Entomologia Experimentalis et Applicata **91:** 233–236, 1999.

Using hybrid and backcross larvae of *Papilio canadensis* and *Papilio glaucus* to detect induced phytochemical resistance in hybrid poplar trees experimentally defoliated by gypsy moths

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Accepted: November 10, 1998

Key words: hybrid genotypes, herbivore defoliation, phytochemical induction, differential growth rates, indirect competition, phenolic glycoside detoxification, *Lymantria dispar*, Papilionidae, tiger swallowtail butterflies

Abstract

Sub-plots of hybrid poplars were experimentally defoliated using 10 million gypsy moth larvae. Half of the defoliated (and undefoliated control) plots were fertilized to see if this would ameliorate the predicted induction of carbon-based phenolic defenses in the regrowth leaves. In order to bioassay the leaves of the four different treatments, we employed a continuum of genotypes (different hybrids and backcrosses of two different species of tiger swallowtail butterflies) with different abilities to detoxify these allelochemicals. Based on our previous studies with phytochemicals from the Salicaceae plant family, *Papilio canadensis* was likely to consume and process all *Populus* spp treatments, whereas *P. glaucus* predicted to either not consume or else quickly die on all *Populus* treatment leaves. Hybrid and backcross larvae of these two butterfly species are known to have intermediate levels of esterase detoxication enzymes and would therefore be likely to provide a continuum or at least varying degrees of sensitivity in bioassays for even the most subtle induction responses in the regrowth leaves. This presumption was supported in the feeding and growth studies conducted at different times post-defoliation during the 1997 growing season in Michigan.

Introduction

Detection of induced phytochemical resistance (rapid or delayed) after insect defoliation will depend upon the species (or genotype) of plant and the abiotic environment, including nitrogen availability and the C/N balance (Bryant et al., 1983; Herms & Mattson, 1992). However, perhaps most significantly, it depends upon the particular herbivore species or genotype encountering this post-defoliation plant, and the degree of behavioral or physiological inducibility in its response capabilities (Dankert et al., 1997).

With plants in the Salicaceae family (aspens, poplars, willows, etc.), we have observed well-adapted herbivores (e.g., *Papilio canadensis* and *P. rutulus*) with esterase detoxification systems (Lindroth et al., 1988; Scriber et al., 1991). Other very closely related congeneric species have low esterase activities and very poor abilities to survive on any Salicaceae species (e.g., *P. glaucus*, *P. alexiares*, and *P. multicaudatus*; Scriber et al., 1995; Scriber, 1996).

Interspecific hybrids and backcross larvae of the Salicaceae-adapted *P. canadensis* and the unadapted *P. glaucus* had intermediate esterase levels and correspondingly intermediate growth rates when challenged with phenolic glycosides from aspen (Scriber et al., 1989). This continuum of swallowtail butterfly genotypes might provide a differentially sensitive bioassay technique with which to detect even subtle changes in phytochemical suitability (induced defense) after severe defoliation of *Populus* hybrids by gypsy moth larvae.

As part of the design, half of the defoliated plots were fertilized to assess the degree to which induced resistance might be ameliorated by N-fertilization. For

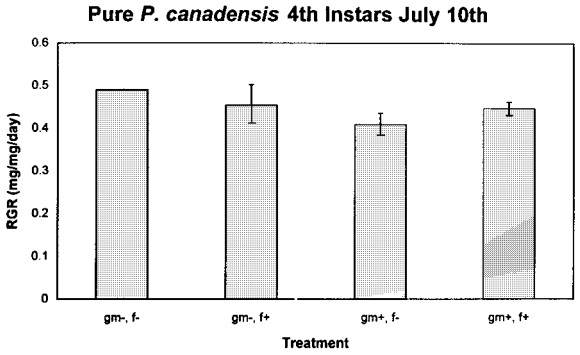

Figure 1. The relative growth rates (mg $\times$ mg^{-1} $\times$ d^{-1}) of penultimate instar *P. canadensis* fed hybrid *Populus* from four experimental treatments (+ with defoliation, + with fertilization, etc.). No significant difference exists for defoliation ($F = 1.39$, $P = 0.30$) or fertilization ($F = 1.23$, $P = 0.33$).

