



Lepidoptera-specific insecticide used to suppress gypsy moth outbreaks may benefit non-target forest Lepidoptera

Rea Manderino*, Thomas O. Crist† and Kyle J. Haynes*

*The Blandy Experimental Farm, University of Virginia, 400 Blandy Farm Lane, Boyce, VA, 22620, U.S.A. and †Institute for the Environment and Sustainability, Miami University, Oxford, OH, 45056, U.S.A.

- Abstract**
- 1 Despite considerable interest in the impacts of forest-defoliating insects and pesticide-based suppression of defoliator outbreaks on non-target arthropods, studies have often been hampered by the unpredictability of outbreaks.
 - 2 We evaluated the long-term impacts of forest defoliation by gypsy moths, and the suppression of their outbreaks with *Bacillus thuringiensis* var. *kurstaki* (Btk), on native moths. Three years after a gypsy moth outbreak, moth diversity and abundance were compared among sites that were defoliated but not sprayed with Btk (defoliated sites), defoliated and sprayed (Btk sites) or neither (undisturbed sites). We conducted separate evaluations of the effects of disturbance history on the overall moth community, taxonomic subgroups (families) and moths differing in their dietary overlap with gypsy moths.
 - 3 Analyses of the full moth community revealed no effects of disturbance history on local (α) moth diversity or diversity of moths among sites (β -diversity). The α - or β -diversities of moths classified by their dietary overlap with gypsy moths (overlapping, partially overlapping, not overlapping) were also not affected by disturbance history. However, taxonomic affiliation was important. Geometridae α -diversity in late summer was significantly lower in defoliated sites than in Btk or undisturbed sites. No effects of disturbance history on moth abundances were found.
 - 4 We conclude gypsy moth defoliation had negative effects on a major moth family (Geometridae), although Btk application may have protected Geometridae from the adverse effects of gypsy moth defoliation. The results of the present study help to clarify the relative, and sometimes countervailing, effects of defoliators and microbial pesticides on forest communities.

Keywords Beta diversity, community resilience, disturbance, diversity partitioning, Lepidoptera, pest management strategies.

Introduction

Outbreaks of forest-defoliating insects can lead to widespread effects, including increased tree mortality and an alteration of microclimates and nutrient cycles (Lovett *et al.*, 2006; Kenis *et al.*, 2009; Gandhi & Herms, 2010). Forest insect communities may also be affected by these outbreaks through a variety of mechanisms, including reductions in the quantity and quality of foliage available to folivores and increased densities of generalist parasitoids (Redman & Scriber, 2000). Potentially compounding the effects of defoliator outbreaks on forest insect communities,

outbreaks are often suppressed with chemical or microbial pesticides that have non-target effects. Both defoliation and suppression of defoliator populations with pesticides are known to adversely affect the abundance and diversity of forest insects (Work & McCullough, 2000; Rastall *et al.*, 2003; Scriber, 2004). However, the relative impacts of defoliation and suppression, and the combined effects of these disturbances, are not well understood. Sample *et al.* (1996) attempted to separate the effects of defoliation and suppression on forest arthropods using an experimental design that relied on being able to predict the locations of defoliator outbreaks. Unfortunately, their method of predicting outbreaks based on the egg mass densities of the defoliating species proved unreliable, limiting their ability to examine defoliation effects.

Correspondence: Rea Manderino. Tel.: +1 316 323 9147; fax: +1 540 837 1523; e-mail: rm4zp@virginia.edu

In addition, existing studies investigating how the diversity of non-target insects is affected by defoliator outbreaks and suppression have not addressed whether the impacts vary from local to regional scales (Sample *et al.*, 1996; Work & McCullough, 2000; Rastall *et al.*, 2003; Boulton *et al.*, 2007; Timms & Smith, 2011). Previous studies have focused on diversity within individual localities (sites) but, as is recognized within community ecology (Whittaker, 1960), regional (γ) diversity results from a combination of both local (α) diversity and variation in diversity across space (β -diversity). Previous work has shown that effects of large-scale disturbances on species diversity may be spatially heterogeneous, such that the disturbance increases β -diversity (Anderson *et al.*, 2011). Understanding the regional effects of defoliation and suppression of defoliator populations will therefore require a consideration of the effects of these disturbances on both local diversity and spatial variability in diversity.

The gypsy moth (*Lymantria dispar* L.) periodically defoliates large swaths of eastern hardwood forests in North America; the mean annual extent of defoliation from 1975 to 2010 was 827 327 ha (U.S. Forest Service Gypsy Moth Digest; <http://www.na.fs.fed.us/fhp/gm/defoliation>). Monophagous and oligophagous folivores with host preferences that overlap with gypsy moths are most strongly affected as a result of the reduced availability of foliage associated with gypsy moth outbreaks (Redman and Scriber, 2000; Work & McCullough, 2000; Timms & Smith, 2011). In addition, gypsy moth feeding may also affect other Lepidoptera by inducing the production of plant defence compounds and the reduction of nitrogen content in leaves. Defences induced by gypsy moths include compounds such as phenolic glycosides and hydrolysable tannins that reduce the digestibility of foliage (Wold & Marquis, 1997; Redman & Scriber, 2000; Kosola *et al.*, 2001). The consumption of both damaged and regrown foliage can adversely affect the growth and survival of herbivores (Redman & Scriber, 2000; Calvo & Molina, 2010). Additional indirect effects of gypsy moth outbreaks on native Lepidoptera include increased densities of generalist parasitoid species introduced to control the gypsy moth, such as *Compsilua concinnata* M. and other tachinids, which have been found to kill over 200 species of native Lepidoptera (Howarth, 2001; Wagner & Van Driesche, 2010). Redman and Scriber (2000) found increased parasitism of swallowtail larvae by tachinid flies when in proximity to high densities of gypsy moth caterpillars.

In recent years, aerial spraying of microbial pesticides has been the primary method of suppressing gypsy moth outbreaks, although chemical pesticides (e.g. difluzuron) are still applied in some areas (White *et al.*, 1981; Tobin & Liebhold, 2011). Two microbial agents, the gypsy moth nucleopolyhedrosis virus (LdNPV) and the soil bacterium *Bacillus thuringiensis* (Bt), have been deployed in gypsy moth suppression efforts. Unlike Bt, LdNPV is only lethal to gypsy moths, although it is generally only used in regions where endangered Lepidoptera are known to occur as a result of the expense and difficulty of application (Hajek & Tobin, 2010). Many strains of Bt exist but the most potent to Lepidoptera is the *kurstaki* group (Hajek & Tobin, 2010). Although effective in reducing immediate defoliation caused by many Lepidoptera species, the long-term toxicity of the bacterium is variable, lasting anywhere from several days to months, and the persistence of the spores depends on

environmental conditions, such as rain and sunlight (Scriber, 2004). The pathogenicity of *Bacillus thuringiensis* subspecies *kurstaki* (Btk) has also been found to vary among Lepidoptera families, with butterfly families being the most sensitive and some moth families largely resistant (Peacock *et al.*, 1998).

