	0.667	0.33	0.958	0.88	0.083	0.45	0.083	0.37	0.16	0.05	0.76	0.27	0.82
Passerina cyanea	NB	1.6	0.66	1.35	0.54	0.458	0.22	1.017	0.59	0.833	0.30	0.783	0.24					
Ovenbird	*Btk*	6.583	1.88	3.667	1.16	3.917	1.74	3.083	1.03	3.333	0.98	2.417	0.94	0.80	<0.0001	0.55	0.70	0.81
Seiurus aurocapillus	NB	7.583	1.39	6.175	0.86	7.583	1.29	4.708	0.77	4.475	0.73	3.967	0.69					
Piliated Woodpecker	*Btk*	0.667	0.25	0.167	0.20	0.167	0.25	0.583	0.23	0.167	0.15	0.667	0.18	0.00	0.18	0.73	0.38	0.23
Dryocopus pileatus	NB	0.517	0.18	0.383	0.15	0.467	0.19	0.508	0.17	0.225	0.11	0.458	0.13					
Pine Warbler	*Btk*	1.5	0.84	0.667	1.04	1.667	1.66	2.917	1.72	0.833	1.08	0.583	0.68	0.07	0.20	0.70	0.29	0.91
Dendroica pinus	NB	1.375	0.47	2.229	0.58	2.667	0.93	2.375	0.96	2.125	0.60	0.896	0.38					
Red-eyed Vireo	*Btk*	11.25	2.36	7	1.56	7.667	1.20	6	1.62	6.083	1.45	5	1.32	0.01	0.00	0.54	0.05	0.79
Vireo olivaceus	NB	8.625	1.67	8.25	1.10	8.25	0.85	6.208	1.15	6.25	1.03	5.208	0.93					
Rose-breasted Grosbeak	*Btk*	1.75	1.02	1.25	0.72	0.333	0.42	0.417	0.69	1.167	0.58	0.417	0.35	0.10	0.24	0.56	0.36	0.43
Pheucticus ludovicianus	NB	0.9	0.74	0.5	0.52	0.5	0.30	0.55	0.50	0.275	0.42	0.475	0.25					
Scarlet Tanager	*Btk*	3.333	0.84	2	0.58	2.5	0.78	2.833	0.39	1.917	0.54	1.083	0.47	0.05	0.02	0.67	0.15	0.09
Piranga olivacea	NB	3.25	0.59	2.625	0.41	2.917	0.55	2.583	0.27	1.458	0.38	2.208	0.33					
White-breasted Nuthatch	*Btk*	1.083	0.58	1.083	0.56	0.583	0.19	0.25	0.33	0.583	0.20	0.75	0.27	0.27	0.19	0.91	0.20	0.54
Sitta carolinensis	NB	1	0.41	1.083	0.40	0.583	0.14	0.625	0.23	0.333	0.14	0.375	0.19					
Wood Thrush	*Btk*	2.25	0.49	1.125	0.31	0.792	0.40	1.083	0.68	0.583	0.26	1	0.36	0.32	0.00	0.66	0.48	0.73
Hylocichla mustelina	NB	1.375	0.31	0.417	0.20	0.708	0.25	0.958	0.43	0.5	0.16	0.25	0.23					
Worm-eating Warbler	*Btk*	5.583	0.76	3.667	1.01	3.167	0.84	3.333	0.77	3.333	0.54	3.333	0.67	<0.0001	0.03	0.29	0.11	0.34
Helmitheros vermivorus	NB	5.271	0.60	5.313	0.80	5.5	0.66	4.604	0.61	3.25	0.43	4.229	0.53					
Yellow-billed Cuckoo	*Btk*	1.125	0.23	0	0.07	0	0.20	0.083	0.20	0	0.07	1	0.30	0.08	<0.0001	0.02	0.04	0.05
Coccyzus americanus	NB	0.375	0.18	0.042	0.05	0.292	0.15	0.417	0.16	0.292	0.05	0.708	0.23					

[1] p values for univariate ANOVA; F=forest (block); T=time; Trt=treatment
[2] Dark-eyed Junco was only detected on MNF therefore no tests related to forest could be conducted

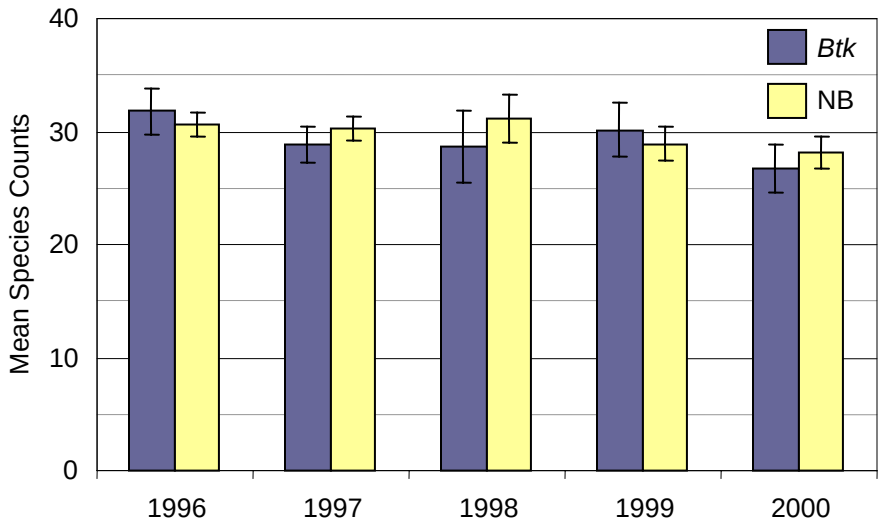

Figure 87. Species richness means for the *Btk* and non-treated (NB) plots from 1996 to 2000. Treatment years were 1997 and 1998. Error bars indicate one standard error.

Abundance

Point count data for the 27 most common species were analyzed for each year as well as between the treatment/non-treatment plots. Two-thirds (18) of these species showed a noticeable decline on treatment plots relative to non-*Bacillus* (NB) treatment plots following the application of *Btk* (Table 7; Figure 88). Most (13) of the species showed the effect during the first year of treatment, while only five exhibited the expected reduction of numbers starting in 1998, the year following the first year of treatment (Figure 88). Three of the affected species (Black-throated Green Warbler, Eastern Tufted Titmouse, and Yellow-billed Cuckoo) were found to show a significant interaction between time and treatment or time, treatment, and national forest (Table 7).

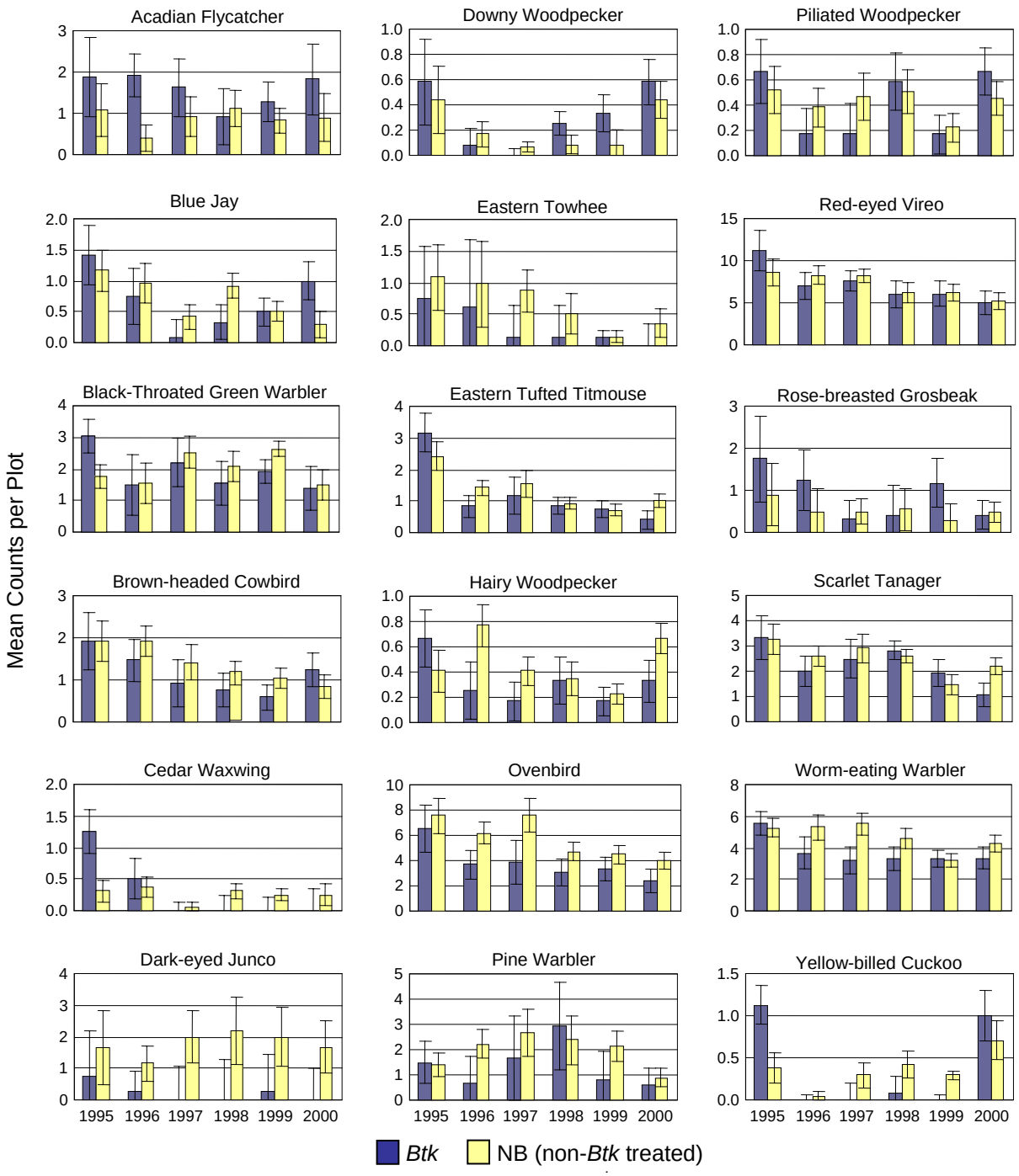

Figure 88 (previous page). Abundance data from point counts on the Monongahela National Forest and the George Washington National Forest during 1996 to 2001, for eighteen of the most common species that exhibited a slight negative trend from the use of *Btk*. Error bars indicated one standard error.