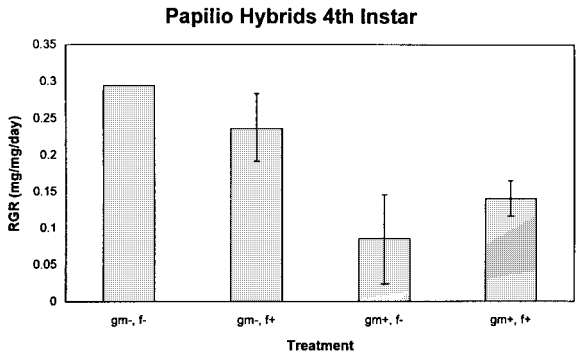

Figure 2. The relative growth rates of penultimate instar hybrid larvae (*P. canadensis* female $\times$ *P. glaucus* male) fed four *Populus* treatments.

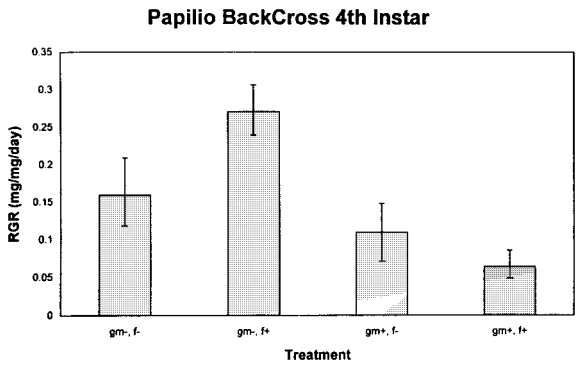

Figure 3. The relative growth rates of penultimate instar backcross larvae (hybrid female $\times$ *P. glaucus* male) fed four *Populus* treatments.

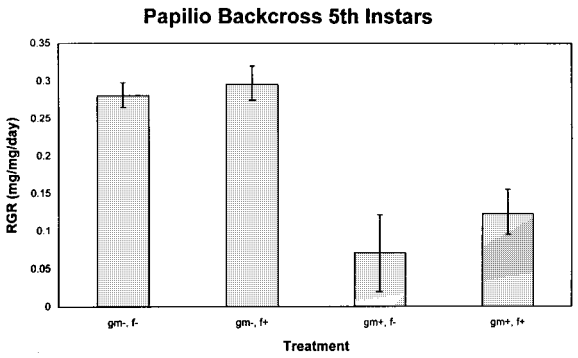

Figure 4. The relative growth rates of final instar backcross larvae (hybrid female X *P. glaucus* male) fed four *Populus* treatments.

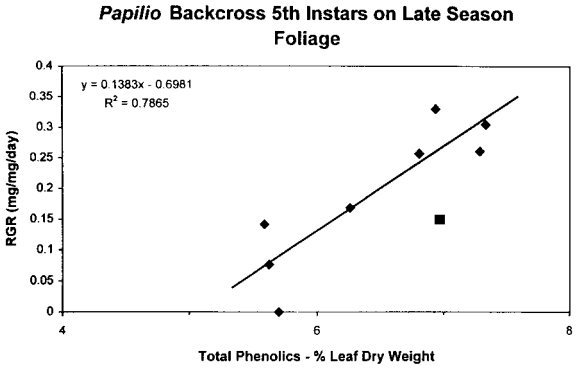

Figure 5. Final instar growth rates of backcross larvae as a function of total foliar phenolics.

comparison, half of the undefoliated plot was also fertilized.

Figure 6. A photograph of the defoliated versus non-defoliated treatments (1997).

