# Fate of Conifer Terpenes in a Polyphagous Folivore: Evidence for Metabolism by Gypsy Moth (Lepidoptera: Lymantriidae)[1]

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**Abstract** Conifers comprise a substantial portion of the host range of the gypsy moth, *Lymantria dispar* L. In this study, the effects and fates of the predominant conifer phytochemicals, monoterpenes and diterpene acids, were determined. Gypsy moth larvae were fed physiological levels of three monoterpenes (bornyl acetate, limonene, and myrcene) and two diterpene acids (isopimaric acid and neoabietic acid) for the duration of their second, third, and fourth stadia. Frass was collected daily, and larvae and exuviae were collected after the completion of the stadium. These samples were chemically analyzed for parent terpenes or their metabolites. Overall, gypsy moth larvae were highly tolerant of all terpenes administered, despite some treatment effects. Limonene, myrcene, isopimaric acid, and neoabietic acid were not recovered suggesting that, once ingested, these terpenes were converted into other products. Bornyl acetate was metabolized in part to borneol and also was recovered in small quantities from frass. Its metabolite borneol was mostly excreted, with a small amount remaining in body tissues. Additional experiments were performed, determining that monoterpenes were effectively ingested, and that monoterpenes were not lost substantially through volatilization from diet or larvae. There was no evidence that terpene metabolism was due to P450 enzymes or esterases, although these results are inconclusive. Gut bacteria do not appear to be primarily responsible for terpene metabolism.

**Key words** Gypsy moth, *Lymantria dispar,* terpenes, metabolism, detoxification, sequestration, excretion, conifers

The gypsy moth, *Lymantria dispar* L., is a folivore native to Eurasia. It was introduced to North America in Massachusetts in 1869, and its geographic range has since expanded southward and westward (Liebhold et al. 1997). The gypsy moth is currently distributed throughout the eastern U.S. from Maine to North Carolina, and west to Wisconsin (Liebhold et al. 1997, USDA APHIS 2001). In addition, there are occasional small populations in the western U.S. due to accidental introductions (Schaefer 1989). The gypsy moth is a highly polyphagous insect, with an estimated host range of 300 to 500 species in North America (Montgomery and Wallner 1988).

Most research on host suitability and relationships with phytochemicals has been performed on broadleaf angiosperms native to the northeastern U.S. because these trees are most affected in the gypsy moth's current range. However, as the gypsy

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moth expands into more western and southern regions, it will encounter forest habitats containing higher proportions of conifers (Miller et al. 1991). Several conifer species can be utilized to varying degrees by gypsy moth. Hosts that can be utilized successfully from eclosion to pupation include *Larix laricina* (Du Roi) K. Koch (Lechowicz and Mauffette 1986, Miller and Hansen 1989), *L. decidua* Mill. (Grijpma 1988, Miller and Hansen 1989), *Cedrus deodora* Loud. (Miller and Hansen 1989), *Picea pungens* Engelm. (Miller and Hansen 1989), *Taxodium distichum* (L.) Richard (Fehrer et al. 1992), and *Pseudotsuga menziesii* (Mirb.) Franco (Moldenke et al. 1992, 1997). Of these, only *L. laricina, L. decidua,* and *C. deodora* are considered preferred hosts. Performance on *Larix* in the laboratory is often equivalent to some of the angiosperm species that are most heavily defoliated in the field, such as *Quercus* and *Populus* (Miller and Hanson 1989, Kruse and Raffa 1997).

Monoterpenes and diterpenes are the most important classes of compounds conferring defense by conifers against insect herbivory (Trapp and Croteau 2001). Although terpenes are present in some angiosperm trees (Staudt et al. 2001), they are generally more characteristic of conifers, in which their concentrations are higher. Terpenes can negatively impact insect herbivores through toxic and deterrent effects (Gershenzon and Croteau 1991, Langenheim 1994). Toxicity may result from several mechanisms, including inhibition of ATP formation, interference with hormone production, and binding of proteins or sterols in the gut (Langenheim 1994). Mechanisms of deterrence are less clear but may involve interactions with sensory receptors (Gershenzon and Croteau 1991). Terpenes are toxic to a broad range of insects, including Lepidoptera (Cates 1996, Lee et al. 1997, Hummelbrunner and Isman 2001), Coleoptera (Regnault-Roger and Hamraoui 1997, Regnault-Roger 1999, Trapp and Croteau 2001), Diptera (Regnault-Roger 1999), Isoptera (Cornelius et al. 1997), Homoptera (Regnault-Roger 1999), and Hymenoptera (Wagner et al. 1983, Larsson et al. 1986), as well as other arthropods (Lee et al. 1997). Terpenes are involved in additional aspects of plant-insect interactions, such as pollination and attraction of entomophagous insects (Langenheim 1994). They also inhibit fungal infection and are allelopathic against soil bacteria and seed germination by plants (Langenheim 1994).

Herbivores are known to process phytochemicals by metabolism, excretion, passive accumulation in body tissues, or sequestration for defense against natural enemies. When phytochemicals are metabolized, the insect may subsequently use one or more of these mechanisms to contend with the resulting metabolites. It is unknown how gypsy moth larvae contend with conifer terpenes. In this study, we evaluated (1) response of gypsy moth to various monoterpenes and diterpene acids, (2) mechanisms gypsy moth larvae use to process varying concentrations of monoterpenes and diterpene acids, and (3) whether specific mechanisms of metabolism were due to enzymes or gut microflora.

## Materials and Methods

Our overall approach to characterizing the effects and fate of terpenes in gypsy moth is depicted in Figure 1. Defined amounts of terpenes were administered in artificial diet to larvae, and their consumption and growth were quantified. The fates of these compounds were determined by chemical analyses. Terpenes were considered excreted if they were recovered from frass (Fig. 1a), accumulated if they were recovered from larvae, or sequestered if they were accumulated in higher concen-

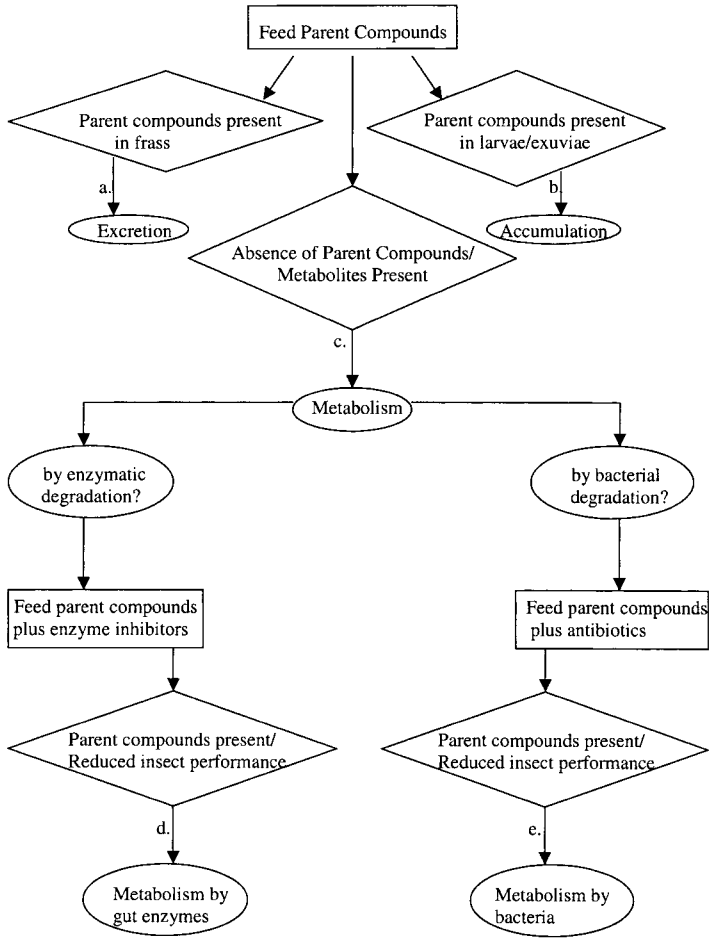


Fig. 1. Overall approach used to evaluate fate of terpenes in gypsy moth. Terpenes were considered excreted if recovered from frass (a), sequestered or accumulated if recovered from larvae (b), and metabolized if not recovered from either, or if metabolites of parent terpenes were present (c). If metabolism appeared to have occurred, further experiments were performed to evaluate the mechanisms involved. These experiments included application of enzyme inhibitors or antibiotics in combination with terpenes. If parent terpenes were present or if insect performance decreased when larvae were fed enzyme inhibitors in combination with terpenes but not terpenes alone, affected enzymes were considered involved in metabolism (d). If parent terpenes were present or if insect performance decreased when larvae were fed terpenes in combination with antibiotics but not terpenes alone, gut microflora were considered involved in metabolism (e).

trations than in the diet (Fig. 1b). Terpenes were considered metabolized if they were not recovered from frass or larvae, or if metabolites of the parent terpenes were present (Fig. 1c). If metabolism occurred, further experiments were performed to evaluate the mechanism. Subsequent experiments included application of enzyme inhibitors of P450 enzymes and esterases (Fig. 1d) and antibiotics to reduce or eliminate gut microflora (Fig. 1e).