Reproductive Success

Nest Success

A total of 927 nests of all four focal species (Red-eyed Vireo, Blue-headed Vireo, Wood Thrush and Worm-eating Warbler) were found on both national forests over the 4-year period. Sample size of nests varied between national forests so that estimation of nest success and some other reproductive parameters was not always possible for some forest/species combinations. Red-eyed Vireos were not as abundant on the GWNF and very few nests were found on that forest. Over the 4-year period, approximately twice as many Blue-headed Vireo nests and three times as many Wood Thrush nests were located on the MNF compared with the GWNF, while three times as many nests of the Worm-eating Warbler were found on the GWNF compared with the MNF. Nest-searching effort was similar on each forest, and both point count and plot-mapping data reflected the same trends in abundance as did the total number of nests found for each species on both plots and forests (R. Cooper, unpubl. data). Due to these differences, yearly nest success and the reproductive variables in MNF were only determined for Red-eyed Vireos, Blue-headed Vireos, and Wood Thrush and for Worm-eating Warblers on the GWNF. We did not observe the predicted trends in nest success due to food reductions for any focal species. Although similar nesting success rates were found on the *Btk* and NB plots in the pre-treatment year (1996) for both Wood Thrush and Red-eyed Vireos, the rates varied between treatments in the following treatment years (1997 and 1998; Table 8) showing no treatment effect. Blue-headed Vireo rates were low on both treatments during all years with no obvious treatment effect. Nesting success rates for Worm-eating Warblers were higher on NB plots during pre-treatment and increased during the first treatment year while rates on *Btk* plots went down slightly. During the second treatment year both treatment rates dropped but NB plots were still higher than *Btk* plots. Post-treatment rates were similar between treatments and higher then other years. Overall probability of nest success for Worm-eating Warblers was lower in 1998 than 1997 and significantly lower than 1999 ($df = 1$, $x^2 = 7.403$, $p = 0.007$) with the treatments combined. Within years, we found no significant differences in the overall probability of nest success for Worm-eating Warblers between the *Btk* and NB plots.

Table 8. Mean reproductive variables (± SE) calculated from nests found and monitored during 1996-1999 for the Blue-headed Vireo, Red-eyed Vireo, and Wood Thrush in the Monongahela National Forest and for the Worm-eating Warbler in the George Washington National Forest.

		1996						1997						1998						1999					
		Btk			NB			*Btk*			NB			*Btk*			NB			*Btk*			NB		
Analysis	Species	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE
Mayfield																									
	Blue-headed Vireo	0.15	13	0.12	0.30	15	0.11	0.16	14	0.09	0.17	20	0.09	0.16	16	0.14	0.26	35	0.08	0.16	25	0.08	0.17	47	0.06
	Red-eyed Vireo	0.34	21	0.12	0.42	27	0.11	0.63	28	0.11	0.37	26	0.10	0.41	21	0.12	0.67	18	0.13	0.51	27	0.11	0.17	33	0.07
	Wood Thrush	0.21	13	0.12	0.20	17	0.10	0.30	17	0.11	0.58	23	0.11	0.73	21	0.10	0.33	13	0.13	0.48	28	0.11	0.58	26	0.11
	Worm-eating Warbler	0.34	13	0.15	0.48	27	0.12	0.33	21	0.15	0.55	40	0.10	0.26	26	0.10	0.34	52	0.07	0.64	20	0.17	0.60	28	0.10
Clutch Size																									
	Blue-headed Vireo	3.83	6	0.17	3.86	7	0.14	3.67	3	0.33	3.67	9	0.17	4.00	3	0.00	3.82	11	0.18	4.00	5	0.00	3.81	16	0.10
	Red-eyed Vireo	3.38	8	0.18	3.17	12	0.11	2.89	9	0.20	3.44	9	0.18	3.08	12	0.15	3.00	8	0.27	3.00	8	0.19	2.92	12	0.08
	Wood Thrush	3.67	6	0.21	3.42	12	0.23	3.55	11	0.21	3.56	18	0.15	3.38	16	0.20	4.00	8	0.19	3.48	21	0.15	3.73	22	0.15
	Worm-eating Warbler	5.00	5	0.32	4.09	11	0.44	4.90	10	0.18	4.79	24	0.12	4.53	17	0.19	4.58	31	0.12	4.67	15	0.16	4.95	20	0.11
Hatching Success																									
	Blue-headed Vireo	1.00	3	0.00	0.92	3	0.08	1.00	2	0.00	0.88	6	0.06	0.83	3	0.08	0.88	8	0.05	0.94	4	0.06	0.94	12	0.03
	Red-eyed Vireo	1.00	5	0.00	0.88	9	0.06	0.97	8	0.03	0.90	6	0.06	0.98	10	0.03	0.83	6	0.11	0.90	7	0.06	1.00	5	0.00
	Wood Thrush	0.83	4	0.10	0.96	8	0.04	0.83	7	0.08	0.92	14	0.05	0.94	14	0.03	0.97	7	0.03	0.94	17	0.03	0.95	20	0.03
	Worm-eating Warbler	0.94	3	0.06	0.88	10	0.07	1.00	7	0.00	0.89	21	0.04	0.87	13	0.04	0.86	24	0.04	0.87	13	0.06	0.89	19	0.04
Productivity																									
	Blue-headed Vireo	4.00	2	0.00	3.67	3	0.33	3.50	2	0.50	3.50	2	0.50	3.00	1	-	3.00	7	0.38	3.50	2	0.50	3.29	7	0.36
	Red-eyed Vireo	3.17	6	0.17	2.75	4	0.25	2.70	10	0.15	3.00	8	0.33	3.20	5	0.20	2.57	7	0.43	2.75	4	0.25	3.00	2	0.00
	Wood Thrush	3.33	3	0.67	3.00	2	1.00	3.67	3	0.33	3.13	8	0.35	3.00	13	0.23	4.00	1	-	3.00	7	0.31	3.21	14	0.21
	Worm-eating Warbler	3.83	6	0.54	3.29	14	0.27	3.75	12	0.41	4.26	23	0.29	3.38	8	0.50	4.18	22	0.16	3.75	16	0.35	4.31	16	0.18
Fledging Success																									
	Blue-headed Vireo	1.00	2	0.00	0.92	3	0.08	1.00	2	0.00	0.88	2	0.13	0.75	1		0.70	5	0.12	1.00	1	-	1.00	5	0.00
	Red-eyed Vireo	1.00	3	0.00	0.89	3	0.11	0.95	5	0.05	0.88	2	0.13	0.94	4	0.06	0.75	4	0.14	1.00	2	0.00	1.00	1	-
	Wood Thrush	0.89	3	0.11	1.00	2	0.00	1.00	2	0.00	0.87	7	0.10	0.95	10	0.03	1.00	1	-	0.88	7	0.06	0.94	12	0.04
	Worm-eating Warbler	0.92	2	0.08	0.87	5	0.13	0.92	5	0.08	0.87	14	0.06	0.87	3	0.13	0.92	10	0.03	0.86	12	0.06	0.86	10	0.04

Reproductive Variables

One hundred seventy-seven Red-eyed Vireo nests were monitored in MNF during this study. Average clutch size was similar between treatments during 1996 but was significantly higher on NB plots during the first treatment year (df = 1, $\Pi^2 = 4.347$, p = 0.037; Table 8). The subsequent treatment and post-treatment years had similar average clutch size. Hatching and fledging success were highest for *Btk* plots for all years except 1999 although not significantly, while the mean number of fledglings (productivity) varied each year (Table 8).

We monitored 155 Blue-headed Vireo nests in MNF throughout the study. For these nests, clutch size varied little over the course of the study and was similar for both treatments (Table 8). Increased hatching and fledging success was found on *Btk* plots during the pre-treatment and first year of treatment, although sample sizes were very low. Treatment years had significantly lower fledging success and productivity than pre-treatment years (df = 1, $\Pi^2 = 4.43$, p = 0.035; df = 1, $\Pi^2 = 5.01$, p = 0.025) for both treatments combined while hatching success fell significantly for just *Btk* plots (df = 1, $\Pi^2 = 4.01$, p = 0.045). Average hatching and fledging success, and productivity levels rose on both treatments during post-treatment.

We monitored 224 Worm-eating Warbler nests on GWNF between May to July of 1996 to 1999. The average clutch size rose almost 1 egg over the course of the study for NB plots while clutch size on *Btk* plots fell close to 0.5 eggs (Table 8). *Btk* plots had elevated hatching success rates during 1996 and 1997 then fell to levels similar to those on NB plots, which had remained constant throughout the study. Additionally, the difference in average clutch size between a successful and failed nest in 1997 to 1999 was lower on *Btk* than NB plots (Figure 89; i.e., nests with larger average clutch sizes failed more often on *Btk* than NB plots). All estimated reproductive variables dropped on *Btk* plots between 1997 and 1998. Average productivity rose significantly on NB plots during treatment years (df = 1, $\Pi^2 = 8.96$, $P = 0.003$) while it dropped on *Btk* plots. Fledging success was similar throughout the study for both treatments.