The present study aimed to assess the impacts of past gypsy moth defoliation and spraying of Btk on the abundance and species diversity of forest moths. Because predicting the timing and location of gypsy moth outbreaks is difficult, we carried out blacklight sampling to compare moth abundance and diversity among areas affected by past defoliation, areas affected by both defoliation and suppression treatment, and areas unaffected by either defoliation or suppression. Surveying was conducted 3 years after defoliation and Btk applications occurred, allowing us to assess the long-term effects of these disturbances on forest moths. Based on previous work (Sample *et al.*, 1996; Work & McCullough, 2000; Rastall *et al.*, 2003; Boulton *et al.*, 2007; Timms & Smith, 2011), we predicted that undisturbed sites (no defoliation or spraying with Btk) would have a higher abundance and local (α) diversity of moths than sites that were defoliated but not sprayed with Btk and sites where defoliation was mitigated by Btk application. We also expected that moths sharing host plants with gypsy moths would be most affected by defoliation (Redman & Scriber, 2000; Work & McCullough, 2000). In addition, we expected moth families known to be relatively insensitive to Btk (particularly Geometridae) to have a higher abundance and α diversity in sites sprayed with Btk compared with defoliated sites (Peacock *et al.*, 1998). Finally, because the effects of defoliation or Btk might vary spatially as a result of local heterogeneities (e.g. canopy cover, terrain, tree and moth species composition), we evaluated whether either disturbance favoured increased β diversity.

Materials and methods

The site of the present study, Shenandoah National Park, is located within the Appalachian Oak Forests biome. Although historically oak–chestnut (*Quercus–Castanea*) over much of its range, subsequent to the introduction of chestnut blight, the forest ecosystem has shifted to dominantly *Quercus* spp. and *Carya* spp. (hickory; Johnson & Ware, 1982). The forest region is now considered to be composed of a mosaic of community types, including oak–hickory, oak–pine and mixed mesophytic forests, which vary with local moisture conditions. The understory is frequently composed of *Lindera benzoin*, *Rosaceae* spp., *Kalmia latifolia* and young *Acer pensylvanicum*. This ecoregion represents the second richest temperate broadleaf forest in the world, with at least 158 tree species, and, in conjunction with the neighbouring Appalachian Mixed Mesophytic forest ecoregion, it contains the richest endemic flora and fauna species in North America (Stephenson *et al.*, 1993; Ricketts *et al.*, 1999).

Moth community composition in these forests shows little variability across regions, remaining consistent in species diversity and changing mainly in the identity of the dominant taxa (Butler *et al.*, 2001; Summerville *et al.*, 2008; Stange *et al.*, 2011). The most critical factor determining the structure of lepidopteran communities in regions of similar biogeographical history is plant community composition, which is largely associated with stand age and structure of the surrounding landscape

(Hammond & Miller, 1998; Usher & Keiller, 1998; Summerville *et al.*, 2003).

Shenandoah National Park (SNP; 38°32'0"N, 78°21'0"W) lies along the Blue Ridge Mountains in north-central Virginia. It is a narrow tract of forest 169 km in length and ranging from < 1 to 15 km in width. Elevation in the park ranges from 300 to 1200 m. Historically a forest dominated (> 75%) by *Quercus prinus* L. and *Quercus rubra* L., the oaks have declined in the past half century to 59% park cover, with an increase in *Liriodendron tulipifera* L. and other hardwood species to 31% (Karban, 1978; Stephenson *et al.*, 1993; McNab, 1994). Conifers such as *Pinus strobus* L. and *Tsuga canadensis* L. are in the minority.

SNP has experienced frequent gypsy moth defoliation since 1986, with millions of trees killed during epidemic years of 1986–1995, when the entire length of the park was defoliated. The most recent defoliation cycle occurred in June of 2007–2009, with the heaviest defoliation in 2008. On 15 May 2008, for the first time since 1995, the National Park Service aerially sprayed *Bacillus thuringiensis* var. *kurstaki* (Btk) over 1012 ha of forested areas along the northern corridor of the park's main roadway. Treatment areas were chosen based on annual gypsy moth egg mass surveys in regions of high cultural value (National Park Service, 2008). Btk application was conducted using a fixed-wing aircraft with an application rate of 20–27 CLU (National Park Service, 2008; R. Gubler, personal communication). Defoliation was documented in the Btk-treated regions in 2008 based on aerial surveys (see below) but, during the next summer, there was also most no defoliation recorded in the treated areas (Fig. 1).

In the spring and summer of 2011, 3 years after the defoliation and Btk-application occurred, we conducted black-light sampling at sites selected using defoliation maps and spray block data provided by the National Park Service. The defoliation maps of were produced by aerial surveys conducted by the Virginia Department of Forestry, where defoliation was defined as > 75% of canopy removed. We established five sites within each of the three types of disturbance history found in the park: areas defoliated and sprayed with Btk (Btk: sites 7, 8, 10, 11 and 14), areas defoliated but not sprayed (defoliated: sites 6, 9, 12, 13 and 15), and areas not defoliated by the gypsy moth since 1995 (undisturbed: sites 1–5) (Fig. 1). Although every defoliated site experienced defoliation in 2008, all sites but two (sites 13 and 7) experienced defoliation in 2007 as well, and two sites (sites 12 and 11) experienced additional gypsy moth defoliation in 2009. To minimize bias as a result of any pre-existing differences among sites, all sampling sites were established with North–North-East–East facing aspects and within a relatively narrow range of elevations (635–1008 m). Furthermore, sampling sites were placed at least 2 km apart to minimize their interdependence. Subject to these constraints, we attempted to place sites away from boundaries between different disturbance histories as a result of the potential for surveyor error in mapping. Defoliated and undisturbed and sites were placed at least 0.5 km from boundaries with different disturbance histories. Because the Btk spraying was limited to an area centered over a roadway, the areas available for placing sites were more restricted given our 2-km minimum distance between sites. Thus, Btk sites were established as close to the centre of the sprayed area as possible at the same time as maintaining the

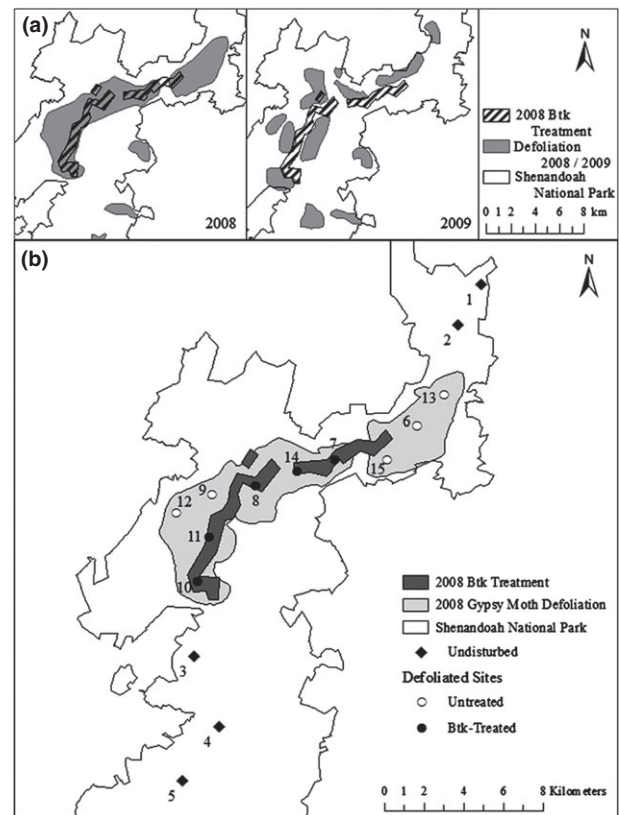


Figure 1 (A) Map of gypsy moth defoliation in 2008 and 2009, as well as the 2008 *Bacillus thuringiensis* subspecies *kurstaki* (Btk) application in Shenandoah National Park. (B) Map of study site locations, as well as areas defoliated and sprayed with Btk in 2008.

distance from the road. The minimum distance from a Btk site to the road or unsprayed defoliated areas was approximately 150 m. The distance between the farthest sites (Site 1–5) was less than 30 km. Full descriptions of each site are provided in the Supporting information (Appendix S1).