**Insects.** Gypsy moth egg masses were obtained from a laboratory strain maintained by the USDA APHIS, Otis AFB, Cape Cod, MA, and placed in cold storage (10°C) until used. Egg masses were surface sterilized in a solution of 2% bleach (Clorox®, 5% hyperchlorite, The Clorox Company, Oakland, CA) in deionized water with Tween® (1%, Sigma, St. Louis, MO) for 3 min, then rinsed 3 times in deionized water. Egg masses were allowed to air dry for 30 min and were placed individually in Petri dishes (14.0 × 3.9 cm, TriState Plastics, Dixon, KY). Upon eclosion, larvae were fed an agar- and wheat germ-based artificial diet formulated for gypsy moths (ICN Pharmaceuticals, Inc., Costa Mesa, CA). Larvae were reared in growth chambers under a photoperiod of 18:6 (L:D) h at 25°C, and were offered fresh diet every other day until used in experiments.

**Monoterpene bioassays.** Larvae showing head capsule "slippage" (i.e., those just before ecdysis) were transferred to Petri dishes (14 × 3.9 cm) without diet. After 24 h, newly-molted larvae were weighed and used in the bioassays. Individual larvae were placed in 30-ml plastic cups (Polar Plastics Inc., Winston-Salem, NC) and fed amended diet. Thin (~1 mm) sections of artificial diet were weighed. Test chemicals or solvent controls were added at rate of 0.75 µl per mg wet weight of diet.

Test monoterpenes were bornyl acetate, limonene, and myrcene (Aldrich Chemical Company, Milwaukee, WI). These monoterpenes were evaluated because they occur in *L. laricina,* a preferred conifer host of the gypsy moth (Lechowicz and Mauffette 1986, Miller and Hansen 1989), and likely an important food source as it becomes more established in the upper Midwest. All monoterpenes were diluted in a 0.75% solution of Triton X 405 (Aldrich Chemical Company, Milwaukee, WI) in distilled water ($dH_2O$) before being added to artificial diet (Powell and Raffa 1999a). Two sets of assays were conducted: (1) 0.01%, 0.1%, or 1% bornyl acetate, limonene, or myrcene, and (2) 0.01%, 0.1%, 1%, or 5% bornyl acetate. There were two controls for each assay, $dH_2O$ and 0.75% Triton solution. The first three concentrations were applied to provide a gradient of concentrations found in *L. laricina* (Powell and Raffa 1999a). The 5% concentration was added to determine the limits at which gypsy moths could contend with this compound. Both bioassays were performed on second, third, and fourth instars, and sample sizes were 9 or 10. A total of 180 larvae were assayed.

Larvae were fed newly-amended diet every 24 h until they reached the next stadium. Frass was collected and frozen daily to insure that only minimal amounts of terpenes were lost through volatilization. Upon completion of the stadium, larvae and exuviae were collected. Larvae were weighed and frozen until chemical analysis. Exuviae were pooled into groups of 2 or 3, depending on the stadium, and frozen until analysis. All uneaten diet was collected, dried, and weighed. Ten subsamples of artificial diet were weighed, dried, and then reweighed to determine the wet weight/dry weight ratio. Total consumption was calculated by multiplying the dry weight of the unconsumed diet by the wet weight/dry weight ratio, and subtracting the product from the sum of the daily wet weights. Duration of each stadium (d), relative consumption rates (g/g/d; RCR) as calculated by: (weight of diet consumed over the

stadium÷weight of insect at the beginning of the stadium) ÷ duration of the stadium, and relative growth rates (g/g/d; RGR) as calculated by: (weight gain over stadium ÷ weight of larva at the beginning of the stadium) ÷ duration of the stadium were determined (Waldbauer 1968).

**Volatilization.** Two experiments were performed to evaluate whether monoterpenes would be lost through volatilization. Details are in Powell (2002). The first experiment was performed to determine if monoterpenes volatilized from artificial diet. We amended artificial diet and analyzed for monoterpene concentrations at 0 h and 24 h. Thirty sections of artificial diet, ranging from 251 mg to 354 mg, were amended with 0.75 µl of either 5% bornyl acetate, limonene, or myrcene per mg wet weight. Half of the amended diet sections were collected and frozen immediately after addition of terpenes, and the other half were collected after 24 h in growth chambers under the experimental conditions described above. After 24 h, all sections were extracted and analyzed for monoterpene content. The second experiment was performed to determine if monoterpenes volatilized from larvae. Newly-molted third instars were offered artificial diet amended with 1% bornyl acetate, limonene, or myrcene. After 24 h, individual larvae were placed in 15-mL glass vials with hole caps fitted with a thermoseal septum (Supelco, Bellefonte, PA), and the atmosphere was allowed to equilibrate for 1 h. We conducted head-space analysis by solid phase microextraction (SPME) (Theodoridis et al. 2000) followed by gas chromatography. The needle of the SPME unit (Supelco, Bellefonte, PA) was inserted through the septum, and the SPME fiber (100 ul polydimethylsiloxane) was exposed for 5 min. The fiber was desorbed in the injection port (220°C) for 30 s.

**Monoterpene degradation.** The time course over which bornyl acetate, limonene, and myrcene could be recovered from various larval tissues was examined to estimate the rates and sites of metabolism. In addition, presence of monoterpenes in guts of larvae established that monoterpenes were effectively ingested (Powell 2002). Concentrations of the above monoterpenes, and borneol, an apparent metabolite of bornyl acetate, were monitored in gut and peripheral larval tissues at four intervals following ingestion of known amounts of parent compounds. Third instars were fed artificial diet discs (~ 2mm diam, 1 mm thick) amended with 1 ul of 1% bornyl acetate, 1% limonene, or 1% myrcene. Once the amended diet was consumed, larvae were fed unamended diet and allowed to feed until being collected for chemical analysis. Larvae were observed over the course of the experiment, and time 0 was set for each larva as the time at which its diet was completely consumed. This ranged from 1.5 h to 6 h after amended diet was offered. If the amended diet disc was not consumed within 6 h, the larva was removed from the experiment. Larvae were destructively sampled at five intervals (0, 1, 2, 3, and 4 h) after consuming the amended diet disc. Larvae were dissected, and gut and peripheral tissues were analyzed separately for monoterpenes and their metabolites. There were 3 to 5 larvae per treatment per interval.

**Enzyme inhibitors.** Enzyme inhibition assays were performed to determine whether P450 enzymes or esterases are involved in terpene metabolism by gypsy moth. Piperonyl butoxide (PBO), an inhibitor of P450 enzymes (Brattsten and Metcalf 1970), and S,S,S-tri-n-butyl phosphorotrithioate (DEF), an inhibitor of esterases (Usmani and Knowles 2001), were evaluated. Preliminary assays demonstrated that our experimental condition yielded synergism when metabolic inhibitors were combined with insecticides having known detoxification pathways (Powell 2002).

The monoterpenes bornyl acetate, limonene, and myrcene were each applied at

1% alone or in combination with 0.1% PBO. A 0.75% Triton solution, $dH_2O$ and a 0.1% PBO solution in 0.75% Triton were applied as controls. This bioassay was performed on second, third, and fourth instars, and sample sizes ranged from 8 to 10. A total of 51 larvae were assayed. Frass, exuviae, and larvae were collected, and RGR, RCR, and development time were calculated as described previously.