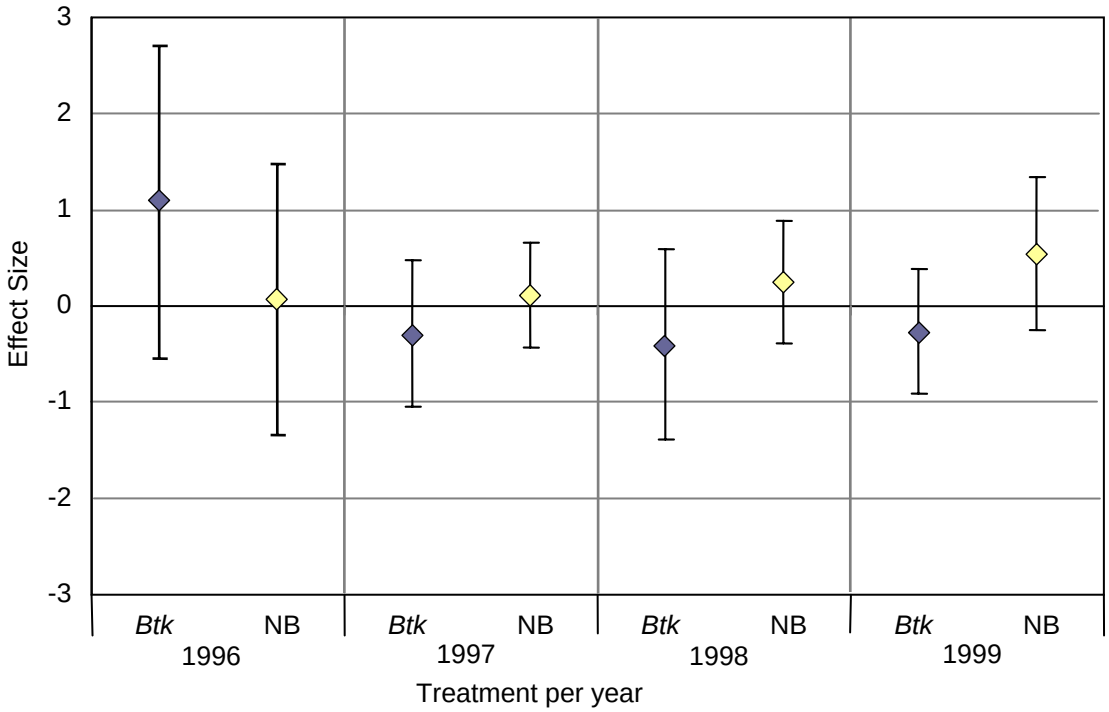

Figure 89. Difference in average clutch size between successful and failed Worm-eating Warbler nests for *Btk* and NB plots in the George Washington National Forest during 1996 to 1999. Bars indicate 95% confidence intervals.

Over the 4-year period, 153 Wood Thrush nests were located and monitored in the MNF. Average clutch size increased on the NB plots throughout the treatment years while averages on the *Btk* plots fell (Table 8). During the second treatment year, clutch size was significantly higher on NB plots and cumulative effect size calculations from 1996 to 1998 showed almost a 1 egg difference between treatments. Hatching success was similar between treatments throughout the study although slightly higher on NB plots. Fledging success and productivity levels varied each year with no obvious trends.

Provisioning Rates

Forty (14 on *Btk* plots, 26 on NB plots) of the 90 Worm-eating Warbler nests found in GWNF during 1998 were videotaped for a total of 126 hours of observations. Of those nests, 35 did not contain a cowbird young and were used for analyses of provisioning rates. Although the trend was for adults to make slightly more total trips/hour/young on the *Btk* plots than the NB plots (Figure 90), this appeared to be mostly influenced by nests with 4 young in it (Figure 91c). There was also a consistent trend for lower provisioning rates for each of the prey categories on *Btk* plots as well as the total number of prey items (Figure 92), but primarily the number of lepidopteran caterpillars/young/hour. In particular, the number of caterpillars/young/hour decreased as the number of young in the nest increased (Figure 91a), and significantly fewer caterpillars were provisioned to nests on *Btk* plots that contained 5 young as compared to NB plots. There was also a trend for nests with both 4 and 5 young to receive fewer prey items/young overall on *Btk* plots, although the precision of all estimates was low (Figure 91b).

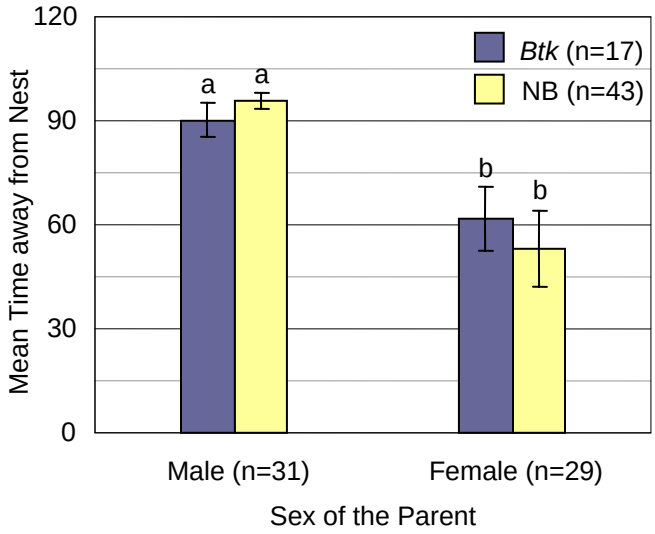

Figure 90. Mean (± SE) proportions of the observation time that each color-marked Worm-eating Warbler parent was away from the nest, summarized by sex and treatment (*Btk* and NB) in the Georgia Washington National Forest during 1998. Different letters above the bars represent significant differences (<0.05).

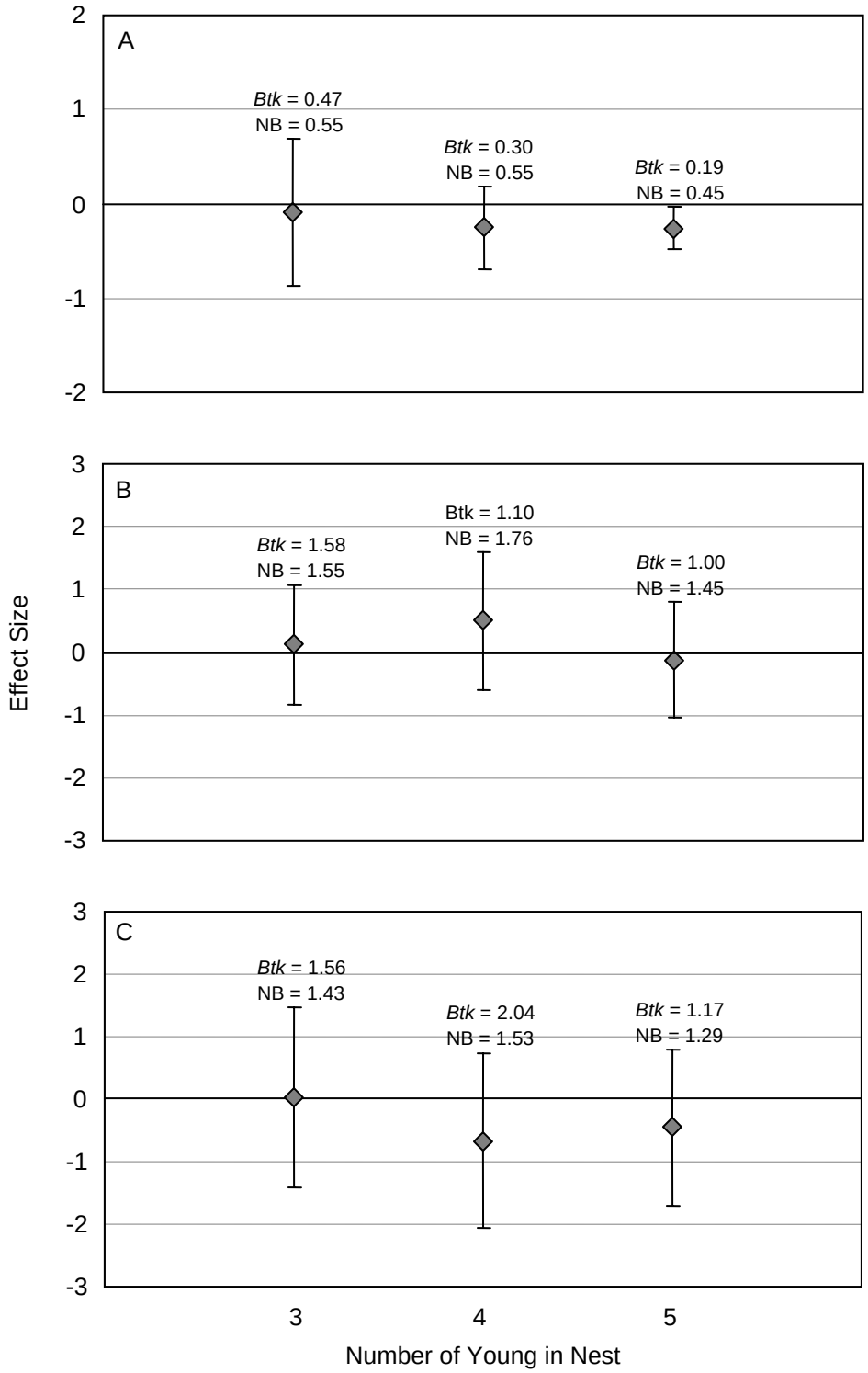

Figure 91. Effect size (± 95% CI) of the difference between treatments (*Btk* - NB) as it varies by clutch size for the number of Lepidoptera prey/young/hour (A), the total prey/young/hour (B), and the total trips/young/hour for Worm-eating Warbler nests (C) found on George Washington National Forest during 1996 to 1999.