Trapping was conducted using Universal blacklight traps (12 W; BioQuip Products, Rancho Dominguez, California) powered by 12-V, 26-Ah batteries. Black-light trapping is the most widely used method for conducting moths surveys as a result of their attraction to ultraviolet light (Southwood & Henderson, 2000). Light trapping does not determine the full composition of the Lepidoptera community, attracting only a subset that fly to lights and whose flight capabilities are sufficient to approach the trap, although it is established as the standard method of ascertaining community structure of adult moths (Young, 1997). The day before a sampling night, we placed a single trap on a 1.5-m platform at each site location. Traps were charged with ethyl acetate as the primary killing agent and DDBT (No Pest Strip; Spectrum Brands, United Industries, Earth City, Missouri) as a backup killing agent. Each trap was equipped with a digital timer switch (ST01C; Intermatic, Inc., Spring Grove, Illinois) used to light the trap from 21.00 h to 05.30 h EDT.

Sampling sessions were conducted approximately every 4 weeks (Session 1: 25–29 May; Session 2: 28 June 28 to 2 July; Session 3: 26–30 July; Session 4: 24 August 24 to 2 September

2011) to analyze the effects of seasonality. Because the sampling efficiency of blacklight trapping is known to be affected by weather and ambient moonlight, optimal conditions restricted trapping to nights of low moonlight intensity (half to new moon phases), no precipitation, wind speeds below 12 km/h, and a minimum temperature $\geq 17^\circ\text{C}$ (Yela & Holyoak, 1997; Young, 2005). We divided trapping between two nights per sampling cycle to minimize travel time between sites; the number of sites sampled per night (9/6, 8/7) changed over the course of the study as needed to account for destroyed stands, manipulated traps and other conditional events. The period between trapping nights ranged from 1 to 7 days. However, each sampling cycle was completed within the confines of the optimal sampling conditions.

Samples were transported to the laboratory on ice, and stored in -20°C freezers before identification. Identifications were based on Covell (1984) and Hodges *et al.* (1972–2008). Given the large number of specimens collected and the difficulty of identifying members from the microlepidopteran families to species, we only identified specimens of the families: Bombycidae, Cossidae, Drepanidae, Erebididae, Geometridae, Lasiocampidae, Limacodidae, Noctuidae, Notodontidae, Saturniidae, Sesiidae, Sphingidae, Uraniidae and Yponomeutida. Specimens not belonging to these families were destroyed. Specimens were identified to species using morphology and wing patterns. Species groups that could not be distinguished by wing pattern without further examining genitalia were counted as single species [*Besma quercivoraria* Guenée paired with *Besma endropiaria* Grote & Robinson (Geometridae), *Hypagyrtis unipunctata* Haworth paired with *Hypagyrtis ester* Barnes (Geometridae), *Xestia dolosa* Franclemont paired with *Xestia c-nigrum* Linnaeus (Noctuidae), *Eulithis diversilineata* Hübner paired with *Eulithis gracilineata* Guenée (Geometridae) and *Probole amicaria* Herrich-Schäffer paired with *Probole alienaria* Herrich-Schäffer (Geometridae)]. Voucher specimens are retained at Blandy Experimental Farm (Boyce, Virginia).

Moth species were classified according to the most recent family designations (Lafontaine & Schmidt, 2010) and by their host plant preferences (Covell, 1984; Wagner, 2005; Robinson *et al.*, 2010). Species were classified as overlapping, non-overlapping or partially overlapping with gypsy moths in host plant preferences. The overlapping class was reserved for species for which all of their host plants are also fed upon by gypsy moths, and species feeding on a mixture of host plants fed on and not fed on by gypsy moths were classified as partially overlapping. Because the 2008 gypsy moth outbreak in SNP was described as causing heavy defoliation and tree mortality by Virginia's Department of Forestry (T. Edgerton, personal communication), it was assumed that gypsy moths used all available suitable hosts and were thus in overlap with the associated lepidopteran herbivores. Species with unknown host plants were excluded from the host plant analysis. These exclusions amounted to 1.8% of total species and 0.5% of total abundance.

To evaluate the effects of past gypsy moth defoliation on tree diversity and tree size, tree surveys were conducted between late July and mid-October 2011. Four 400-m² quadrats were established at each plot, with the intersection of the four centred on the trap stand. Two of these quadrats were randomly chosen and, within these quadrats, trees larger than 137 cm were counted.

Individuals were generally identified to species, although a few individuals known to easily hybridize and confound identification (i.e. *Crataegus* spp.) were only identified to genus. Diameter at breast height (DBH) was measured to determine tree size.

We considered how differences in site defoliation histories (defoliated, defoliated and sprayed with Btk, and undisturbed) may have affected the size (mean DBH) and diversity of trees (using the Shannon Diversity Index, H'_{tree}) using one-way analysis of variance (ANOVA) (PROC GLM in SAS, version 9.2; SAS Institute, Cary, North Carolina). To examine how differences in tree communities affect moth communities, we used a multiple regression to evaluate relationships between H'_{tree} , mean tree size (DBH) and moth diversity (H'_{moth}). This was performed using the PROC REG procedure in SAS.

The local moth diversity (α -diversity) of each collection site was calculated both as species richness (number of species) and the Shannon Index of diversity (H'_{moth}). Because species richness was strongly correlated with H'_{moth} ($R^2 = 0.74$), subsequent analyses were based only on H'_{moth} . For each sampling session and site history, we calculated the total H'_{moth} across sites (γ -diversity) and H'_{moth} among sites (β -diversity) using the additive model of β -diversity (Lande, 1996), where $\beta = \gamma - \alpha$. Additive partitioning was selected as it provides a measure of heterogeneity (β -diversity) in the same units as α - and γ -diversities, facilitating comparisons of the contributions of α - and β -diversity to γ -diversity. We note, however, that the Shannon Index (H') has the special property among diversity metrics where the additive partition of the Shannon Index ($H'_\alpha + H'_\beta = H'_\gamma$) is equivalent to a multiplicative partition of Shannon diversity ($H_\alpha H_\beta = H_\gamma$), where H is expressed the effective numbers of species per sample, $H_\alpha = \exp(H'_\alpha)$ (Anderson *et al.*, 2011). An interpretation of the H'_β mean value of the Shannon Index is not found in a given sample but found among all other samples in the overall data. It is therefore a single-value metric of turnover of species composition among samples that depends on the variation in relative abundance of species among sites (Anderson *et al.*, 2011).