The monoterpenes bornyl acetate, limonene, and myrcene were each applied at 1% alone or in combination with a 0.01% DEF solution in Triton. A 0.75% Triton solution, $dH_2O$, and a 0.01% DEF solution in 0.75% Triton were applied as controls. Three groups of larvae were tested. One group (N = 10) was treated for the duration of the stadium. Frass, larvae, and exuviae were collected, and RGR, RCR, and development time were calculated. Two groups were treated for 2 d. One group (N = 4 to 5) was starved for 24 h to insure that the gut was empty at the time of chemical analysis. Frass was collected daily, and larvae were collected and frozen on d 3. The last group of larvae (N = 4 to 5) was collected on d 2. A total of 38 larvae were assayed. Larvae were dissected, and the gut and the body tissues were analyzed separately. Frass was collected daily.

**Gut bacteria.** To determine if gut bacteria contribute to metabolism of monoterpenes, we fed antibiotics to treated and control larvae. The antibiotic mixture contained equal parts of penicillin, streptomycin, chloramphenicol, and chlortetracycline (Andrews and Spence 1980) dissolved in 0.75% Triton. Concentrations were 12 mg antibiotics/ml 0.75% Triton. Another experiment using 6 mg antibiotics/ml yielded similar results and so are not reported here (Powell 2002).

Third instars were fed artificial diet amended with the following treatments: 1% bornyl acetate, 1% bornyl acetate + 12 mg antibiotic/ml, 2% bornyl acetate, 2% bornyl acetate + 12 mg antibiotic/ml, 12 mg antibiotic/ml, $dH_2O$, and 0.75% Triton. A total of 39 larvae was assayed. Frass was collected daily and frozen, and larvae and exuviae were collected and frozen upon molting to the fourth instar. RGR, RCR, and development time were calculated. After 2 d of feeding, a subsample of 3 larvae per treatment was harvested and dissected, and their gut contents were analyzed for the presence of bacteria. The crop and midgut of each larva were placed in 1 mL of phosphate buffer saline (PBS) in a 1.5-mL microcentrifuge tube. Individual guts in PBS were sonicated for 45 s, diluted, and drop-plated on 1/10-strength tryptic soy agar and incubated at 28°C. Colonies were counted after 24, 48, and 72 h (Broderick et al. 2000).

**Diterpene acid bioassays.** Bioassays were performed on third instars using diet amended with the diterpenes, isopimaric acid and neoabietic acid. These were used because they are found in biologically significant quantities in *L. laricina* and have strong biological activity against conifer sawflies (Wagner et al. 1983). General conditions were as described for the monoterpene bioassays. The diterpene acids (Helix Biotechnologies, Westminster, B.C., Canada) were dissolved in HPLC grade methanol (MeOH; Fisher Scientific, Milwaukee, WI) before adding to artificial diet (Powell and Raffa 1999a). Excess methanol was evaporated under a gentle stream of nitrogen before being fed to larvae. The concentrations were 25 ug/ml and 125 ug/ml. MeOH and $dH_2O$ served as controls. All treatments and controls were added at a rate of 0.75 µl solution/mg artificial diet (Powell and Raffa 1999a). Sample sizes ranged from 4 to 5. A total of 19 larvae was assayed. Frass, exuviae, and larvae were collected for chemical analysis, and development time, RGR, and RCR were calculated.

**Chemical analyses.** Larvae were macerated before analysis. Neither frass nor exuviae required any preparation. The concentrations and composition of monoterpenes were determined by gas liquid chromatography following previously described procedures (Raffa and Steffeck 1988, Powell and Raffa 1999b). Each sample was extracted in 250 to 1000 µl of hexane, depending on the size of the sample. After 24 h, the extract was removed and filtered through glass wool. The sample was rinsed in 200 to 500 µl hexane, the rinsate was removed and the sample subsequently filtered through glass wool. Each sample was rinsed twice to insure recovery of all extracted terpenes. The volume of extract was brought to 250 to 1000 µl by addition of hexane, or by evaporation under a gentle stream of nitrogen. Depending on final volume of sample, 2.5 to 10 µl of 10% para-cymene (Aldrich Chemical Company, Milwaukee, WI) was added to each extract as an internal standard. Para-cymene was used as an internal standard because it is not found in *L. laricina* foliage (Powell and Raffa 1999b).

All separations were performed on a Shimadzu GLC 17A, fitted with an AOC 20i autosampler (Shimadzu Scientific Instruments, Inc. Columbia, MD), using a 25 m × 0.25 mm bonded fused silica open tubular polyethylene glycol column (Alltech Assoc., Deerfield, IL). Oven temperature was 60°C for the first 10 min, and increased 10°C each min for 10 min until a final temperature of 160°C was reached. The carrier gas, helium, was maintained at 30 cm per second. The concentration of each monoterpene was determined by integrating peak areas using Class-VP software (Shimadzu Scientific Instruments, Inc., Columbia, MD). All compounds were quantified by comparing their percentage of the total with the percentage the internal standard, para-cymene.

Diterpene acids were analyzed using the direct injection HPLC method modified from Chow and Shepard (1996). Each sample was extracted in 200 µl of HPLC grade MeOH. After 24 h, the extract was removed and filtered through glass wool. The sample was rinsed in 100 µl MeOH, the rinsate was removed, and filtered through glass wool. Each sample was rinsed twice to insure removal of all extracted diterpene acids. The volume of the extract was brought to 250 µl by addition of MeOH or evaporation under a gentle stream of nitrogen. Samples were analyzed using a Shimadzu LC-10AS HPLC fitted with a Zorbax Rx-C8 column (4.6mm × 25 cm; Alltech Assoc., Deerfield, IL), and a 20 µl sample loop. The UV-Visible Light detector (Shimadzu SPD-10a) was set at 200 nm (Chow and Shepard 1996). The mobile phase consisted of 65% acetonitrile/35% 0.1% acetic acid (Fisher Scientific, Milwaukee, WI) at 2 ml/min.

**Statistical analyses.** Insect performance was analyzed for each stadium using a 1-way ANOVA, where the potential source of variation was treatment. Treatments included each concentration of each terpene and the solvent controls. Variables were tested to satisfy assumptions of normality and homogeneity of variance by graphical analysis of residuals. No insect performance data deviated from a normal distribution, and thus no transformations were required.

All chemical data are represented as the percentage of terpenes recovered from the amount consumed. Only those compounds found exclusively in frass or larval tissue of larvae fed terpenes (i.e., parent terpenes or their metabolites) were statistically analyzed.

Chemical data from experiments 1, 3, and 4 were analyzed using two methods: ANOVA and logistic regression of binomial data. ANOVAs were 1-way when experiments were performed on one stadium (Treatment), or 2-way ANOVA when experi-

ments were performed on two or more stadia (Treatment, Instar, Treatment*Instar). Chemical data were not distributed normally, and moreover could not be transformed due to the high number of zeroes. Therefore, interpretation of P-values had to be done with caution. To verify interpretations of P-values, all chemical data were re-analyzed using logistic regression of binomial data to test the significance of treatment, instar, and their interaction. Here, data were transformed into binomial data (presence of compound = 1, absence of compound = 0), and 4 possible models were tested: (1) treatment instar treatment*instar, (2) treatment, (3) instar, and (4) treatment instar. Likelihood ratio tests were used to select the best model using $\alpha < 0.05$. Once the best model was determined, the results of the binomial analyses were compared to ANOVA analyses on untransformed data. In all cases, the best binomial model agreed with the ANOVA analysis. Therefore, *P*-values from ANOVA analyses are reported. A significance level of $P < 0.05$ was used for all insect performance and chemical data analyses.

Chemical data from the monoterpene degradation experiment were analyzed using 1-way ANOVA, where the potential source of variation was time. Where a significant time effect was observed, we applied a curve-fitting program using Cricket Graph (Computer Associates, Malvern, PA).

## Results

**Monoterpene bioassays.** Overall, monoterpene treatments had little or no effect on gypsy moth performance when compared to $dH_2O$ and 0.75% Triton controls. Complete statistics are in Powell (2002). In the first monoterpene assay among second instars, mean RGR (range 0.8 to 1.0 g/g/day), mean RCR (range 1.2 to 1.9 g/g/day), and mean development time (range 3.6 to 4.2 d) did not vary with treatment. In the second monoterpene assay, mean RGR was lower among larvae fed 5% bornyl acetate (0.4 g/g/day) than all other treatments and controls (0.5 to 0.6 g/g/day) ($F = 4.690$; df = 5, 52; $P = 0.0001$). Mean RCR (range 1.1 to 1.5 g/g/day) did not vary with treatment. Mean development time was statistically longer among larvae fed 5% bornyl acetate (5.8 d) than all other treatments and controls (4.8 to 4.9 d) ($F = 10.805$; df = 5, 52; $P = 0.0001$). In the second monoterpene experiment, insect performance was not affected by 0.01%, 0.1%, or 1.0% bornyl acetate, which represent levels of bornyl acetate found in preferred host trees of gypsy moth.