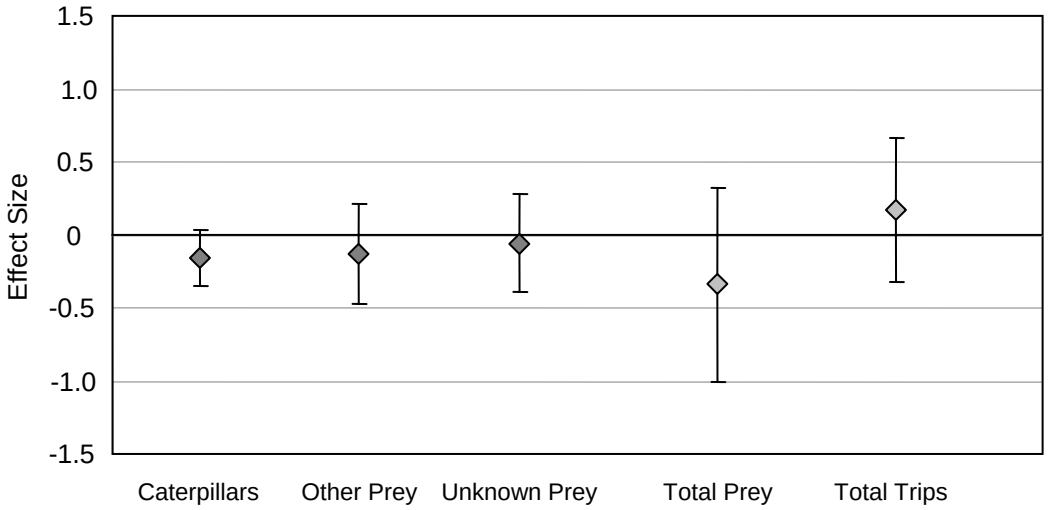

Figure 92. Effect size (± 95% CI) of the difference between treatments (*Btk* - NB) for the amount of each prey type brought to the nest and total number of trips for Worm-eating Warbler nests found on Georgia Washington National Forest during 1998.

Males spent significantly more time away from their nests than females (df = 1,56, F = 9.18, p = 0.004) when treatments were combined. However, when comparing within sex there were no significant differences between treatments (df = 1,56, F = 0.02, p = 0.667, Figure 90), or a significant interaction between sex and treatment (df = 1,56, F = 0.70, p = 0.406). There was a slight trend for females on *Btk* plots to spend a greater percentage of time away from the nests than females on NB plot.

Nestling Weights

A total of 144 nestlings were weighed from 35 Worm-eating Warbler nests (12 on *Btk* plots, 23 on NB plots) between 6 May and 14 July 1998. Nestlings from nests on *Btk* plots were lighter than nestlings on NB plots (Figure 93). On NB plots a trend of decreasing average weight was detected as the number of young in the nest increased from 3 to 5. However, there was no similar trend on the *Btk* plots, with nests containing 4 young having the larger average weights. There was also a greater variation in size of nestlings within broods on *Btk* plots, although not significantly. In nests without cowbird nestlings, the variation within nest increased with clutch size on *Btk* plots. The average difference between the lightest and heaviest nestling in a nest with 5 nestlings was more than 1½ g higher on *Btk* plots than NB although the sample size was very low.

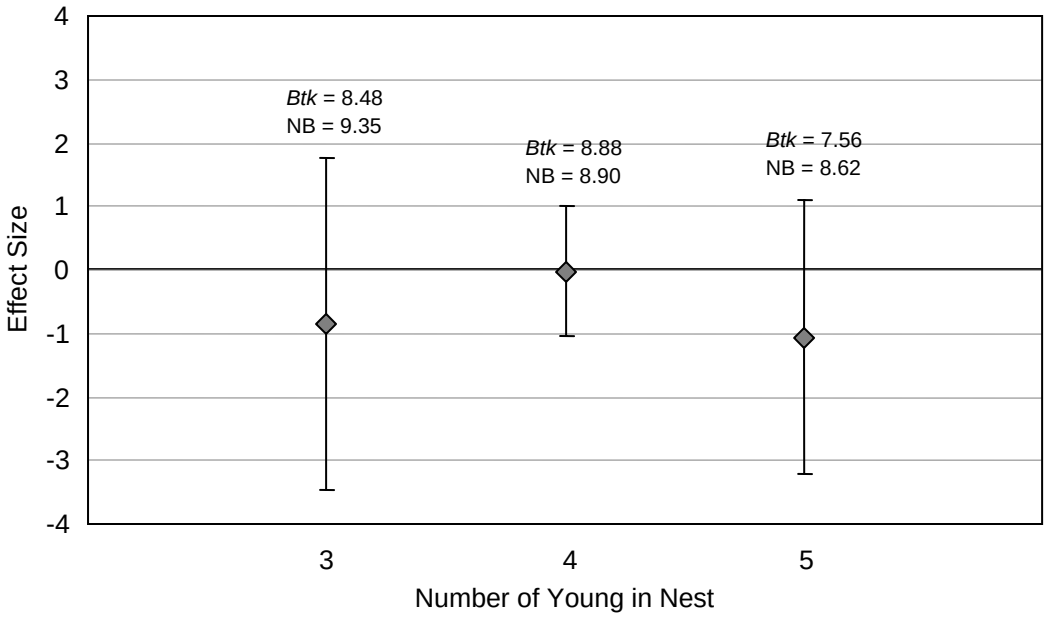

Figure 93. Difference between treatments (*Btk* - NB) for average nestling weight (g) as it varies by clutch size for Worm-eating Warbler nests found on George Washington National Forest during 1998. Bars indicate 95% confidence intervals.

DISCUSSION

We hypothesized that decreases in caterpillar abundance caused by *Btk* should not result in fewer birds or species of birds in the first year of application. Rather, if any effect were to be noted, it should be during the year after treatment. Specifically, *Btk* is applied in the spring after birds have established territories, and often after birds have laid eggs. Thus, there should be no effect of *Btk* application on bird abundance in both the pretreatment year (1996) and the first treatment year (1997). However, if caterpillar abundance remains low in the spring on the second treatment year, that is when bird abundance should be reduced on treatment plots. A similar or larger effect should be seen in the first post-treatment year (1999) for the same reason. After that, numbers should begin to recover provided that caterpillar numbers also recover. Of the most common species, only the Black-throated Green Warbler, Acadian Flycatcher, and Rose-breasted Grosbeak exhibited the hypothesized trend. Several other species, including the Eastern Towhee and Dark-eyed Junco, also exhibited a decrease in 1998 but the numbers on *Btk* plots never returned to that of the NB in the post-treatment years. One species, Yellow-billed Cuckoo, was a unique situation because it was common during the first two years of the project (1995 and 1996) when gypsy moth populations were high, then was an uncommon species until the last year of the project, when gypsy moth populations again increased. Many species exhibited the expected decrease during the first treatment year (1997), a faster response than we predicted and possibly related to the very cold spring of 1997, which delayed nesting and perhaps other aspects of the breeding cycle like territory establishment and pair formation. Because caterpillar abundance then stayed low on *Btk* plots for some time, territory abandonment could have occurred and abundance then could have been altered. Again, only three of the above ‘trends’ were significant with regard to treatment effect.

Unlike abundance, we hypothesized treatment effects on reproductive parameters in the first year of treatment, with effects growing more pronounced in the second treatment year and the first post-treatment year. The probability of nest success was not affected by treatment for any of the four species studied, which is consistent with similar studies conducted elsewhere (Nagy and Smith 1997; Holmes 1998). However, a closer look at two of the species studied more intensively, the Red-eyed Vireo and Worm-eating Warbler, showed what we think are biologically significant albeit sometimes subtle effects on their reproductive ecology. Although not reported here, Red-eyed Vireos delayed the onset of breeding in treatment years and the post-treatment year on *Btk* plots, shortening the breeding season by 3 to 5 days, which translated to a decrease of 0.15 to 0.25 young per female per year (Marshall et al. 2002). Of the four focal species, only Worm-eating Warblers showed the predicted response of a food reduction on nesting success. Although nest success was unaffected by treatment, Worm-eating Warblers showed decreased clutch size, nestling weight, and fledglings produced per nest on *Btk* plots in at least some years. Nestling weights were approximately 16% less on *Btk* plots with greater variability within nests compared to those on NB plots. This was likely related to the decreased amount of food brought to nests by adults compared with unsprayed plots, especially for nests with a larger clutch size. Fledgling survival has been shown to be related to nestling weight at the time of fledging (Perrins 1965), and combined with decreased productivity on *Btk* plots would likely result in decreased recruitment into the breeding population the following year.

We therefore urge caution when considering the application of *Btk* over larger spatial scales, repeatedly in the same area, or in locations of bird species of concern where even a modest reduction in seasonal productivity could be detrimental.

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CHAPTER 7. SALAMANDER STUDIES

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INTRODUCTION

Salamanders belonging to the family Plethodontidae (lungless salamanders) are the most diverse and abundant members of the order Caudata. They play a vital role in woodland ecosystems and represent a large portion of animal biomass in aquatic and terrestrial habitats. Salamanders serve as a link between higher vertebrates and detritivores, and provide measurable responses to determine ecologic trends due to environmental disturbances (Pauley 1995a, Stebbins and Cohen 1995, Duellman and Trueb 1986). It is known that amphibian populations are prone to large fluctuations (Stebbins and Cohen 1995), but the extent of the relationship between natural climatic events, human activities, and the current global amphibian decline is not known. The natural history and unique physiology of salamanders may render them susceptible to direct and indirect effects of aerial sprays used to control pest species. In the past, chemical and biological pesticides have been shown to cause negative impacts in amphibian activities and life cycles (Beebee 1996, Hayes et al. 2002, Ouellet et al. 1997, Russell et al. 1995, Sparling et al. 2001).

Treatments intended to target gypsy moth caterpillars may disrupt the food web by impacting nontarget caterpillars that are available to salamanders as food items. These caterpillars may be ones that feed on leaf litter, or foliage feeding caterpillars moving to the soil to pupate, or may represent treatment-affected caterpillars that fall to the forest floor. This only creates a short term "wind fall" of small, young caterpillars available as a food resource. In addition, pesticides washed from foliage may eventually enter the surface water. Because salamanders are long-lived, studies to determine impact of natural or human activities must be relatively long term.

Salamanders as Ecological Study Models

Amphibian skin serves many purposes, including protection against disease and injury, respiration, and absorption of water (Stebbins and Cohen 1995). In 1974, Gatz et al. found that 85% of total gas exchange in the Northern Dusky Salamander (*Desmognathus fuscus*) took place through the skin. The gelatinous eggs of salamanders are also permeable (Vitt et al. 1990). Chemicals introduced into the environment, including biological and chemical pesticides, may be absorbed through the permeable skin and into the eggs of amphibians. Many studies have been conducted to determine the potential for salamanders to act as indicators of environmental contamination (Southerland et al. 2002). And several studies have assessed the impacts of pesticides upon amphibians and noted detrimental effects (Bridges and Semlitsch 2000, Sanders 1970, Bridges 2000). Bridges (2000) also showed that amphibians are more susceptible to pesticides during different developmental stages. Boone and Semlitsch (2001) demonstrated that pesticides can alter community dynamics in ways that strict dose-response laboratory experiments can not illustrate, suggesting the importance of community level studies.