A permutation test was used to test for differences in α - and β -diversity of the overall moth community among disturbance histories for each sampling session. Randomization enables an unbiased comparison of diversity across sites where unequal numbers of individuals were sampled and allows us to determine whether the observed diversity of those sites could have been obtained by a random allocation of samples among sites. In addition, randomization is necessary for hypothesis tests on observed values of β -diversity (either single-value metrics or multivariate dissimilarities) because of the dependence of β -diversity on the patterns of species distributions among samples, which will also be present in the null distributions of randomized samples (Anderson *et al.*, 2011). Using code developed by Diekötter and Crist (2013) and run in the R language (R Development Core Team, Austria), for each sampling session, we calculated the observed variation (F -statistic) in H'_{moth} among defoliated, Btk and undisturbed sites and then randomly reshuffled samples across the site histories to produce a pseudo F -statistic. The samples were reshuffled 9999 times. Pseudo F -statistics were computed after each randomization and the percentage of reshuffled pseudo F -statistics exceeding the observed F -statistic was used to test for significance. Sampling sessions were analyzed

individually to remove time as a variable because it is already established that moth diversity changes over time. The effects of disturbance history on the H'_{moth} in each host plant classification were also tested using permutation (as above). The same procedure was used to test the effects of disturbance history on each moth family, although families not present in every trapping session were excluded from this analysis. Sessions featuring sites with zero specimens of certain families or host plant classes were transformed by the addition of a pseudo species with an abundance of 1 at each site for the purposes of running the Diekötter and Crist (2013) R code.

We adjusted the significance level for each set of analyses on moth diversity to account for non-independent analyses. Trapping sessions were considered independent given that the moth community largely turns over within the 1-month timeframe between our sampling sessions (Scoble, 1992; Burford *et al.*, 1999; Summerville & Crist, 2003). To account for non-independence of α - and β -diversity (Stegen *et al.*, 2012), however, the significance level for analyses on the diversity of the overall moth community was set at $\alpha = 0.025$ ($0.05/2$). Because of potential non-independence among moth families arising from interspecific interactions, and non-independent partitions of α - and β -diversity, the significance level for the analyses on moth families was adjusted to $\alpha = 0.0083$ [$0.05/(3 \text{ families} \times 2 \text{ diversity partitions})$]. The same correction ($\alpha = 0.0083$) was applied for the analyses of moth diversity in different host plant use classes (overlapping, non-overlapping, partially overlapping).

Effects of defoliation history on overall moth abundance, abundance within families and abundance within host plant classes were assessed using repeated measures factorial ANOVAs (SAS, PROC MIXED) using trapping session as the repeated factor. Site variables that were found to be significantly related to moth abundance variables (based on multiple regressions) were included as covariates in the ANOVA models.

Results

Site descriptions

The identity of the dominant tree species (as measured by relative frequency) varied among sites, although the majority of canopy trees were *Quercus* spp., *Carya* spp., *Ostrya virginiana*, *Robinia pseudoacacia* and *Betula lenta*. Sub-canopy and understory trees were composed mainly of *A. pensylvanicum*, *Hamamelis virginiana* and *L. benzoin*. Lists of dominant canopy and understory trees for each site are provided in the Supporting information (Appendix S1). Mean DBH ranged from 6.4 to 17.5 cm. There were no significant differences in H'_{tree} ($F_{2,12} = 0.38$, $P = 0.6931$) or DBH ($F_{2,12} = 0.30$, $P = 0.7479$) among site histories.

Moth community

A total of 8471 moth specimens representing 284 species of the selected 14 families were collected. The most abundant species (595 individuals), *Halysidota tessellaris* J.E. Smith (Erebidae), was found at every site during Session 2. Almost half (137) of all species were represented by seven or fewer individuals. Fifty-two species were found only once in a single site and

Table 1 Species richness and abundance of moth families sampled over four trapping sessions at 15 sites ($n = 58$) from 25 May to 9 September 2011 in Shenandoah National Park, Virginia

Family	Richness	Abundance
Bombycidae	2	60
Cossidae	1	1
Drepanidae	4	57
Erebidae	74	2054
Geometridae	88	4036
Lasiocampidae	1	20
Limacodidae	7	109
Noctuidae	66	1262
Notodontidae	23	546
Saturniidae	8	174
Sesiidae	1	2
Sphingidae	7	134
Uraniidae	1	11
Yponomeutidae	1	5
Total	284	8471

session (singletons), including *Citheronia regalis* Fabricius (Saturniidae), *Callasomia promethea* Drury (Saturniidae) and *Eumorphia pandorus* Hübner (Sphingidae). We identified 22 singletons in the undisturbed region, 20 occurring in the unsprayed defoliated sites and 10 in the Btk-sprayed sites. The full list of collected species by trapping session, site and host plant classification is provided in the Supporting information (Appendix S2). Two samples were lost to bears attacking the trap: an undisturbed site (site 1) in Session 3 and a defoliated site (site 15) in Session 4.

Geometridae was the most abundant and diverse family, with 4036 individuals representing 88 species. The other common families were Erebidae (2054 individuals; 74 species), Noctuidae (1262 individuals; 66 species) and Notodontidae (546 individuals; 23 species). Eight Saturniidae species constituted 174 individuals, and both the Sphingidae and Limacodidae families had seven species with 134 and 109 individuals, respectively (Table 1). Of the selected families, only geometrids, erebids and noctuids were sampled in every trapping session; many families were not sampled in Session 4 or were sampled at such low abundance that they could not be analyzed. Most species were classified as overlapping with the gypsy moth in host plants (6050 specimens from 179 species), whereas non-overlapping and partially overlapping species were almost evenly represented, with 1189 specimens from 57 species and 1194 specimens from 42 species, respectively.

Moth abundance

Based on the repeated measures factorial ANOVA, overall moth abundance differed significantly among trapping sessions (see Supporting information, Table S1), increasing from Sessions 1 to 3, and then lower in Session 4. Undisturbed sites tended to have higher abundance than both defoliated or Btk sites in every session, whereas defoliated sites had a higher mean abundance than Btk sites in Sessions 1 and 2 and Btk sites had a higher mean abundance than defoliated sites in Sessions 3 and 4 (Fig. 2). However, there were no significant differences in overall

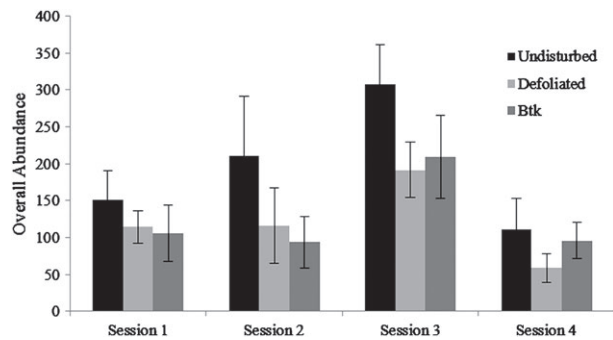


Figure 2 Mean overall abundance of moth species according to site history and trapping session. Error bars represent the SE.

moth abundance among defoliation histories (see Supporting information, Table S1).