In the first monoterpene assay among third instars, mean RGR was lower among larvae fed 1% bornyl acetate and 0.01% limonene (0.4 g/g/day) than $dH_2O$ or Triton controls (0.5 g/g/day) ($F = 2.925$; df = 10, 97; $P = 0.0030$). RCR was statistically higher among larvae fed 0.1% myrcene (2.1 g/g/day) than $dH_2O$ (1.7 g/g/day) ($F = 2.080$; df = 10, 93; $P = 0.0337$). Mean development time (range 5.0 to 5.5 d) did not vary with treatment. In the second monoterpene assay among third instars, mean RGR was statistically lower among larvae fed 5% bornyl acetate (0.3 g/g/day) than Triton (0.5 g/g/day) ($F = 4.74$; df = 5, 53; $P = 0.0012$). Mean RCR was statistically lower among larvae fed 5% bornyl acetate (0.5 g/g/day) than $dH_2O$ (1.1 g/g/day) ($F = 2.595$; df = 5, 53; $P = 0.0358$). Mean development time was statistically lower among larvae fed 0.01% bornyl acetate (4.3 d) than $dH_2O$ and Triton (4.9 d), and lower among larvae fed 0.1% bornyl acetate (4.5 d) than Triton (4.9 d). Mean development time was longer among larvae fed 5% bornyl acetate (5.5 d) than $dH_2O$ and Triton (4.9 d) ($F = 6.851$; df = 5, 53; $P = 0.0001$).

In the first monoterpene assay among fourth instars, mean RGR (range 0.2 to 0.3 g/g/day; $F = 0.635$; df = 10, 90; $P = 0.7800$), mean RCR (range 0.8 to 1.1 g/g/day),

and mean development time (range 6.7 to 8.2 d) did not vary with treatment. Likewise, in the second monoterpene assay among fourth instars, mean RGR (range 0.2 to 0.3 g/g/day), mean RCR (range 0.7 to 0.9 g/g/day), and mean development time (range 6.6 to 7.4 d) did not vary with treatment.

Although several compounds were detected in the extracts of frass and larval tissues, only two were recovered exclusively from larvae fed monoterpenes. Bornyl acetate and borneol, its likely metabolite, were recovered only from frass and larval tissues of larvae fed bornyl acetate. Neither limonene nor myrcene were recovered from frass or larval tissues. No compounds were recovered exclusively from frass or larvae fed limonene or myrcene. Figure 2 shows representative chromatograms of extracts from frass of larvae fed Triton (2a) and $dH_2O$ (2b), while Figure 2c is a chromatogram of extract from frass of larvae fed bornyl acetate. The first 4 compounds (retention times (RT) = 2.7, 4.5, 5.8, and 10.2 min) are present in all frass regardless of treatment, and are not described hereafter. However, bornyl acetate (RT = 17.1) and borneol (RT = 18.8) were recovered only from the frass of larvae that were fed bornyl acetate.

The amount of bornyl acetate recovered from frass in the first assay varied with treatment ($F = 31.640$, df = 2.55; $P = 0.0001$). There was no effect due to instar. The amount of bornyl acetate recovered from frass was higher among larvae fed 1% bornyl acetate than 0.1% bornyl acetate, and among larvae fed 0.1% bornyl acetate than 0.01% bornyl acetate (Table 1). In the second assay, bornyl acetate was recovered only from the frass of second instars fed 5% bornyl acetate.

The amount of borneol recovered from frass varied among treatments and instars in both monoterpene experiments. In the first assay, the amount of borneol recovered from frass increased with increasing concentration of bornyl acetate ($F = 25.05$; df = 2, 55; $P = 0.0001$), and with increasing stadium ($F = 30.08$; df = 2, 55; $P = 0.0001$). The amount of borneol recovered was highest among fourth instars fed 1% bornyl acetate. In the second assay, the amount of borneol recovered from frass was highest among third instars fed 5% bornyl acetate (Instar: $F = 7.099$; df = 2, 61; $P = 0.0013$/ Concentration: $F = 4.985$; df = 3, 61; $P = 0.0030$).

In addition to bornyl acetate and borneol, an unknown (retention time = 22.3 min) was recovered in small amounts (0.03%) in the frass of fourth instars fed 1% bornyl acetate in the first monoterpene bioassay. It did not appear in any other treatments.

The amount of borneol recovered from larvae varied with instar ($F = 6.589$; df = 2, 55; $P = 0.0025$) but not with treatment in the first assay (Table 1). The amount of borneol recovered was higher among third instars than second and fourth instars (Table 1). In the second assay, the amount of borneol recovered from larvae varied with both treatment ($F = 7.737$; df = 3, 61; $P = 0.0008$) and instar ($F = 21.812$; df = 2, 61; $P = 0.0001$). Borneol was recovered only from larvae fed 1% and 5% bornyl acetate. The amount of borneol recovered was highest among third instars fed 5% bornyl acetate (Table 1). Borneol was recovered in small amounts (0.09%) from one exuviae sample of larvae fed 5% bornyl acetate.

**Volatilization.** Mean recoveries of monoterpenes from amended artificial diet was 84% for bornyl acetate, 72% for limonene, and 87% for myrcene at 0 h. Mean recoveries at 24 h were 57% for bornyl acetate, 59% for limonene, and 66% for myrcene. No monoterpenes were recovered from the head-space of larvae fed monoterpenes.

**Monoterpene degradation.** The time course of bornyl acetate degradation differed considerably from that observed for limonene and myrcene. Bornyl acetate and