Because of the high permeability of their skin, salamanders must remain in moist areas. For woodland salamanders, taking refuge under damp logs, rocks, and leaf litter allows them to avoid

desiccation. Leaf litter retains moisture (litter moisture) and provides refuge for invertebrates that are the main food source of salamanders. Litter and fallen debris on the forest floor also provide shelter and breeding habitat for terrestrial salamanders. Under most forest canopies, soil temperatures are normally lower and relative humidity higher than in areas without forest cover (Smith 1980). Therefore, the moist environmental conditions of the Appalachian Mountains provide excellent habitats for these woodland species.

Larval and adult salamanders are carnivorous. Appalachian plethodontids feed on a variety of live animals, including many arthropods, annelids, mollusks, and other amphibians (Raimondo 1999). However, the majority of their diet consists of adult and larval insects (Raimondo et al. 2003). Environmental disturbances, such as aerially applied pesticides may greatly affect the amount and type of prey available for salamanders.

Plethodontid salamanders have very limited home ranges, often spending the majority of their lives in or around a single object or cover site that offers them prey and protection from desiccation and predation (Gergits and Jaeger 1990). In one example, Northern Dusky Salamanders (*Desmognathus fuscus*) were found to have a mean activity radius of only 1.1 m during summer months (Barthalmus and Bellis 1972). Because salamanders are long lived and relatively sedentary (Kleeberger and Werner 1982), they can be used to study the short and long term effects of insecticide treatments on nontarget organisms.

Importance of Salamanders in a Woodland Ecosystem

Within the deciduous forests of the Appalachian Mountain Range, salamanders of the family Plethodontidae are among the most common vertebrate species. In West Virginia, 26 species of salamanders have currently been identified in this family (Pauley 2004). Plethodontid salamanders in the Appalachian forest have been recorded at high densities and comprise a large proportion of the total vertebrate biomass.

Due in part to their ubiquity, plethodontid salamanders are a vital part of food webs in forest and stream ecosystems. Salamanders consume small prey and efficiently assimilate the biomass of this prey into their own tissue, which can then be utilized by larger predators, such as fish, snakes, birds, and invertebrates (Pough 1983).

Through predation, salamanders help regulate the population size and structure of invertebrate communities. In terrestrial ecosystems, salamanders feed upon leaf litter fragmenters, such as millipedes and insect larvae (Wyman 1998). In aquatic ecosystems, salamanders play an important role: in first and second order streams, they replace fish as top predators moderating invertebrate population size and diversity (Petranka 1998).

Potential Effects of Gypsy Moth Insecticides

The application of the microbial insecticides in this study raised two concerns: one, the possibility of directly affecting the salamanders and, two, the potential for indirect effect through the reduction of their food. A decrease in food alters the amount of tail fat storage that is used as energy in females which could result in a decrease in reproductive capacity and subsequent decrease in the population.

In a five-year study in the Fernow Experimental Forest in West Virginia, effects of the insect growth regulator, diflubenzuron (Dimilin®), on aquatic salamanders were evaluated (Pauley 1995b). *Desmognathus monticola, Desmognathus ochrophaeus*, and *Plethodon cinereus* were examined for stomach content, tail fat, carcass fat, total fat, and number of follicles present. Results suggested that diflubenzuron caused a shift in diets of *D. monticola*, but not an overall reduction in food consumption or energy levels.

While interest has been expressed in possible effects of *Btk* on amphibian populations (USDA 1995), there have been no previous studies on the effects of Gypchek or *Btk* on salamander populations.

Life Histories of Study Salamanders

Two subfamilies of Plethodontidae, the Desmognathinae and Plethodontinae, were observed during this study. Members of the Desmognathinae, represented by members of the *Desmognathus*, have a unique jaw mechanism that allows the upper jaw to pivot while the lower jaw is stationary. These species are generally semi-aquatic, often residing in streams or on stream banks, and return to water to deposit eggs (Green and Pauley 1987). The other species included in the study are highly variable in life history and all belong to the Plethodontinae, having a typical vertebrate jaw mechanism in which the lower jaw opens downward. These salamanders are terrestrial and directly develop from the egg without a post-emergent aquatic larval stage. They do not return to streams in order to deposit eggs, however, eggs are deposited in moist areas. The nine most commonly sampled salamanders species are shown in Figure 94.

Desmognathus fuscus (Northern Dusky Salamander)

Plethodon cinereus (Red-backed Salamander)

Gyrinophilus p. porphyriticus (Northern Spring Salamander)

Desmognathus monticola (Seal Salamander)

Plethodon glutinosus (Northern Slimy Salamander)

Eurycea bislineata (Northern Two-lined Salamander)

Desmognathus ochrophaeus (Allegheny Mountain Dusky Salamander)

Plethodon hoffmani (Valley and Ridge Salamander)

Pseudotriton ruber ruber (Northern Red Salamander)

Figure 94. The nine most commonly sampled salamander species.

METHODS

Study Site Selection

The original salamander study design included all 18 study plots in the GWNF and MNF. However, in the fall of 1996, the minute numbers of salamanders observed in the GWNF study plots (19 in total as compared to 302 on the MNF) compelled us to discontinue work on the

GWNF. This disparity between forests was attributed to lower leaf litter moisture and relative humidity, and higher soil temperatures on the GWNF.

Eliminating the GWNF sites allowed a more in-depth study of the MNF by including aquatic salamanders which, like terrestrial salamanders, are known to be predators on a wide variety of invertebrates (Petranka 1998). Aquatic and semi-aquatic salamanders prey on invertebrates in the stream and in the adjacent riparian areas, a community that may be influenced by gypsy moth sprays. The addition of aquatic study sites allowed for a more complete look into the role of salamanders in the food chain within the forest community.

Survey Methods

All nine study plots in the MNF contained a terrestrial and aquatic component, each with specific survey methods (Heyer et al. 1994) conducted along multiple transects (Jaeger and Inger 1994) along elevation gradients (Figure 95).

It is important to use multiple transects rather than one single long transect for statistical analysis (Jaeger and Inger 1994). In salamander terrestrial studies, vertical transects (i.e., positioned along an incline) are desirable as the changing elevation gradients are helpful in monitoring potential niche partitioning shifts due to habitat or environmental alterations. It is also necessary to use randomized sequential sampling to reduce the effects of short-term temporal changes in the sampling area. Short-term temporal changes include such things as microclimate conditions.

Terrestrial Studies

For terrestrial salamander studies, sampling was conducted along three 100 m vertical transects for set up on each plot to collect baseline data on species richness, species abundance, and species densities. The first and second transects on each plot were monitored during the day and the third transect at night. The first transect extended 100 m from the stream to the ridge in each study plot and contained 10 1 m x 1 m sites positioned every 10 m (Figure 95). Two additional sites were placed at the end of each transect along the ridge. Each of the 12 sites of transect 1 had 12 pine coverboards (15 cm x 8 cm x 2.5 cm) (Figure 96) positioned in a 3 by 4 matrix with an approximate 5 mm gap between boards to monitor surface abundance of salamanders (Pauley 1995a). At each sampling period, species and abundance of salamanders under coverboards were recorded. Population numbers were expressed as the surface count of adults, subadults, and juveniles within each 100 m section by time or area. Environmental data are recorded at each coverboard site.

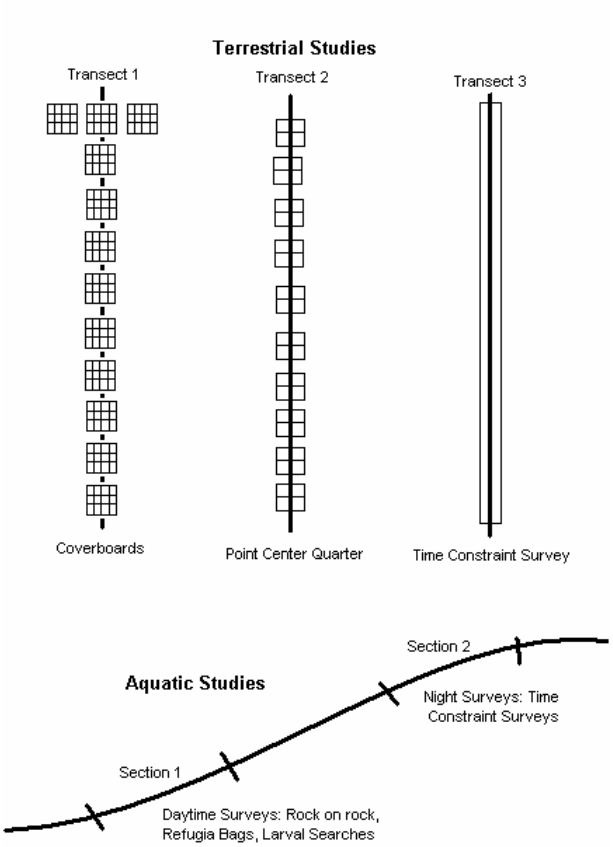

Figure 95. Diagram of salamander study transects.

Figure 96. Cover board array.

The second transect in each study plot (Figure 95) consisted of 10 sets of four 1 m x 1 m quadrats positioned every 10 m. These transects were also vertically arranged from the stream to the ridge. Every 10 m a small tree was flagged and using the point centered quarter method (Warde and Petranka 1981), two imaginary lines were drawn at right angles through this tree

(Figure 97). From these lines four 1 m x 1 m quadrats were created. Each quadrat was labeled rotating clockwise and each month a different quadrat was searched. In each quadrat, a complete removal census of surface salamanders was conducted. All cover objects and litter were removed from the site and all salamanders were identified and counted. This is an especially effective method for sampling juvenile salamanders (Heyer et al. 1994).