Based on the lack of a significant family \times disturbance history interaction in the repeated measures ANOVA, we found no differences in the effects of disturbance history on moth abundance among families (see Supporting information, Table S1 and Fig. S1). Similarly, no significant differences were found for host plant class between disturbance histories (see Supporting information, Table S2 and Fig. S2).

Moth diversity

Based on permutation analysis, there were no differences in α - and β -diversity of the overall moth community among disturbance histories (see Supporting information, Table S3). However, there was weak evidence for differences in α -diversity among disturbance histories in Session 4 ($F = 3.118$, $P = 0.0883$), in which α -diversity appeared to be slightly higher in undisturbed and Btk sites than in defoliated sites (see Supporting information, Fig. S3). Moth diversity increased significantly with tree diversity but was unrelated to mean tree size (see Supporting information, Table S4).

In Session 4, α -diversity of Geometridae was significantly different among disturbance histories ($F = 8.560$, $P = 0.005$) (Table 2). Local α -diversity of this family was approximately 1.8-fold higher in undisturbed and Btk sites than in defoliated sites (Fig. 3a). No other family exhibited significant differences in mean α -diversity or β -diversity among disturbance histories (Fig. 3b,c and Table 2), nor did any moth host plant class (Fig. 4; see also Supporting information, Table S5).

Discussion

Based on light trap sampling in SNP during the summer of 2011, there were no major differences in the moth communities among regions defoliated by the gypsy moth in 2008, sprayed with Btk that same summer in addition to experiencing defoliation, and regions with no such disturbances since 1995. The present study is the first to examine the long-term effects of both gypsy moth defoliation and Btk applications on adult moth communities, and also the first to show lingering effects of defoliation but no negative effects of Btk on adult forest moth abundance and diversity.

Table 2 Results of permutation tests examining effects of disturbance history on Shannon Diversity (H'_{moth}) of moth families for each trapping session

Family	Trapping session	α -diversity	β -diversity
Geometridae	S1	$F = 0.694$, $P = 0.533$	$F = 0.685$, $P = 0.589$
	S2	$F = 0.729$, $P = 0.553$	$F = 0.479$, $P = 0.659$
	S3	$F = 0.686$, $P = 0.584$	$F = 0.496$, $P = 0.620$
	S4	$F = 8.56$, $P = 0.005$	$F = 0.552$, $P = 0.346$
Erebidae	S1	$F = 1.17$, $P = 0.351$	$F = 2.09$, $P = 0.050$
	S2	$F = 0.425$, $P = 0.661$	$F = 1.65$, $P = 0.440$
	S3	$F = 1.41$, $P = 0.319$	$F = 0.540$, $P = 0.123$
	S4	$F = 2.51$, $P = 0.126$	$F = 0.223$, $P = 0.923$
Noctuidae	S1	$F = 0.047$, $P = 0.953$	$F = 0.245$, $P = 0.863$
	S2	$F = 0.702$, $P = 0.528$	$F = 1.56$, $P = 0.284$
	S3	$F = 1.71$, $P = 0.219$	$F = 0.329$, $P = 0.635$
	S4	$F = 0.288$, $P = 0.741$	$F = 0.939$, $P = 0.240$

Significant results at the $P = 0.0083$ [0.05/(3 families \times 2 diversity partitions)] level are shown in bold.

Studies examining the effects of gypsy moth presence on larval and adult forest moths have taken place during and directly after outbreak events with inconsistent defoliation among sampled sites (Sample *et al.*, 1996; Work & McCullough, 2000; Timms & Smith, 2011). They did not find significant effects of gypsy moth presence on overall native moth communities, although there was some evidence of reduced abundance and diversity of specialist feeders of *Quercus* spp. (Work & McCullough, 2000). Miller (1990) found larval abundance to have recovered 3 years after Btk application, although diversity remained significantly lower in sprayed sites compared with unsprayed sites. Boulton *et al.* (2007) sampled larval Lepidoptera up to 4 years after Btk application and found the system's diversity mostly recovered, although rare species were the slowest to recover. In a 7-year study of non-target Btk effects on native arthropods in Virginian and West Virginian forests, Lepidoptera whose phenology was determined to be most sensitive to the Btk application experienced significantly decreased abundance during treatment years but returned to pre-treatment and comparable control levels 2 years after treatment (Strazanac & Butler, 2005). If defoliation and Btk application did negatively affect the moth community in SNP during or in the years immediately after the disturbances, the community has largely recovered. One notable exception, as explored below, is the abundant and speciose family Geometridae.

The lack of differences in the size and diversity of trees among disturbance histories may help explain why defoliation had only limited long-term effects on the moth community. Consistent with previous work showing that the species composition of the dominant canopy trees is an important driver of moth community composition (Usher & Keiller, 1998; Summerville *et al.*, 2003; Summerville & Crist, 2008), we found positive correlations between tree diversity and the abundance (see Supporting information, Table S1) and diversity (see Supporting information, Tables S2 and S3) of moths. Although tree diversity and size in 2011 appeared to be largely unaffected by the 2007–2009 outbreak, this does not rule out the possibility of short-term effects of defoliation on the native moth community