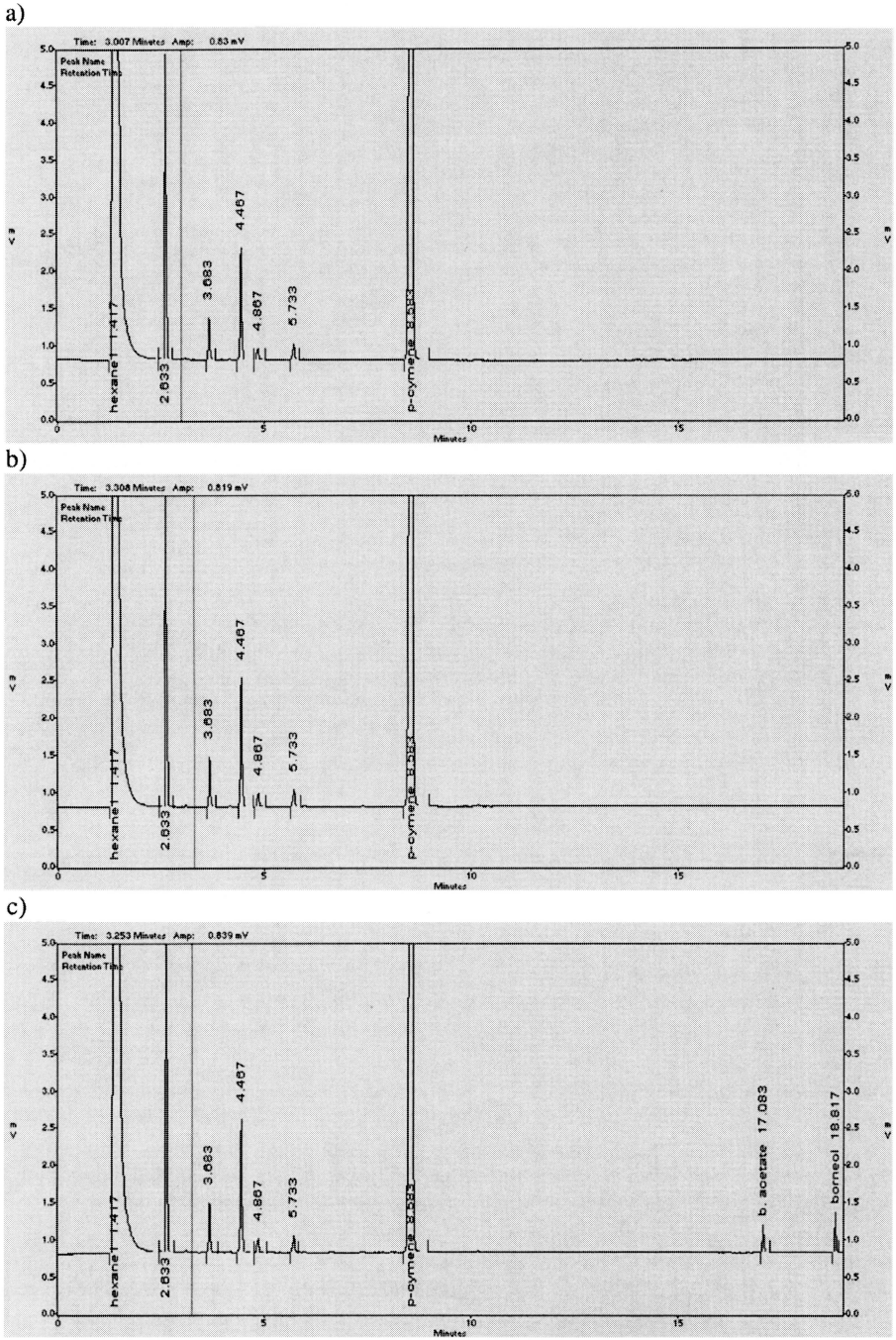


Fig. 2. Representative gas chromatographic (GC) spectra of frass of gypsy moth larvae fed (a) triton, (b) $dH_2O$, and (c) bornyl acetate. GC spectra of frass of gypsy moth larvae fed limonene and myrcene are similar to (a) and (b) and, therefore, are not shown.

**Table 1. Percentage of bornyl acetate and borneol recovered from frass and larvae of gypsy moths fed varying concentrations of bornyl acetate**

| Experiment | Instar | Concentration | N | % bornyl acetate | % borneol | |
|---|---|---|---|---|---|---|
| | | | | frass* | frass | larvae |
| 1 | 2 | 0.01 | 9 | 0.0 (0.0) | 0.0 (0.0)a | 0.0 (0.0) |
| | | 0.1 | 9 | 1.4 (0.5) | 0.0 (0.0)a | 0.1 (0.1) |
| | | 1.0 | 8 | 1.7 (0.3) | 0.4 (0.2)ab | 0.5 (0.1) |
| | 3 | 0.01 | 9 | 0.0 (0.0) | 0.0 (0.0)a | 1.9 (1.9) |
| | | 0.1 | 8 | 0.8 (0.4) | 1.4 (0.6)b | 1.9 (0.7) |
| | | 1.0 | 7 | 1.3 (0.2) | 3.6 (0.8)c | 2.6 (0.6) |
| | 4 | 0.01 | 8 | 0.0 (0.0) | 0.0 (0.0)a | 0.0 (0.0) |
| | | 0.1 | 7 | 0.4 (0.3) | 3.6 (0.9)c | 0.0 (0.0) |
| | | 1.0 | 8 | 1.9 (0.2) | 5.6 (1.0)d | 0.4 (0.2) |
| 2 | 2 | 0.01 | 10 | 0.0 (0.0)a | 0.0 (0.0)a | 0.0 (0.0)a |
| | | 0.1 | 10 | 0.0 (0.0)a | 0.0 (0.0)a | 0.0 (0.0)a |
| | | 1.0 | 9 | 0.0 (0.0)a | 1.7 (0.5)ab | 4.6 (0.9)b |
| | | 5.0 | 9 | 0.5 (0.2)b | 2.0 (0.3)ab | 4.3 (0.9)b |
| | 3 | 0.01 | 10 | 0.0 (0.0)a | 0.0 (0.0)a | 0.0 (0.0)a |
| | | 0.1 | 10 | 0.0 (0.0)a | 4.0 (1.9)ab | 0.0 (0.0)a |
| | | 1.0 | 10 | 0.0 (0.0)a | 5.4 (1.3)b | 3.3 (1.6)bc |
| | | 5.0 | 10 | 0.0 (0.0)a | 12.7 (5.0)c | 9.1 (1.8)d |
| | 4 | 0.01 | 9 | 0.0 (0.0)a | 0.0 (0.0)a | 0.0 (0.0)a |
| | | 0.1 | 7 | 0.0 (0.0)a | 3.0 (1.3)ab | 0.0 (0.0)a |
| | | 1.0 | 8 | 0.0 (0.0)a | 4.1 (0.7)ab | 1.0 (0.2)c |
| | | 5.0 | 5 | 0.0 (0.0)a | 2.6 (0.4)ab | 1.4 (0.4)c |

* Means (±SE) followed by different lower case letters are significantly different at $P < 0.05$ (Fisher's Protected LSD).

borneol were recovered from both gut and peripheral larval tissues; whereas, limonene and myrcene were recovered only from gut tissues (Fig. 3). The presence of bornyl acetate in peripheral larval tissues diminished to very low levels almost immediately after ingestion. The degradation of limonene and myrcene occurred more gradually, and extended over 3 to 4 h. Higher concentrations of limonene and myrcene, than of bornyl acetate, were recovered.

The amounts of bornyl acetate and borneol recovered did not vary among time periods in either larval tissues or gut tissues. The mean amount of bornyl acetate recovered from larval tissues ranged from 1.1% to 3.2%, and the amount of borneol recovered from larval tissues ranged from 0% to 3.2% (Fig. 3a). The mean amount of bornyl acetate recovered from guts ranged from 0% to 3.4%, and the amount of borneol recovered from guts ranged from 0.6% to 3.2% (Fig. 3a).

The amount of limonene and myrcene recovered from guts decreased with time (Fig. 3b, 3c). Both showed relationships that could be closely approximated with a simple polynomial curve. The mean amounts of limonene recovered were statistically higher among guts sampled at 0 h (11.9%) and 1 h (11.8%) than at 3 h (3.7%) and 4 h (0.9%) ($F = 7.024$; df = 4, 12; $P = 0.0037$). The amount of myrcene recovered was higher among guts sampled at 0 h (6.5%) and 1 h (5.3%) than guts sampled at 3 h (1.4%) and 4 h (0.8%) ($F = 5.191$; df = 4, 13; $P = 0.0101$).

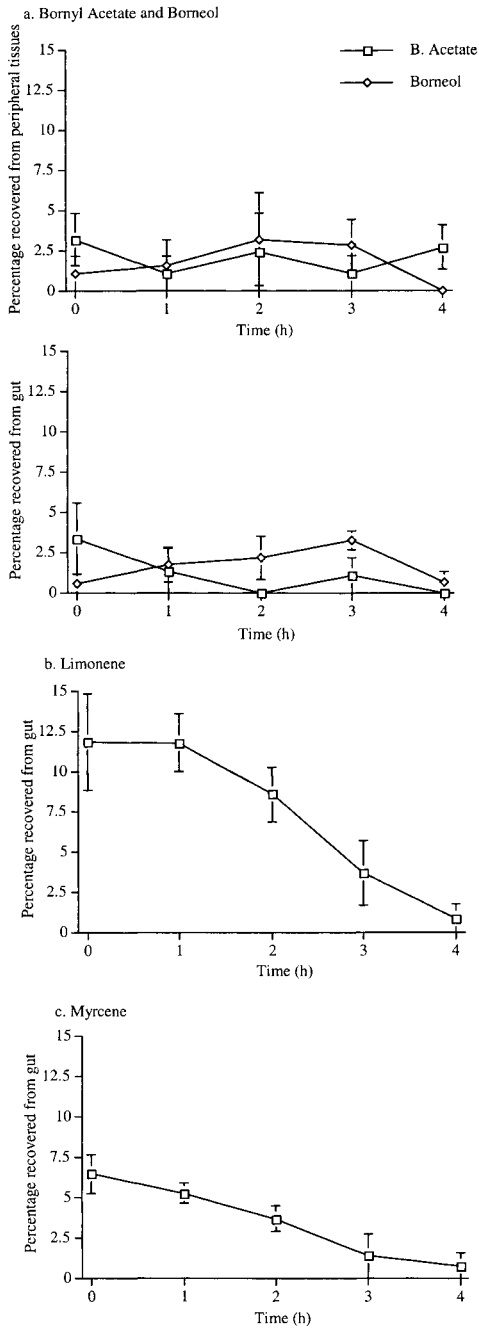


Fig. 3. Percentages of bornyl acetate, borneol, limonene, and myrcene recovered from gut and larval tissues of gypsy moth larvae at 0, 1, 2, 3, and 4 h after consumption of 1 ul of (a) bornyl acetate, (b) limonene, and (c) myrcene. Limonene and myrcene showed relationships that could be closely approximated with a second-degree polynomial curve (limonene: $y = -0.2507x^2 - 0.981x + 12.386$, $r^2 = 0.97$; myrcene: $y = 0.036x^2 - 1.673x + 6.671$, $r^2 = 0.98$).