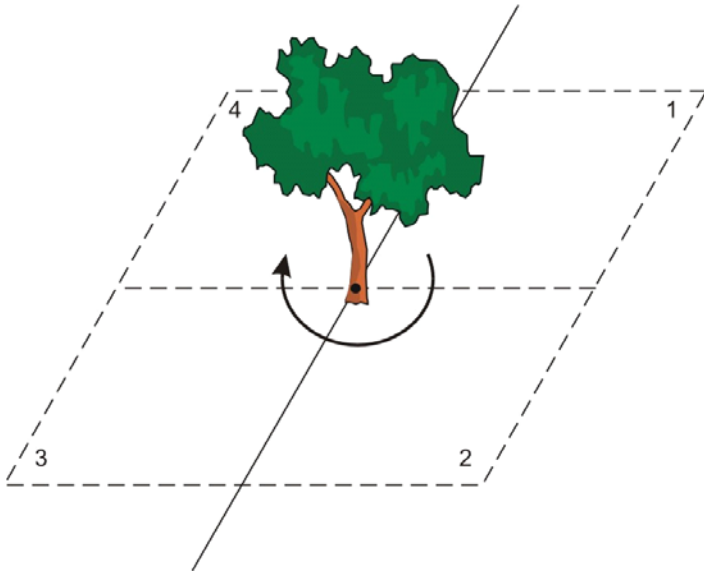

Figure 97. Illustration of point-quarter method.

The third 100 m vertical transect (Figure 95) on each plot also extended from the stream uphill and was used to conduct time constraint searches at night. An area approximately 1 m on each side of transect 3 was searched with headlamps by 2 or 3 investigators after dusk during rain or within 48 hours of rainfall. Statistically, at least 50 sampling sites should be used for an entire area (Jaeger and Inger 1994). Our study used 32 terrestrial sites per plot and 288 sites throughout the entire MNF study area.

Aquatic Studies

For the aquatic studies, two randomly selected 50 m sections along a stream in each study plot were used. The first 50 m stream section was examined during the day, while the second was examined at night. Environmental data were collected during each survey. For the day study, 10 refugia bags made of plastic netting and filled with leaf litter from the surrounding area (Pauley and Little 1998) were placed at the head of small pools within the stream to capture juvenile and larval salamanders. For adult surveys, 10 survey sites were constructed using flat rocks (approximately 35 cm x 35 cm) on another rock of equal size or larger (Pauley, unpublished data). This method has been termed “rock on rock” (Pauley 1995b). These two survey methods were used together, with one refugia bag paired with one rock on rock site and positioned every 5 m within the 50 m section (Figure 95). The abundance of stream species was expressed as the surface counts of adults, larvae, or juveniles observed within the 50 m section.

Also, during the day study, 1-hour time constraint searches for salamander larvae were conducted using aquarium nets and tea strainers within the first 50 m stream section. This involved searching all habitats that were likely to harbor larvae including pools, riffles, and mossy areas along rocks in water. Substrate was also disturbed to dislodge larvae hiding in gravel or leaf litter. Larvae were captured, held in a plastic bin and then processed and released after completion of the survey time period.

Night surveys involved time constraint searches with 2 or 3 researchers searching the entire width of the stream within the second 50 m section. All species observed were captured, measured, and recorded, and environmental data were taken at the beginning, middle, and end of the stream section. Population numbers of stream species were expressed as the surface counts of adults, juveniles, and larvae observed during time period or section area.

Sampling Schedule

Because various species of amphibians are active during different seasons, sampling was conducted at least once per month: spring (May and June), summer (July and August), and autumn (September and October). At each sampling period, salamander surveys were conducted and soil and litter samples were taken. Every month, three males and three females of each salamander study species were collected from all study plots for stomach contents studies.

Environmental Data Collection and Analysis

During each sampling period on terrestrial transects environmental and habitat data, and beginning and ending sampling times were recorded. Air temperature and relative humidity at ground level were measured with a thermo-hygrometer. Temperature of the first 3 cm of soil was measured with a Reotemp® Bimetal Pocket Thermometer. Soil samples consisting of about 7 to 9 g of soil no more than 3 cm deep were taken from each sample site, placed in plastic sealable bags and frozen as soon as possible for laboratory analysis of percent moisture and pH. Percent of sunlight reaching the forest floor was measured with an Extech Instruments® light meter, and the percent canopy coverage was taken monthly, May through October, with an ocular densiometer. In aquatic transects, date, and beginning and ending times of sampling were recorded. Water temperature was measured with an armored thermometer and water pH with a pH Testr™ meter.

Litter weight was determined no later than 48 hours after litter collection or the samples were frozen. An Ohaus® balance was used to weigh each sample. Samples were then placed into a drying oven set to approximately 105° C for 24 hours and were reweighed. Percent moisture was determined by subtracting the dry weight from the wet weight then dividing by the wet weight.

All frozen soil samples were warmed to room temperature before weighing. Rocks and twigs were removed from the samples. About 5 to 8 g of soil from each sample were added to a petri dish, weight was recorded, and then dried at 105° C for 24 hours. Soil samples were reweighed and dry weight recorded. Percent moisture was calculated. To determine soil sample pH, a 9:1 distilled water to soil slurry was made in a small beaker and a calibrated pH meter was used.

Diet Composition

Food items were obtained from stomachs dissected out of salamanders and from stomach pumping salamanders in the field. Salamanders that were stomach-pumped were not taken from the transects but were found by overturning natural cover objects in terrestrial and stream habitats in other areas. Stomachs were pumped in the field with a 10 cc syringe fitted with 18-gauge Nalgene® tubing (Figure 98). Stomach contents were immediately placed into 70% ethyl alcohol (Fraser 1976). Each plot was thoroughly searched once a month from May through September 1997 and from May to October 1998. Search times varied for each site visit depending on number of salamanders captured and time spent processing each salamander. An effort was made to sample the same number of each salamander species on each plot. Salamanders were weighed, snout vent length and cranial width measured, and returned to the original cover object. Each month, six additional adults (three male, three female) of each species were taken back to the laboratory for dissection. Specimens were frozen and placed in formalin, stomachs were dissected and prey items were removed and integrated with items taken by stomach pumping.

Figure 98. Salamander stomach pumping equipment: 10 cc syringe fitted with 18-guage Nalgene® tubing, water bottle, containment tray, and calipers.

Arthropods making up the diets of salamanders were identified to family when possible and to order when only remnants of whole prey were present (Fraser 1976). Length and width of whole prey items were measured with an ocular micrometer. Prey size was measured as volume, calculating the item as a prolate spheroid (Caldwell and Vitt 1999). Empty stomachs, plant material, rocks, and mostly-digested, unidentifiable organic matter were not included in the analyses. Prey items were separated into morphotypes based on order, life stage (adult/larva) and in the case of Hymenoptera, apterous (Formicidae) or winged (Caldwell and Vitt 1999). For each salamander species, seven prey categories were established: the five most abundant morphotypes, lepidopteran larvae, and miscellaneous taxa (Fraser 1976, Caldwell and Vitt 1999).

Analysis

Between the spring of 1995 and the fall of 2001, 4,641 salamanders were observed within all 18 study plots. Of the 4,639 found on the MNF, 2,051 were found in plots treated with Gypchek, 1,313 were found in plots treated with *Btk*, and 1,275 were found in plots not treated. These data were analyzed for relationships between gypsy moth treatments and salamander counts, species richness, and prey items. Relationships between treatments and environmental factors were also examined as well as the persistence of any treatment effects over time. One-way ANOVA was used to determine statistical significance between treatments. Diet composition was compared separately for each species using multivariate analysis of variance (MANOVA). Predator-prey size correlation was conducted using a linear regression analysis of salamander cranial width plotted against prey volume (Jaeger et al. 1995).

RESULTS

Coverboards provide more accurate assessments of salamander populations when compared to point transects and terrestrial night surveys (Johnson et al. 2003). Salamander species recorded under terrestrial cover boards from 1995 to 2001 included *Desmognathus ochrophaeus* (Alleghany Mountain Dusky Salamander), *Desmognathus fuscus* (Northern Dusky Salamander), *Eurycea bislineata* (Northern Two-lined Salamander), *Notophthalmus v. viridescens* (Eastern Red-spotted Newt), *Plethodon cinereus* (Eastern Red-backed Salamander), *Plethodon glutinosus* (Northern Slimy Salamander), *Plethodon hoffmani* (Valley and Ridge Salamander), and *Plethodon wehrlei* (Wehrle's Salamander).

When yearly total counts of species found under coverboards were grouped by treatments, *Btk* and Gypchek plots followed similar trends (Figure 99). Both *Btk* and Gypchek plot means increased the second year of sampling, peaking the following year in 1997. Yearly means decreased to their lowest level in 2000, and again increased in 2001. Mean numbers of salamanders on the *Btk* and Gypchek plots were 5.2 salamanders and 12.4 salamanders respectively. The control plots had the highest yearly mean of the treatment groupings with 16.1 salamanders in 1995, the first year of sampling. From this high point, control plot salamander mean counts decline, and after a slight increase for two years, continue to decline until the last year of sampling in 2001. There was not a significant change in mean counts of terrestrial salamanders on the *Btk* plots, or on the Gypchek plots. However, there was a significant change in salamander count means on the control plots ($p<0.05$).

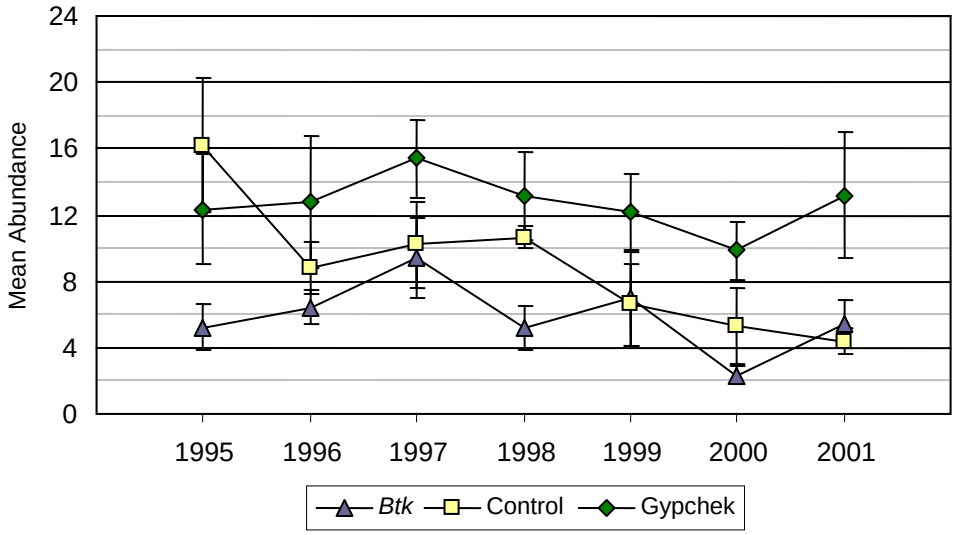

Figure 99. Yearly mean counts of terrestrial salamanders surveyed under coverboards grouped by plot treatment. Error bars indicate one standard error.