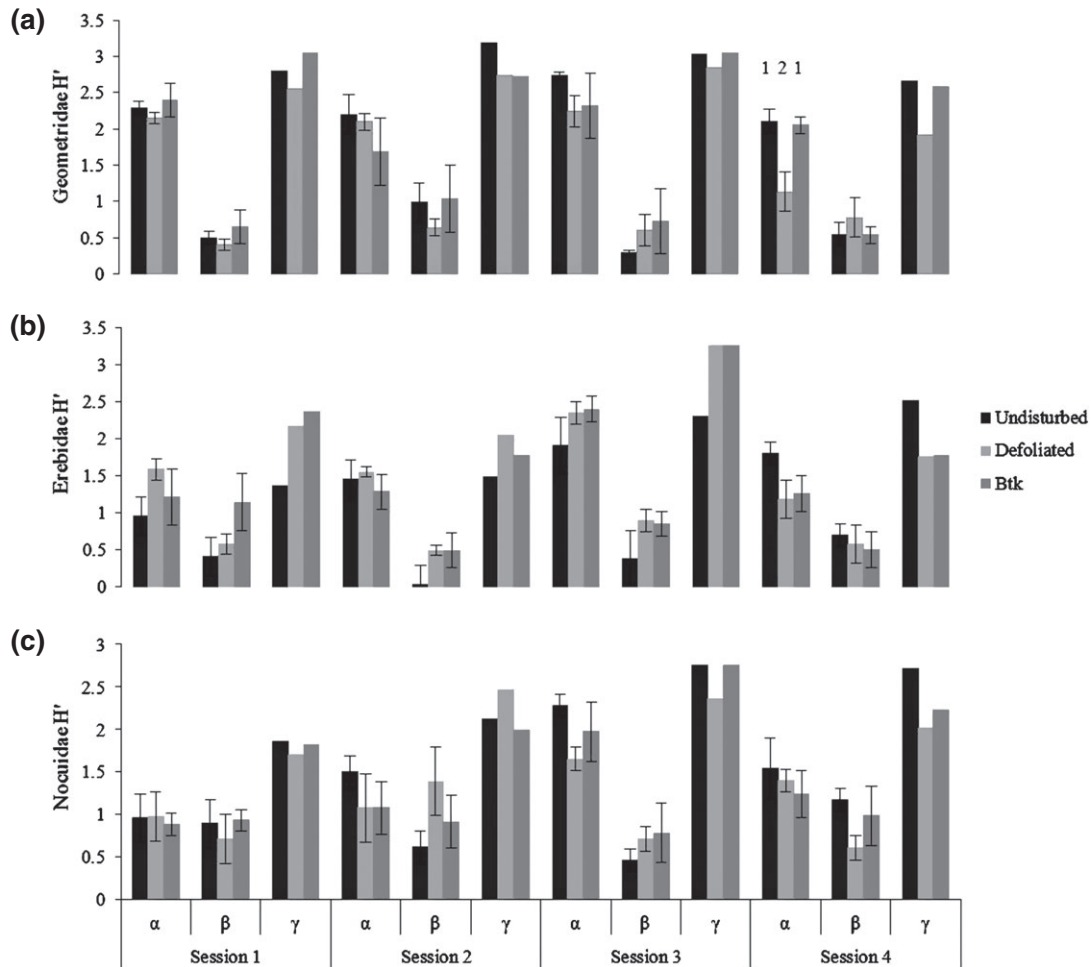


Figure 3 Mean Shannon Diversity (H') of (a) Geometridae, (b) Erebidae and (c) Noctuidae for each disturbance history and each trapping session. Diversity was partitioned as local diversity (α), diversity among locations (β) and regional diversity (γ). Error bars represent the SE. Significant differences in Geometridae are identified by numbers specifying significantly different groups.

as a result of increased competition for foliage or other indirect effects.

Although defoliation and Btk application did not affect the overall diversity of moths, α -diversity of Geometridae adults in late summer was negatively impacted by defoliation but was approximately equal in unaffected sites and sites sprayed by Btk. The reduced diversity of adult geometrids in late summer, subsequent to the defoliation by gypsy moths in early summer, could have stemmed from the lower survival of geometrid larvae as a result of a reduced availability of forage. The fact that geometrid α -diversity was very similar between Btk sites and undisturbed sites, and that geometrids are known to be less sensitive to Btk than other families (Peacock *et al.*, 1998), suggests that Btk had an overall protective effect for geometrids. Application of Btk may have reduced gypsy moth numbers to a sufficient extent to prevent competitive exclusion of geometrid species, at the same time as not causing high levels of Btk-induced mortality in geometrids. This result is consistent with the findings by Rastall *et al.* (2003), who found no significant differences in geometrid abundance in the year after Btk application, although this is the first study to provide

evidence that Btk application protects against loss of geometrid diversity as a result of gypsy moth defoliation.

Notwithstanding the long-lasting effects of defoliation on Geometridae, the apparent recovery of moth communities in defoliated and Btk-sprayed areas could be a result of recolonization. Because the areas affected by the 2007–2009 gypsy moth outbreak were surrounded by undisturbed forest refugia, recolonization may have proceeded rapidly. The lower diversity of geometrids in defoliated than undisturbed sites may be the result of slow recolonization by this family as a result of its poor dispersal ability. Geometrids are known to be weak fliers compared with other macrolepidoptera families and their recolonization of disturbed areas has been found to be slower than stronger fliers such as noctuids and sphingids (Usher & Keiller, 1998). The recovery of moth communities after defoliation or Btk application may also have been aided by species that persisted locally through these disturbances and subsequently exhibited rapid population growth.

Although, in the present study, moth families other than Geometridae appear to have recovered after a gypsy moth defoliation event, we cannot rule out any additional long-term

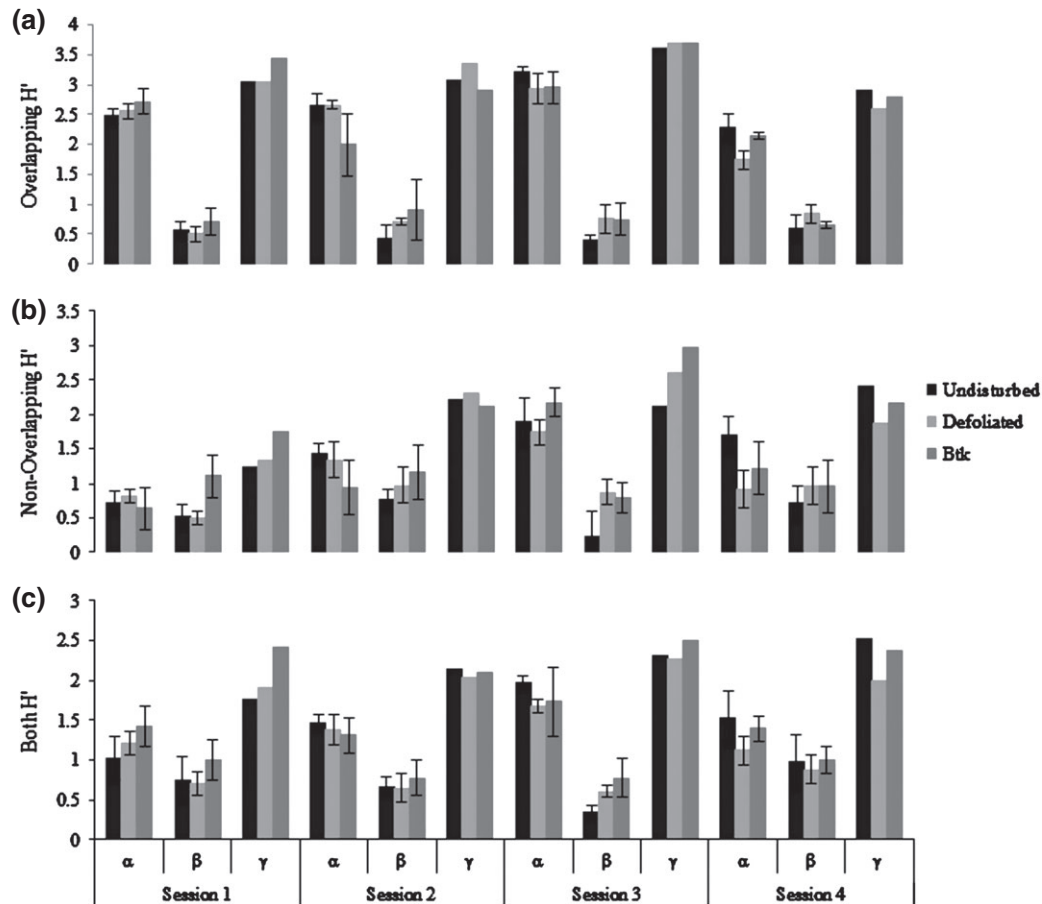


Figure 4 Mean Shannon Diversity (H') of (a) overlapping, (b) non-overlapping and (c) both at each site history for each trapping session. Diversity was partitioned as local diversity (α), diversity among locations (β) and regional diversity (γ). Error bars represent the SE.

effects of this invasive insect on native moth communities. Some evidence, particularly at the leading edge of the gypsy moth's invasion, suggests that elevated gypsy moth abundance, even at densities insufficient to cause defoliation, is a driver of native moth community change as a result of effects on host foliage quality and changes in the composition of natural enemies (Sample *et al.*, 1996; Work & McCullough, 2000; Timms & Smith, 2011). Thus, the moth community in SNP may have been affected by the presence of gypsy moths even before any defoliation events first occurred. Moreover, the defoliation of the entirety of the park in 1995 could have had major effects on the native moth community, and regions of the Northern Corridor of the park (the region examined in the present study) have experienced over 10 seasons of defoliation between 1984 and 2009 (Edgerton, 2010). An important avenue of future research would be to monitor native moth communities both before and after the initial invasion of the gypsy moth.