**Enzyme inhibitors.** Addition of PBO to monoterpene treatments did not affect RGR among second instars. RGR was higher among larvae fed $dH_2O$ and Triton (0.9 g/g/day[1]) than bornyl acetate (0.7 g/g/day) or bornyl acetate + PBO (0.6 g/g/day) ($F$ = 2.612; df = 8, 76; $P$ = 0.0140). Likewise, RCR was higher among larvae fed $dH_2O$ (5.2 g/g/day) than bornyl acetate (3.9 g/g/day), bornyl acetate + PBO (3.9 g/g/day), myrcene (4.3 g/g/day), and myrcene + PBO (4.3 g/g/day) ($F$ = 2.386; df = 8, 73; $P$ = 0.0241). Mean development time (range 3.6 to 4.7 d) did not vary with treatment. Among third instars, mean RGR, mean RCR (range 1.3 to 1.8 g/g/day), and mean development time (range 6.4 to 7.2 d) did not vary with treatment. Likewise, mean RGR (range 0.2 to 0.3 g/g/day), mean RCR (range 0.7 to 1.3 g/g/day), and mean development time (range 7.3 to 8.7 d) did not vary with treatment among fourth instars.

The amounts of bornyl acetate ($F$ = 27.310; df = 2, 37; $P$ = 0.0001) and borneol ($F$ = 63.621; df = 2, 37; $P$ = 0.0001) recovered from frass varied with instar but not treatment. The percentage of bornyl acetate and borneol recovered from frass increased with increasing instar (Table 2) The amount of borneol recovered from larvae did not differ among instars or treatments (Table 3).

In addition to bornyl acetate and borneol, an unknown (retention time = 17.9) was recovered from fourth instars. The amount of the unknown recovered was 0.03% and 0.06% from larvae fed bornyl acetate and bornyl acetate + PBO, respectively.

Addition of DEF to monoterpene treatments did not affect insect performance. Mean RGR (range 0.6 to 0.8 g/g/day) and mean development time (range 6.3 to 7.1 d) did not vary with treatment. RCR was higher among larvae fed DEF (1.8 g/g/day) than all other treatments or controls (0.7 to 1.4 g/g/day) ($F$ = 3.422; df = 4, 42; $P$ = 0.0165).

The amount of bornyl acetate recovered from frass, and the amounts of borneol recovered from frass and larvae, did not vary with treatment among larvae fed amended diet for the duration of the stadium. The amount of bornyl acetate recovered from frass and the amount of borneol recovered from larvae did vary with treatment among larvae that were treated for 2 d then starved for 1 d. The amount of borneol recovered from frass was higher among larvae fed bornyl acetate than larvae fed bornyl acetate + DEF ($F$ = 6.921; df = 1, 7; $P$ = 0.0339). In the group of larvae dissected after 2 d of treatment, the amount of bornyl acetate recovered from frass and the gut, and the amount of borneol recovered from frass, the gut, and larval tissues did not vary with treatment.

**Table 2. Percentage of bornyl acetate and borneol recovered from frass and larvae of gypsy moths fed bornyl acetate + piperonyl butoxide (PBO)**

| | | | % bornyl acetate | % borneol | |
|---|---|---|---|---|---|
| Instar | Treatment | N | frass | frass | larvae |
| 2 | 1% Bornyl Acetate | 10 | 0.5 (0.3) | 0.3 (0.1) | 0.5 (0.2) |
| | 1% Bornyl Acetate + PBO | 8 | 0.3 (0.1) | 0.1 (0.1) | 0.5 (0.3) |
| 3 | 1% Bornyl Acetate | 7 | 1.7 (0.3) | 2.1 (0.2) | 0.2 (0.1) |
| | 1% Bornyl Acetate + PBO | 8 | 1.7 (0.4) | 3.2 (0.6) | 0.4 (0.1) |
| 4 | 1% Bornyl Acetate | 8 | 3.8 (0.6) | 5.9 (0.8) | 0.5 (0.1) |
| | 1% Bornyl Acetate + PBO | 10 | 3.1 (0.6) | 5.8 (0.6) | 0.6 (0.2) |

**Table 3. Percentage of bornyl acetate and borneol recovered from frass, guts, and larval tissues of fourth instar gypsy moths fed bornyl acetate + DEF**

| | | | % bornyl acetate | | % borneol | | | |
|---|---|---|---|---|---|---|---|---|
| Experiment* | Treatment | N | frass | gut only | frass** | larvae | larvae, no gut | gut only |
| 1 | 1% Bornyl acetate | 10 | 1.1 (0.2) | | 4.0 (0.6) | 1.0 (0.2) | | |
| | 1% Bornyl acetate + DEF | 10 | 1.5 (0.4) | | 3.7 (1.6) | 0.7 (0.2) | | |
| 2 | 1% Bornyl acetate | 4 | 0.9 (0.2) | | 2.5 (0.5)a | 0.6 (0.3) | | |
| | 1% Bornyl acetate + DEF | 5 | 0.8 (0.2) | | 0.8 (0.4)b | 0.7 (0.7) | | |
| 3 | 1% Bornyl acetate | 4 | 0.6 (0.2) | 0 | 0.9 (0.3) | | 39.9 (14.0) | 0.5 (0.2) |
| | 1% Bornyl acetate + DEF | 5 | 0.9 (0.1) | 0.2 (0.2) | 1.5 (0.7) | | 7.35 (7.35) | 0.6 (0.3) |

* Experiment 1 = Larvae fed amended diet for the duration of the instar; Experiment 2 = Larvae fed amended diet for two d, then starved for 1 d; Experiment 3 = Larvae fed amended diet for two d, then dissected.

** Means (±SE) followed by different lower case letters are significantly different at $P < 0.05$ (Fisher's Protected LSD).

**Gut bacteria.** Guts dissected from control larvae had an average of 2200 colony forming units (CFU)/gut (range 208 to 26,800). Guts dissected from larvae fed antibiotics had an average of 12 CFU/gut (range 2 to 44).

Mean RGR was lower among larvae fed 1% bornyl acetate + antibiotics, 2% bornyl acetate, and 2% bornyl acetate + antibiotics (0.2 g/g/day) than the RGR of larvae fed Triton and $dH_2O$ (0.3 g/g/day) ($F = 3.912$; df = 6, 61; $P = 0.0023$). Neither mean RCR (range 0.9 to 1.3 g/g/day) nor mean development time (range 6.5 to 7.5 d; $F = 3.50$; df = 6, 611; $P = 0.0049$) varied with treatment.

The amount of bornyl acetate recovered from frass and the amount of borneol recovered from larval tissues were higher among larvae fed 1% bornyl acetate than 1% bornyl acetate + antibiotics ($F = 14.00$; df = 3, 19; $P = 0.0001$). The amount of borneol recovered from frass did not vary with addition of antibiotics for either bornyl acetate concentration (Table 4).

**Diterpene bioassay.** Mean RGR (range 0.7 to 1.1 g/g/day), mean RCR (range 2.9 to 4.3 g/g/day), and mean development time (range 3.6 to 4.5 d) did not vary with treatment. No diterpene acids were recovered from frass or larvae.

## Discussion

These results suggest that gypsy moth is tolerant of a broad range of mono- and di- terpenes. Metabolism appears to be the primary mechanism by which gypsy moth larvae contend with the monoterpenes bornyl acetate, limonene, and myrcene, and the diterpenes isopimaric acid and neoabietic acid. Excretion is partially involved, as bornyl acetate was recovered from frass of larvae fed bornyl acetate. Bornyl acetate also appears to be converted in part to borneol, as this apparent metabolite was recovered from both frass and larval tissues of larvae fed bornyl acetate. Limonene, myrcene, isopimaric acid, and neoabietic acid were never recovered from frass or larval tissues, suggesting that these terpenes were converted entirely to other products. Volatilization cannot explain the absence of these compounds. Their products could not be identified, suggesting that ingested compounds were degraded into compounds, such as glycosylated monoterpenes (Figueiredo et. al. 1996) and isoprene, that would not be detectable under our conditions. Future studies using radiolabeled compounds could help identify derivative products.