Of the eight species sampled from under coverboards, the Eastern Red-backed Salamander (*Plethodon cinereus*) represented the majority of terrestrial salamanders recorded. This species' yearly mean counts grouped by treatment (Figure 100) had trends similar to those of all the species when grouped (Figure 99). Results of statistical analyses were similar as well when comparing the grouped species to the Eastern Red-backed Salamander. There was no significant change in the mean counts of Eastern Red-backed Salamanders on the *Btk* plots or Gypchek plots, but again, there was significant change on control plots ($p<0.05$) over time. If treatments impacted yearly abundance trends, they may be detectable at single species and species assemblage levels.

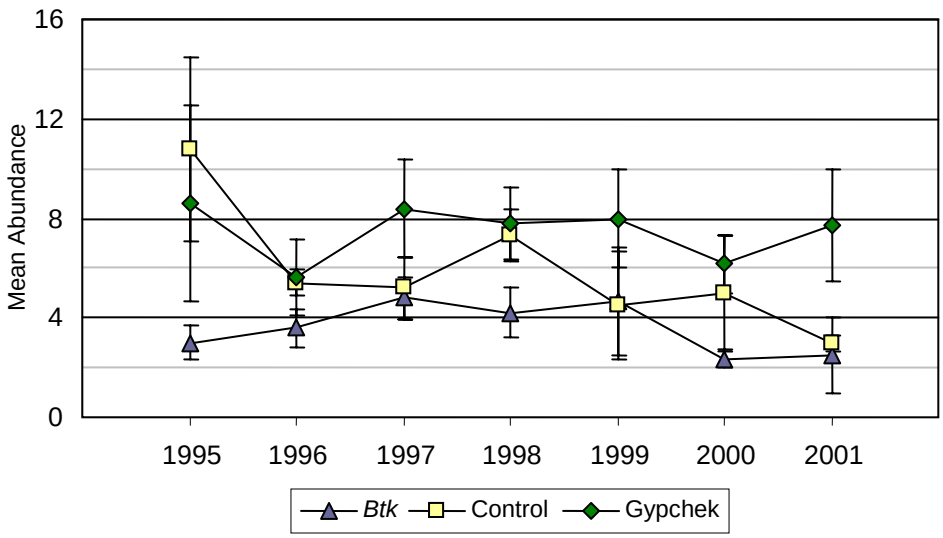

Figure 100. Yearly mean counts of the Eastern Red-backed Salamander (*Plethodon cinereus*) under coverboards grouped by treatment. Error bars indicate one standard error.

Nine species of salamanders were sampled during aquatic surveys, including *Desmognathus monticola* (Seal Salamander), *Gyrinophilus p. porphyriticus* (Northern Spring Salamander), *Pseudotriton r. ruber* (Northern Red Salamander), Alleghany Mountain Dusky Salamander, Northern Dusky Salamander, Northern Two-lined Salamander, Eastern Red-backed Salamander, Northern Slimy Salamander, and Eastern Red-spotted Newt. When these species were grouped by year and treatment, their year to year trends were very similar (Figure 101). The mean counts for each treatment were approximately 1.5 times lower in 1999 when compared to 1997. After 1999, mean counts for all treatment groupings increased near linearly through 2001. There was not a significant change during the study in the mean counts of aquatic salamanders on the *Btk*, Gypchek, or control plots.

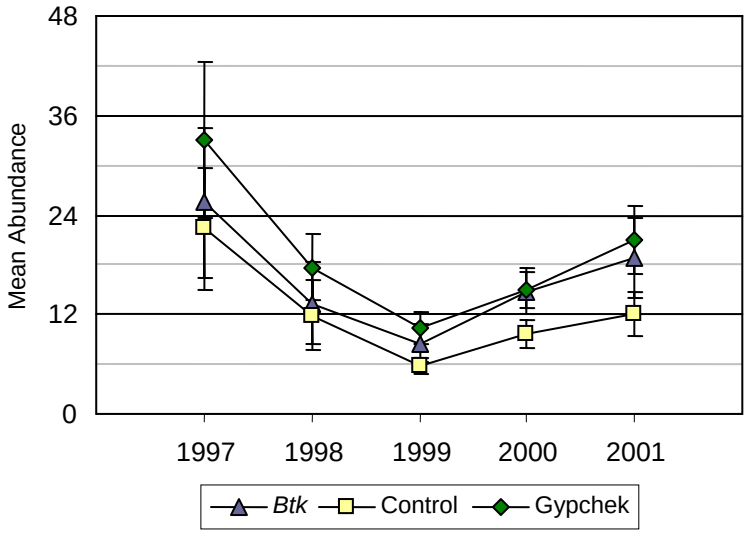

Figure 101. Yearly mean counts of aquatic salamanders grouped by treatment. Error bars indicate one standard error.

The Seal Salamander (*Desmognathus monticola*) was the most abundant stream salamander found during the aquatic surveys. This species had yearly overall trends (Figure 102) similar to those of the grouped aquatic species (Figure 101). There was no significant difference following treatment in the average number of Seal Salamanders for the *Btk* plots, or in the Gypchek plots. However, unlike the aquatic species analyzed as a group, there was a significant change in the mean counts of Seal Salamanders on the control plots ($p = 0.04$).

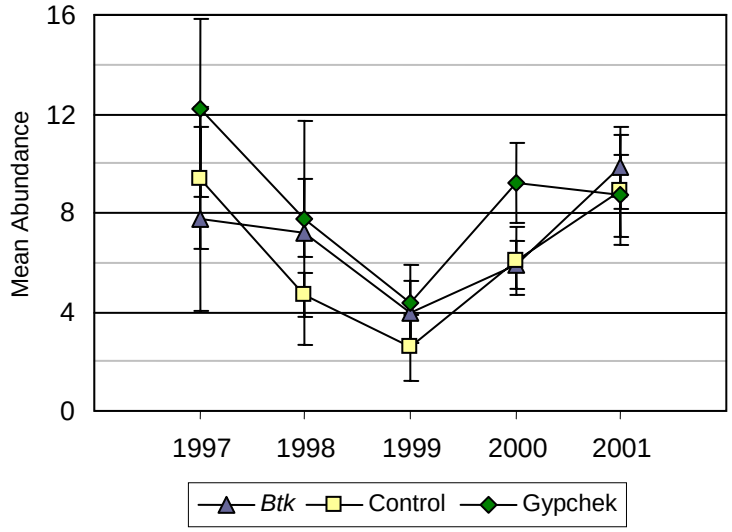

Figure 102. Yearly mean counts of Seal Salamander (*Desmognathus monticola*) grouped by treatment. Error bars indicate one standard error.

The terrestrial salamander year to year mean counts on Gypchek plots were the highest compared to the other two treatments, with the exception of 1995. A similar trend occurred in year to year

species richness means as well (Figure 103). Mean species richness on the Gypchek plots ranged between 3.4 to 4.4. The *Btk* plots, which had generally the lowest yearly mean counts, had the widest range of yearly species richness means, from 2.0 to 4.3. The control plots which had the greatest range of year to year mean counts had the narrowest year to year species richness means at 2.4 to 3.0. The control and Gypchek plot changes in species richness were not statistically significant. The increases in mean species richness on the *Btk* plots during the study was a significant change ($p<0.05$).

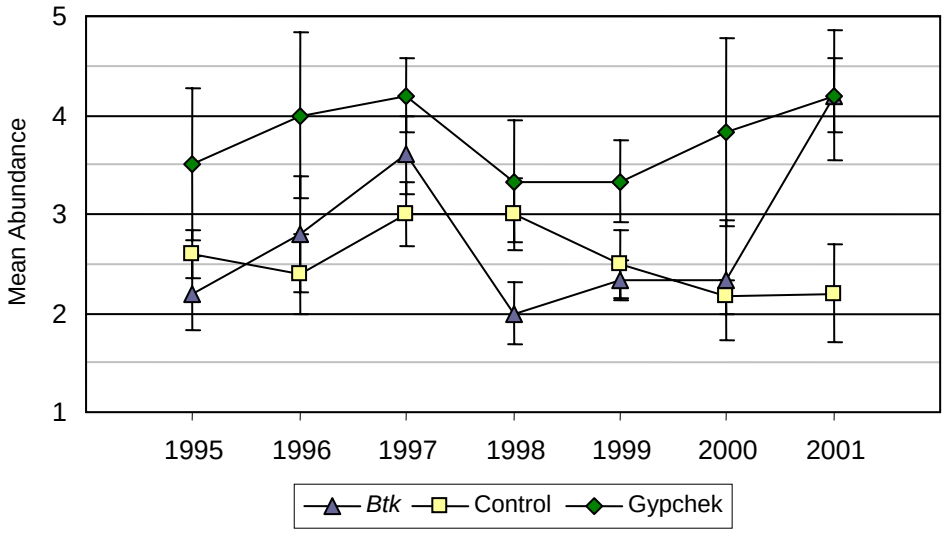

Figure 103. Yearly means of species richness under coverboards of all terrestrial salamanders on plots grouped by treatments. Error bars indicate one standard error.