Previous research into the effects of gypsy moth outbreaks and defoliation effects on other forest Lepidoptera suggests that impacts may be restricted to species feeding on *Quercus* spp. and the aspen species of *Populus*, which are the preferred hosts of the gypsy moth in North America (Redman & Scriber, 2000; Work & McCullough, 2000; Timms & Smith, 2011). Because of the severity of the defoliation in SNP from 2007 to 2009, with

aerial surveys suggesting complete defoliation of all palatable plants, we classified moths in terms of their overlap with any of the host plants of the gypsy moth, rather than focusing whether they fed upon *Quercus*. This may have hidden some effects of the gypsy moth on *Quercus* feeders, although it allowed a test of competitive effects of the gypsy moth on a broader segment of the native moth community. In addition, the unimportance of degree of overlap in host use with the gypsy moth could reflect the fact that gypsy moths can interact with other moth species not only by affecting the availability and quality of forage, but also through shared natural enemies (Redman & Scriber, 2000; Timms & Smith, 2011).

We found no evidence of long-term negative impacts of Btk application on overall forest Lepidoptera abundance and diversity. Indeed, it appears that Btk application protected Geometridae from a loss of diversity caused by gypsy moth defoliation. Our exclusive sampling of moths, however, does not allow us to address the effects on butterfly families, which are generally more sensitive to Btk (Peacock *et al.*, 1998; Scriber, 2004). Long-term monitoring of butterfly families, along with surveys of Lepidoptera both before and after Btk application, is necessary to fully evaluate the effects of Btk on lepidopteran non-target species. Our findings for the geometrid family offer support for the assertion made by Scriber (2004) suggesting that the negative

effects of Btk on non-target Lepidoptera may be outweighed by its benefits.

Acknowledgements

We thank Jonathan Walter, Sang Mee Ko, Hilary Wayland, Clare Rodenburg, Josh Whelan, Andy Allstadt, Steven Waner, Tim Park and Laurel Cepero for their assistance in the field and laboratory, as well as David Carr, T'ai Roulston, Todd Scanlon, Rolf Gubler, Larry Nichols and Todd Edgerton for their assistance with revisions to earlier drafts of this work. The authors extend additional gratitude to the three anonymous reviewers. Funding was provided by the University of Virginia's Blandy Experimental Farm.

Supporting information

Additional Supporting information may be found in the online version of this article under the DOI reference: 10.1111/afe.12066

Appendix S1: Details for 15 sites in Shenandoah National Park. Dominant species defined as species cumulating > 50% stems surveyed July to October 2011.

Appendix S2: Collected moth species in Shenandoah NP in Summer 2011 by trapping session, site and host plant classification.

Figs S1 to S3 and Tables S1 to S5: Additional figures and tables.

References

- Anderson, M.J., Crist, T.O., Chase, J.M. *et al.* (2011) Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. *Ecology Letters*, **14**, 19–28.
- Boulton, T.J., Otvos, I.S., Halwas, K.L. & Rohlf, D.A. (2007) Recovery of nontarget Lepidoptera on Vancouver Island, Canada: one and four years after a gypsy moth eradication program. *Environmental Toxicology and Chemistry*, **26**, 738–748.
- Burford, L.A., Lacki, M.J. & Covell, C.V. (1999) Occurrence of moth among habitats in a mixed mesophytic forest: implications for management of forest bats. *Forest Science*, **45**, 323–332.
- Butler, L., Kondo, V. & Strazanac, J. (2001) Light trap catches of Lepidoptera in two central Appalachian forests. *Proceedings of the Entomological Society of Washington*, **103**, 879–902.
- Calvo, D. & Molina, J.M. (2010) Differences in foliage affect performance of the lappet moth, *Streblote panda*: implications for species fitness. *Journal of Insect Science*, **10**, 1–14.
- Covell, C.V. (1984) *A Field Guide to the Moths of Eastern North America*. Houghton Mifflin, Boston, Massachusetts.
- Diekötter, T. & Crist, T.O. (2013) Quantifying habitat-specific contributions to insect diversity in agricultural mosaic landscapes. *Insect Conservation and Diversity*, **6**, 607–618.
- Edgerton, T. (2010) Gypsy moth cumulative defoliation (1984–2009) (Shenandoah National Park) [map]. Scale not given. Virginia Department of Forestry *Forest Health Review*, May 2010.
- Gandhi, K.J.K. & Herms, D.A. (2010) Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of Eastern North America. *Biological Invasions*, **12**, 389–405.
- Hajek, A.E. & Tobin, P.C. (2010) Micro-managing arthropod invasions: eradication and control of invasive arthropods with microbes. *Biological Invasions*, **12**, 2895–2912.
- Hammond, P.C. & Miller, J.C. (1998) Comparison of the biodiversity of Lepidoptera within three forested ecosystems. *Annals of the Entomological Society of America*, **91**, 323–328.
- Hodges, R.W., Dominick, T., Davis, D., Ferguson, D., Franclemont, J., Munroe, E. & Powell, J. (1972–2008) *The Moths of American North of Mexico*. Wedge Entomological Foundation, Eugene, Oregon.
- Howarth, F.G. (2001) Environmental issues concerning the importation of non-indigenous biological control agents. *Balancing Nature: Assessing the Impact of Importing Non-Native Biological Control Agents (An International Perspective)* (ed. by J. A. Lockwood, F. G. Howarth and M. F. Purcell), pp. 70–99. Entomological Society of America, Lanham, Maryland.
- Johnson, G.G. & Ware, S. (1982) Post-chestnut forest in the central Blue Ridge of Virginia. *Castanea*, **47**, 329–343.
- Karban, R. (1978) Changes in an oak-chestnut forest since the chestnut blight. *Castanea*, **43**, 221–228.
- Kenis, M., Auger-Rozenburg, M.-A., Roques, A. *et al.* (2009) Ecological effects of invasive alien insects. *Biological Invasions*, **11**, 21–45.
- Kosola, K.R., Dickmann, D.I., Paul, E.A. & Parry, D. (2001) Repeated insect defoliation effects on growth, nitrogen acquisition, carbohydrates, and root demography of poplars. *Oecologia*, **129**, 65–74.
- Lafontaine, J.D. & Schmidt, B.C. (2010) Annotated check list of the Noctuoidea (Insecta, Lepidoptera) of North America north of Mexico. *ZooKeys*, **40**, 1–239.
- Lande, R. (1996) Statistics and partitioning of species diversity, and similarity among multiple communities. *Oikos*, **76**, 5–13.
- Lovett, G.M., Canham, C.D., Arthur, M.A., Weathers, K.C. & Fitzhugh, R.D. (2006) Forest ecosystem responses to exotic pests and pathogens in Eastern North America. *Bioscience*, **56**, 395–405.
- McNab, W.H. (1994) *Ecological Subregions of the United States*. USDA Forest Service, Washington, District of Columbia.
- Miller, J.C. (1990) Field assessment of the effects of a microbial pest control agent on nontarget lepidoptera. *American Entomologist*, **36**, 135–140.
- National Park Service. (2008) Shenandoah National Park to employ aerial spraying against gypsy moth. [press release], 1 May 2008. [WWW document]. URL <http://www.nps.gov/shen/parknews/shenandoah-national-park-to-employ-aerial-spraying-against-gypsy-moth.htm> [accessed on 7 November 2010].
- Peacock, J.W., Schweitzer, D.F., Carter, J.L. & Dubois, N.R. (1998) Laboratory assessment of the effects of *Bacillus thuringiensis* on native Lepidoptera. *Environmental Entomology*, **27**, 450–457.
- Rastall, K., Kondo, V., Strazanac, J.S. & Butler, L. (2003) Lethal effects of biological insecticide applications on nontarget Lepidoptera in two Appalachian Forests. *Environmental Entomology*, **32**, 1364–1369.
- Redman, A.M. & Scriber, J.M. (2000) Competition between the gypsy moth, *Lymantria dispar*, and the northern tiger swallowtail, *Papilio canadensis*: interactions mediated by host plant chemistry, pathogens, and parasitoids. *Oecologia*, **125**, 218–228.
- Ricketts, T.H., Dinerstein, E., Olson, D.M. *et al.* (1999) *Terrestrial Ecoregions of North America: A Conservation Assessment*. Island Press, Washington, District of Columbia.
- Robinson, G.S., Ackery, P.R., Kitching, I.J., Beccaloni, G.W. & Hernández, L.M. (2010) *HOSTS – A Database of the World's Lepidopteran Hostplants*. Natural History Museum, U.K. [WWW document]. URL <http://www.nhm.ac.uk/hosts> [accessed on 26 March 2013].
- Sample, B.E., Butler, L., Zivkovich, C., Whitmore, R.C. & Reardon, R. (1996) Effects of *Bacillus thuringiensis* Berliner var. *kurstaki* and defoliation by the gypsy moth [*Lymantria dispar* (L.) (Lepidoptera: Lymantriidae)] on native arthropods in West Virginia. *The Canadian Entomologist*, **128**, 573–592.