The time course experiment revealed substantially different patterns in the me-

**Table 4. Percentage of bornyl acetate and borneol recovered from frass and larvae of gypsy moths fed bornyl acetate + antibiotics**

| Treatment | N | % bornyl acetate | % borneol | |
|---|---|---|---|---|
| | | frass* | frass | larvae |
| 1% bornyl acetate | 10 | 3.3 (0.5)a | 4.2 (1.4)a | 15.6 (4.9)a |
| 1% bornyl acetate + antibiotics | 10 | 0.9 (0.1)b | 2.0 (0.5)ab | 5.3 (0.9)b |
| 2% bornyl acetate | 9 | 1.1 (0.2)b | 2.1 (0.5)ab | 3.2 (0.1)b |
| 2% bornyl acetate + antibiotics | 10 | 1.1 (0.2)b | 0.8 (0.2)b | 7.2 (4.0)ab |

* Means (±SE) followed by different lower case letters are significantly different at $P < 0.05$ (Fisher's Protected LSD).

tabolism of bornyl acetate, an oxygenated monoterpene, versus limonene and myrcene, which are pure hydrocarbon monoterpenes (Fig. 3). First, bornyl acetate was recovered from both gut and peripheral larval tissues; whereas, limonene and myrcene were recovered only from guts. This suggests that neither limonene nor myrcene move through the gut epithelium into peripheral larval tissues, but rather are metabolized within the alimentary canal. In contrast, recovery of borneol from both gut and peripheral larval tissues suggests that either a portion of the bornyl acetate ingested moves across the gut epithelium, whereupon it is metabolized to borneol, or that bornyl acetate is metabolized in the gut to borneol, which then moves into peripheral larval tissues. Borneol appears to reach its peak in gut tissues approximately 3 h after ingestion. A second difference was in the rate at which these compounds were degraded. Nearly all of the bornyl acetate is degraded within 1 h after ingestion; whereas, nearly half of the limonene and myrcene recovered at 0 h are present at 2 h, and an additional 2 h are required for near complete degradation. Future studies should focus on differences in oxygenated versus non-oxygenated compounds during the first 3 h after ingestion, and on using radio-labelled compounds or injection of monoterpenes directly in the hemocoel.

While it has been suggested that ecological factors such as host feeding breadth or feeding guild may select for mechanisms by which various insects contend with phytochemicals, several authors have emphasized the physical and chemical properties of the ingested compounds (Duffey 1980, Gardner and Stermitz 1988, Shapiro 1991). For example, many detoxifying enzymes act on relatively lipophilic compounds, rendering them more hydrophilic, thus facilitating excretion (Brattsten 1986, Lindroth 1991). In our study, 100% of the 2 more lipophilic monoterpenes, limonene and myrcene, were metabolized. The relatively hydrophilic compound, bornyl acetate, was metabolized in part to borneol, and was excreted in small amounts. Borneol was subsequently both accumulated and excreted. Thus, our results support the view that the chemical and physical properties of various terpenoids are important in predicting the mechanisms by which herbivores process them.

Little is known about the enzymes involved in terpene metabolism by gypsy moth. However, it is known that P450 titers are affected by terpenes. For example, P450 content is higher in gypsy moth larvae fed Douglas fir foliage, which contains terpenes, than alder foliage, which does not (Moldenke et al. 1992). In contrast, P450 activity was similar between larvae fed artificial diet amended with the monoterpene $\alpha$-pinene and controls (Sheppard and Friedman 1989). Tolerance of gypsy moth larvae to some insecticides is also affected by terpenes and is higher when larvae feed on Douglas fir than alder foliage (Moldenke et al. 1992, 1997; Berry et al. 1993). Increased P450 activity induced by terpenes can increase tolerance to some, but not all, insecticides (Moldenke et al. 1992, 1997).

Induction of P450 enzymes by monoterpenes has been observed in several lepidopteran species (Brattsten 1986, Ranasinghe et al. 1997), marsupials (Pass et al. 1999) and mammals (Debersac et al. 2001). P450 enzymes or esterases in terpene metabolism by gypsy moth cannot be ruled out, as these enzymes exist in multiple isoforms, some of which may not be inhibited by piperonyl butoxide (Scott 1999), some of which may not be inhibited by piperonyl butoxide or DEF.

We found little evidence for involvement of gut microflora in terpene metabolism, despite their importance in other systems (Miyazawa et al. 1996). Antibiotics added to 1% bornyl acetate reduced the amount of borneol recovered from larval tissues, which supports the involvement of gut microorganisms. However, more bornyl ac-

etate was recovered from frass of larvae fed 1% bornyl acetate than 1% bornyl acetate with antibiotics, which is contrary to what would be expected if gut microorganisms were involved. Gut microorganisms were not found to be responsible for terpene metabolism in a relative of the gypsy moth, the Douglas fir tussock moth (*Orgyia pseudotsugata* McDunnough) (Lepidoptera: Lymantriidae) (Andrews and Spence 1980).

Ability of the gypsy moth to exploit such a broad range of host trees appears to arise largely from metabolic processes. The accompanying relatively indiscriminant feeding behavior contributes to the gypsy moth's invasion success, escape from natural enemies, and eruptive population dynamics (Liebhold 1997). In turn, its resulting economic and environmental impacts affect a broad range of forest product, aesthetic, nursery, and homeowner values (Montgomery and Wallner 1988).

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## References Cited

**Andrews, R. E. and K. D. Spence. 1980.** Action of Douglas fir tussock moth larvae and their microflora on dietary terpenes. Appl. Env. Microbiol. 40: 959-963.

**Berry, R. E., A. F. Moldenke, J. C. Miller and J. G. Wernz. 1993.** Toxicity of diflubenzuron in larvae of gypsy moth (Lepidoptera: Lymantriidae): Effects of host plant. J. Econ. Ecol. 86: 809-814.

**Brattsten, L. B. 1986.** Fate of ingested plant allelochemicals in herbivorous insects, Pp. 211-255. *In* Brattsten, L. B. and Ahmad, S. [eds.], Molecular aspects of insect-plant associations. Plenum Press, NY.

**Brattsten, L. B. and R. L. Metcalf. 1970.** The synergistic ratio of carbaryl with piperonyl butoxide as an indicator of the distribution of multifunction oxidases in the Insecta. J. Econ. Entomol. 63: 101-104.

**Broderick, N. A., R. M. Goodman, K. F. Raffa and J. Handelsman. 2000.** Synergy between Zwittermicin a and *Bacillus thuringiensis* subsp. *kurstaki* against gypsy moth (Lepidoptera: Lymantriidae). Environ. Entomol. 29: 101-107.

**Cates, R. G. 1996.** The role of mixtures and variation in the production of terpenoids in conifer-insect-pathogen interactions. Rec. Adv. Phytochem. 30: 179-216.

**Chow, S. Z. and D. Shepard. 1996.** High performance liquid chromatographic determination of resin acids in pulp mill effluent. Tech. Assoc. Pulp and Paper Indus. J. 79: 173-180.

**Cornelius, M. L., J. K. Grace and J. R. Yates. 1997.** Toxicity of monoterpenoids and other natural products to the formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 90: 320-325.

**Debersac P., J. M. Heydel, M. J. Amiot, H. Goudonnet, Y. Artur, M. Suschetet and M. H. Siess. 2001.** Induction of cytochrome P450 and/or detoxication enzymes by various extracts of rosemary: description of specific patterns. Food Chem. Tox. 39: 907-918.

**Duffey, S. S. 1980.** Sequestration of plant natural products by insects. Ann. Rev. Entomol. 25: 447-477.

**Fehrer, M. D., P. H. Wanner, M. C. Taylor and W. L. Grogan. 1992.** Bald cypress, *Taxodium disticum* (Coniferales, Taxodiaceae), a primary food source of gypsy moth, Lymantria dispar, in Maryland. Proc. Entomol. Soc. Washington 94: 119-122.

**Figueiredo A. C., M. J. Almendra, J. G. Barroso, and J. J. C. Scheffer. 1996.** Biotransformation of monoterpenes and sesquiterpenes by cell suspension cultures of *Achillea millefolium* L, subsp. *millefoliu*. Biotech. Letters 18: 863-868.