Yearly species richness count means for aquatic (Figure 104) compared to the terrestrial salamanders (Figure 103) were usually higher and had narrower ranges. Aquatic species richness means were highest on the *Btk* plots in 2000 with yearly means ranging from 3.8 to 5.0. Species richness within the control and Gypchek plots remained fairly consistent with values ranging from 3.8 to 4.2 and 4.2 to 4.6, respectively. Changes in species richness during the study for the aquatic salamanders were not statistically significant on the control, *Btk*, or Gypchek plots. The changes in mean counts of the grouped aquatic species are not strongly reflected in the species richness means, including the clear decline into 1999. This is contrary to the terrestrial species, where some year to year mean count changes can possibly be identified with species richness mean changes for the same year.

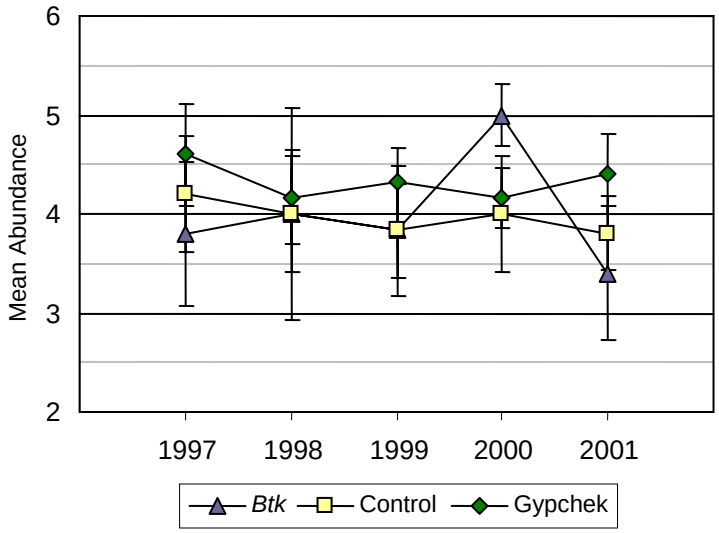

Figure 104. Yearly means of species richness for aquatic salamanders on plots grouped by treatments. Error bars indicate one standard error.

When salamander gut contents were tallied for presence or absence of food items, there was little difference among the three treatments. The greatest difference was just 2% between *Btk* (92%) and Gypchek (90%) plots, with control plots (91%) in the middle. Therefore, it is apparent that *Btk* and Gypchek have little effect on prey item selection of forest salamanders.

Environmental factors that may influence salamander population levels include air temperature, soil temperature, and moisture. Over seven years of field work, mean air temperature ranged from 16.7° to 29.8° C (Table 9). August was the hottest month during the study. Soil temperature during August ranged from 16.2° to 25.0° C (Table 10). Percent soil moisture monitored from 1995 through 2000 ranged from 13.2 to 55.6 (Table 11). There were no correlations found between control, *Btk*, or Gypchek plots and environmental conditions.

Table 9. Mean air temperature (°C) during August from 1995 through 2001 grouped by plot treatment. One standard error is given in parentheses.

	1995	1996	1997	1998	1999	2000	2001
Control	26.8 (1.4)	22.2 (1.3)	16.7 (1.6)	22.0 (1.0)	22.7 (1.4)	19.7 (0.3)	29.8 (0.7)
Btk	24.3 (2.3)	23.5 (0.8)	19.1 (0.8)	21.5 (2.0)	21.3 (0.7)	19.8 (0.2)	27.5 (0.5)
Gypchek	21.2 (2.5)	23.0 (1.1)	19.2 (1.7)	22.3 (0.9)	20.3 (1.4)	18.2 (1.2)	25.2 (1.1)

Table 10. Mean Soil Temperature (°C) during August from 1995 through 2001 grouped by plot treatment. One standard error is given in parentheses.

	1995	1996	1997	1998	1999	2000	2001
Control	25.0 (1.6)	19.0 (1.0)	16.4 (1.6)	19.6 (0.9)	17.6 (0.8)	17.2 (0.4)	21.7 (0.8)
Btk	23.3 (2.2)	19.3 (0.6)	18.3 (0.9)	19.5 (1.7)	16.2 (0.4)	17.8 (0.3)	21.2 (0.5)
Gypchek	21.2 (0.5)	20.2 (1.2)	19.7 (1.7)	19.2 (1.3)	16.4 (0.9)	16.5 (0.7)	19.2 (1.0)

Table 11. Mean Percent Soil Moisture during August from 1995 through 2000 grouped by plot treatment. One standard error is given in parentheses.

	1995	1996	1997	1998	1999	2000
Control	15.7 (7.7)	30.7 (8.3)	36.5 (3.9)	39.4 (4.0)	24.9 (2.9)	53.8 (6.1)
Btk	18.7 (4.2)	41.6 (8.6)	51.4 (4.6)	42.1 (7.3)	21.1 (6.3)	55.6 (15.9)
Gypchek	13.2 (1.8)	29.5 (4.7)	46.1 (3.7)	22.4 (4.8)	22.3 (6.7)	56.1 (17.4)

Diet analysis indicated that the proportion of types of prey items in the stomachs of the salamanders varied among species and treatments. For example, for both *D. fuscus* and *D. monticola*, flies (Diptera) were present in the greatest proportion on all treatment plots. Spiders (Araneae), beetles (Coleoptera), and ants (Hymenoptera: Formicidae) also were abundant prey items in these two salamander species, but their proportions in the diets were variable among treatments. Stomach contents of *D. ochrophaeus* were primarily mites (Acari), beetles, flies, and ants. Lepidoptera larvae were found in the stomachs of all five species. Based on the proportion of all prey taxa, caterpillars represented 9.0% and 6.1% of the diets of *D. monticola* and *P. glutinosus*, respectively (Raimondo et al. 2003). For all species, there were no significant differences in the percentage of caterpillars found in the stomachs between treatments (MANOVA), $p<0.05$.

For all species, proportions of prey taxa were highly variable among treatments, however, similarities did occur in the diets of salamander species occupying similar habitats. For example, terrestrial salamanders had high numbers of mites and ants in their diets, whereas semi-aquatic species had high quantities of adult Diptera. Prey size (volume) was positively correlated with salamander cranial width (linear regression: $r^2 = 0.9$, $p<0.05$). Salamander species were pooled for this regression analysis due to small sample sizes.

DISCUSSION

A decline in caterpillar counts as the result of two consecutive years of *Btk* and Gypchek applications was hypothesized to affect salamander abundance and species richness. Indeed, a decline of early season caterpillars did occur on the *Btk* plots during some treatment and post-treatment years (see Arthropod Results). The sample counts and species richness indicate fluctuations occurred in salamander populations, but analyses of this data gives little or no support to *Btk* and Gypchek impacting forest salamander assemblages. The only negatively significant population change occurred in the control sites. These declines may have been due to fluctuations in environmental conditions. The declines may also be attributed to repeated sampling in the study area, though most decreases in salamander counts were accompanied by an equal increase in subsequent sampling years. The ability to separate population changes caused by anthropogenic factors and natural causes is difficult without long-term studies (Pechmann et al. 1991).

Physiological factors of salamander life history make them susceptible to fluctuations in environmental regimes. The most obvious factor is their moist permeable skin with little resistance to desiccation (Spotila and Berman 1976). Feder (1983) suggested that hydric relationships affect salamanders by restricting feeding and reproduction. Although moisture greatly influences salamander movements, rarely does a salamander expire from desiccation alone (Feder 1983). Jaeger (1980) found that salamander habitats, not salamander densities, changed during rainfall events, and, as rainfall increased, the amount of foraging salamanders increased, while those present under cover objects decreased. Salamanders tend to inhabit cool, shaded areas with a thick, moist litter layer. Moist shaded areas are most common on northeastern exposures and lower areas of inclines in the higher elevations of the Appalachians (Harper and Guynn 1999). Various studies have shown that some amphibian declines are linked strongly to moisture regimen (Wake 1991). Although salamander density was not linked to surface abundance in this study, moisture may have resulted in low numbers of salamanders overall. The declines in aquatic salamander abundance seen in Figures 101 and 102 were probably due to near drought conditions in the summer of 1999.

The feeding ecology of forest salamanders may make them susceptible to some pest-management spraying practices. Elimination of a major food source has the potential of causing salamander population declines. If lepidopterans were a major food source, then *Btk* and Gypchek could have detrimental indirect effects. However, forest salamanders are opportunistic in feeding behavior and feed on a variety of insects. Detritivores comprise a majority of the diet for forest salamanders (Harper and Guynn 1999, Wyman 1998). Pauley (1978) analyzed the stomach contents of *P. cinereus* (n=86) and found a majority of the contents to be mites and ants. In a similar study Davidson (1956) found 42% of *P. glutinosus* (n=100) stomach contents were ants and other Hymenoptera and only 0.6% lepidopteran caterpillars. Based on the total of all prey taxa, lepidopteran caterpillars represented 9.0% and 6.1% of the stomach contents of *D. monticola* and *P. glutinosus*, respectively (Raimondo et al. 2003). There were no differences in the diets of salamanders in the three treatment areas, indicating that spraying had no effect upon feeding ecology.

The data presented suggest that *Btk* and Gypchek had little or no effect upon terrestrial and aquatic salamander density, species richness, or feeding ecology. However, it is believed that environmental regimes did play an important role in population fluctuations. Maintaining a stable ecosystem with little variation in forest canopy cover is most important in providing salamanders with ample foraging opportunities and suitable moist cover objects, necessary for survival during drought conditions.

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Conclusions

The above summarizes the results of a seven-year field study on the impacts of *Bacillus thuringiensis kurstaki* (*Btk*) and Gypchek® (a nucleopolyhedrosis viral product) on nontarget organisms when applied to control gypsy moth. The now naturally occurring (often through human intentional spreading) exotic gypsy moth fungal pathogen *Entomophaga maimaiga* was also examined in regards to its life history and pathogencity concerning nontaget Lepidoptera. Based on our study, these control options for gypsy moth would be ordered as having greatest to least impact on nontarget organisms as Gypchek, *E. maimiaga*, then *Btk.* This equivalent to ordering the control options from most to least specificly lethal to gypsy moth.

MORE TO COME. . . .