- Scoble, M.J. (1992) *The Lepidoptera: Form, Function, and Diversity*. Oxford University Press, New York, New York.
- Scriber, J.M. (2004) Non-target impacts of forest defoliator management options: decision for no spraying may have worse impacts on non-target Lepidoptera than *Bacillus thuringiensis* insecticides. *Journal of Insect Conservation*, **8**, 241–261.
- Southwood, T.R.E. & Henderson, P.A. (2000) *Ecological Methods*, 3rd edn. Blackwell, U.K.
- Stange, E.E., Ayres, M.P. & Bess, J.A. (2011) Concordant population dynamics of Lepidoptera herbivores in a forest ecosystem. *Ecography*, **34**, 772–779.
- Stegen, J.C., Freestone, A.L., Crist, T.O. *et al.* (2012) Stochastic and deterministic drivers of spatial and temporal turnover in breeding bird communities. *Global Ecology and Biogeography*, **22**, 202–212.
- Stephenson, S.L., Ash, A.N. & Stauffer, D.F. (1993) Appalachian oak forests. *Biodiversity of Southeastern United States: Upland Terrestrial Communities* (ed. by W. H. Martin, S. G. Boyce and A. C. Echternacht), pp. 225–303. Wiley, New York, New York.
- Strazanac, J. S. & Butler, L. (eds) (2005) Long-term Evaluation of the Effects of *Bacillus thuringiensis kurstaki*, Gypsy Moth Nucleopolyhedrosis Virus Project Gypcheck, and *Entomophaga maimaiga* on Non-target Organisms in Broadleaf-Pine Forests in the Central Appalachians, FHTET-2004-14. Forest Health Technology Enterprise Team, Morgantown, West Virginia.
- Summerville, K.S. & Crist, T.O. (2003) Determinants of lepidopteran community composition and species diversity in eastern deciduous forests: roles of season, eco-region and patch size. *Oikos*, **100**, 134–148.
- Summerville, K.S. & Crist, T.O. (2008) Structure and conservation of lepidopteran communities in managed forests of northeastern North America: a review. *The Canadian Entomologist*, **140**, 475–494.
- Summerville, K.S., Boulware, M.J., Veech, J.A. & Crist, T.O. (2003) Spatial variation in species diversity and composition of forest Lepidoptera in eastern deciduous forests of North America. *Conservation Biology*, **17**, 1045–1057.
- Summerville, K.S., Dupont, M.M., Johnson, A.V. & Krehbiel, R.L. (2008) Spatial structure of forest lepidopteran communities in oak hickory forests of Indiana. *Environmental Entomology*, **37**, 1224–1230.
- Timms, L.L. & Smith, S.M. (2011) Effects of gypsy moth establishment and dominance in native caterpillar communities of northern oak forests. *The Canadian Entomologist*, **143**, 479–503.
- Tobin, P.C. & Liebhold, A.M. (2011) Gypsy moth. *Encyclopedia of Biological Invasions* (ed. by D. Simberloff and M. Rejmanek), pp. 298–304. University of California Press, Berkeley, California.
- Usher, M.B. & Keiller, S.W.J. (1998) The macrolepidoptera of farm woodlands: determinants of diversity and community structure. *Biodiversity and Conservation*, **7**, 725–748.
- Wagner, D.L. (2005) *Caterpillars of Eastern North America*. Princeton University Press, Princeton, New Jersey.
- Wagner, D.L. & Van Driesche, R.G. (2010) Threats posed to rare or endangered insects by invasions of nonnative species. *Annual Review of Entomology*, **55**, 547–568.
- White, W.B., McLane, W.H. & Schneeberger, N.F. (1981) Pesticides. *The Gypsy Moth: Research Toward Integrated Pest Management* (ed. by C. C. Doane and M. L. McManus), Technical Bulletin 1584, pp. 423–442. USDA Forest Service, Washington, District of Columbia.
- Whittaker, R.H. (1960) Vegetation of the Siskiyou Mountains, Oregon and California. *Ecological Monographs*, **30**, 279–338.
- Wold, E.N. & Marquis, R.J. (1997) Induced defense in white oaks: effects on herbivores and consequences for the plant. *Ecology*, **78**, 1356–1369.
- Work, T.T. & McCullough, D.G. (2000) Lepidopteran communities in two forest ecosystems during the first gypsy moth outbreaks in northern Michigan. *Environmental Entomology*, **29**, 884–900.
- Yela, J.L. & Holyoak, M. (1997) Effects of moonlight and meteorological factors on light and bait trap catches of noctuid moths (Lepidoptera: Noctuidae). *Population Ecology*, **26**, 1283–1290.
- Young, M. (1997) *The Natural History of Moths*. T & AD Poyer Ltd, U.K.
- Young, M. (2005) Insects in flight. *Insect Sampling in Forest Ecosystems* (ed. by S. Leather), pp. 116–145. Blackwell Science Ltd, Maldon, Massachusetts.

Accepted 19 March 2014