**Gardner, D. R. and F. R. Stermitz. 1988.** Host plant utilization and iridoid glycoside sequestration by *Euphydryas anicia* (Lepidoptera: Nymphalidae). J. Chem. Ecol. 14: 2147-2168.

**Gershenzon, J. and R. Croteau. 1991.** Terpenoids, Pp. 165-208. *In* Rosenthal, G. A. and Berenbaum, M. R. [eds.], Herbivores: Their interactions with secondary plant metabolites. 1: The chemical participants. Academic Press, Inc., NY.

**Grijpma, P. J. 1989.** Overview of research on lymantriids in Eastern and Western Europe, Pp. 21-50. *In* Wallner, W. E. and McManus, K. A. [eds.], Proc.: Lymantriidae: A comparison of features of new and old world tussock moths. General Technical Report NE-123, USDA Forest Service Northeastern Forest Experiment Station, Hamden, CT.

**Hummelbrunner, L. A. and M. B. Isman. 2001.** Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). J. Agric. Food Chem. 49: 715-720.

**Kruse, J. J. and K. F. Raffa. 1997.** Effects of selected midwestern larval host plants on performance by two strains of the gypsy moth (Lepidoptera, Lymantriidae) parasitoid *Cotesia melanoscela* (Hymenoptera, Braconidae). Environ. Entomol. 26: 1155-1166.

**Langenheim, J. H. 1994.** Higher plant terpenoids: a phytocentric overview of their ecological roles. J. Chem. Ecol. 20: 1223-1280.

**Larsson, S., C. Bjorkman and R. Gref. 1986.** Responses of *Neodiprion sertifer* (Hym., Diprionidae) larvae to variation in needle resin acid concentration in Scots pine. Oecologia 70: 77-84.

**Lechowitz, M. J. and Y. Mauffette. 1986.** Host preferences of the gypsy moth in eastern North American forests versus European forests. Revue Entomol. Queb. 31: 43-51.

**Lee, S., R. Tsao, C. Peterson and J. R. Coats. 1997.** Insecticidal activity of monoterpenoids to western corn rootworm (Coleoptera: Chrysomelidae), twospotted spider mite (Acari: Tetranychidae), and house fly (Diptera: Muscidae). J. Econ. Entomol. 90: 883-892.

**Liebhold, A. M., K. W. Gottschalk, D. A. Mason and R. R. Bush. 1997.** Forest susceptibility to the gypsy moth. J. Forestry 95: 20-24.

**Lindroth, R. L. 1991.** Differential toxicity of plant allelochemicals to insects: Roles of enzymatic detoxication systems, Pp. 1-33. *In* Bernays, E. A. [ed.], Insect-Plant Interactions. CRC Press, Boca Raton, FL.

**Miller, J. C. and P. E. Hanson. 1989.** Laboratory studies on development of gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), larvae on foliage of gymnosperms. Can. Entomol. 121: 425-429.

**Miller, J. C., P. E. Hanson and D. N. Kimberling. 1991.** Development of the gypsy moth (Lepidoptera: Lymantriidae) on Douglas fir. J. Econ. Entomol. 84: 461-465.

**Miyazawa, M., T. Wada and H. Kameoka. 1996.** Biotransformation of alpha-terpinene in common cutworm larvae (*Spodoptera litura* Fabricius). J. Agric. Food Chem. 44: 2889-2893.

**Moldenke, A. F., R. E. Berry, J. C. Miller, R. G. Kelsey, J. G. Wernz and S. Venkateswaran. 1992.** Carbaryl susceptibility and detoxication enzymes in gypsy moth (Lepidoptera: Lymantriidae): Influence of host plant. J. Econ. Entomol. 85: 1628-1635.

**Moldenke, A. F., R. E. Berry, J. C. Miller and J. G. Wernz. 1997.** Toxicity of acephate to larvae of gypsy moth as a function of host plant and bioassay method. Entomol. Exp. Appl. 84: 157-163.

**Montgomery, M. E. and W. E. Wallner. 1988.** The gypsy moth: a westward migrant, Pp. 353-375, *In* Berryman, A. A. [ed.], Dynamics of forest insect populations: patterns, causes, and implications. Plenum Press, NY.

**Pass, G. J., S. Mc Lean and I. Stupans. 1999.** Induction of xenobiotic metabolising enzymes in the common brushtail possum, *Trichosurus vulpecula,* by *Eucalyptus* terpenes. Comp. Biochem. Physiol. C. 124: 239-246.

**Powell, J. S. 2002.** The fate of conifer terpenes in generalist and specialist Lymantriidae (Lepidoptera). Ph.D. Thesis, Univ. Wisconsin, Madison.

**Powell, J. S. and K. F. Raffa. 1999a.** Effects of selected *Larix laricina* terpenoids on *Lymantria dispar* (Lepidoptera: Lymantriidae) development and behavior. Environ. Entomol. 28: 148-154.

**1999b.** Sources of variation in concentration and composition of foliar monoterpenes in tamarack (*Larix laricina*) seedlings: Roles of nutrient availability, time of season, and plant architecture. J. Chem. Ecol. 25: 1771-1797.

**Raffa, K. F. and R. J. Steffeck. 1988.** Computation of response factors for quantitative analysis of monoterpenes by gas liquid chromatography. J. Chem. Ecol. 14: 1385-1390.

**Ranasinghe, C., M. Headlam and A. A. Hobbs. 1997.** Induction of the mRNA for CYP6B2, a pyrethroid inducible cytochrome P450, in *Helicoverpa armigera* (Hübner) by dietary monoterpenes. Arch. Insect Biochem. Physiol. 34: 99-109.

**Regnault-Roger, C. 1999.** Diversification of plants protection strategies: interest of monoterpenes. Acta Botanica Gallica 146: 35-41.

**Regnault-Roger, C. and A. Hamraoui. 1997.** Defense against phytophagic insects by aromatic plant allelochemicals. Acta Botanica Gallica 144: 401-412.

**SAS Institute. 1988.** SAS User's Guide: Statistics. SAS Institute, Cary, N.C.

**Schaefer, P. W. 1989.** Diversity in form, function, behavior and ecology. An overview of the Lymantriidae (Lepidoptera) of the world, Pp. 1-20. *In* Wallner, W. E. and McManus, K. A. [eds.], Proc.: Lymantriidae: A comparison of features of new and old world tussock moths. General Technical Report NE-123, USDA Forest Service Northeastern Forest Experiment Station, Hamden, CT.

**Scott, J. G. 1999.** Cytochromes P450 and insecticide resistance. Insect Biochem. Molec. Biol. 29: 757-777.

**Shapiro, J. P. 1991.** Phytochemicals at the plant-insect interface. Arch. Insect. Biochem. Phys. 17: 191-200.

**Sheppard, C. A. and S. Friedman. 1989.** Endogenous and induced monooxygenase activity in gypsy moth larvae feeding on natural and artificial diets. Arch. Insect. Biochem. Phys. 10: 47-56.

**Staudt, M., N. Mandl, R. Joffre and S. Rambal. 2001.** Intraspecific variability of monoterpene composition emitted by *Quercus ilex* leaves. Can. J. For. Res, 31: 174-180.

**Theodoridis, D., E. H. M. Koster and D. J. De Jong. 2000.** Solid-phase microextraction for the analysis of biological compounds. J. Chrom. B. 745: 49-82.

**Trapp, S. and R. Croteau. 2001.** Defensive resin biosynthesis in conifers. Ann. Rev. Plant Physiol. Molec. Biol. 52: 689-724.

**USDA APHIS. 2001.** European gypsy moth (*Lymantria dispar*) quarantine. July 31, 2002 <http://www.aphis.usda.gov/ppq/maps/gypmoth.pdf>

**Usmani, K. A. and C. O. Knowles. 2001.** DEF sensitive esterases in homogenates of larval and adult *Helicoverpa zea, Spodoptera frugiperda,* and *Agrotis ipsilon* (Lepidoptera: Noctuidae). J. Econ. Entomol. 94: 884-891.

**Waldbauer, G. P. 1968.** The consumption and utilization of food by insects. Adv. Insect Physiol., 5: 229-289.

**Wagner, M. R., D. M. Benjamin, K. M. Clancy and B. A. Schuh. 1983.** Influence of diterpene resin acids on feeding and growth of larch sawfly, *Pristiphora erichsonii* (Hartig). J. Chem. Ecol. 9: 119-127.