Materials and methods

Creating outbreak (defoliation) and control (undefoliated) plots. In the hybrid poplar plantation plots of Michigan State University's Kellogg Biological Station's L.T.E.R. 'Agroecology' Site, we supplemented natural gypsy moth infestations in 1996 (2 million formalin-treated eggs) and 1997 (10 million treated eggs) in order to simulate outbreak dynamics, but also to create experimentally controlled adjacent plots without defoliation. The formalin killed the transovariole virus (NPV) that would likely have had major disruptive effects on the gypsy moth damage levels we were trying to create. Two large plots (600 m^2) were used in a randomized split plot design with defoliation in the main plots and fertilization (100 kg ha^{-1} of N each year) in the subplots.

Defoliation-free plots were created without the use of pesticides by using tanglefoot protective sticky barriers, and intensive caterpillar removal from burlap-banded control trees as well as general caterpillar 'wrangling' and transport back to defoliation plots. Frass fall in traps correlated very closely with gypsy moth defoliation levels in all 16 treatment plots ($R^2 = 0.75$, $P < 0.01$; see Parry et al., 1998). Defoliation in these two plots was estimated at 30–40% in 1996 and 95–100% in 1997.

Leaf chemical analyses. Leaves (1997) for chemical analyses were collected June 28 and August 8, 1997. Each plot and subplot had 14 leaves sampled from upper crown branches using pole pruners. Leaves were immediately flash frozen in liquid nitrogen and transported in ice chests from the field. A methanolic extract of phenolics was quantified using a Folin-Denis procedure and a Rapid–Flow Analyzer. Condensed tannins were quantified using a modified sulfuric acid procedure (J. N. Nitao et al., unpublished) on the same equipment and are included in the total phenolics.

Larval rearing and bioassays. Larval bioassays were conducted in individual Petri dishes (150 × 25 mm) with a Poplar leaf from one of the 4 treatments supported in a water-filled aqua-pic in a controlled environment chamber at 25 °C and an 18:6 photoperiod. Neonate larval survival was recorded at 6 days. Later instars (4th and 5th) were reared on black cherry (a favorite common host for both *P. canadensis* and *P. glaucus*) until the experimental treatment leaves were introduced to freshly molted caterpillars. Standard gravimetric techniques were used to assess rates and efficiencies of larval consumption and growth for 72 h. Virtually identical size (weight) larvae were used to avoid some of the potential gravimetric technique problems described by Simpson & Raubenheimer (1995) and Scriber (1996b). Hybrids and backcrosses were obtained by hand-pairing lab-reared adult females with lab or field males. *Papilio glaucus* were obtained from Georgia (Clarke Co.) and *P. canadensis* from northern Michigan (Cheboygan, Charlevoix, and Emmet Counties). Oviposition was achieved as described by Scriber (1993).

Results

Pure *Papilio canadensis* larvae fed leaves from the 4 treatment plots in mid-July (3 weeks after peak defoliation by gypsy moths) showed no significant treatment differences in relative growth rates (RGR = $mg \times mg^{-1} \times \text{day}^{-1}$) for either the penultimate (4th; Figure 1) or final (5th) instars. In contrast 4th instar offspring larvae of *P. glaucus* × *P. canadensis* exhibited poor growth (RGR = 0.085) and the highest 3-day mortality (66%) on July 4 leaves from the Defoliated/Unfertilized (gm^+, f^-) plots (Figure 2). In neonate studies, none of the *P. glaucus* larvae survived. There was a significant defoliation effect detected in F1 hybrid larvae ($F = 7.3$, $P = 0.05$), but not pure *canadensis* which were bioassayed simultaneously ($F = 1.7$, $P = 0.26$). Unlike the hybrids, these *P. canadensis* larvae showed 100% survival on all treatments. In mid-August, 6–7 weeks after defoliation, backcross progeny (Pc × Pg) × Pg showed significant reduction in growth on the two defoliated treatments compared to the two control plots for both penultimate and final instars (Figure 3 penultimate instar $F = 8.5$, $P = 0.04$: Figure 4 final instar $F = 42.6$, $P = 0.003$). Defoliation caused a significant elevation in the levels of total phenolics ($F_{1,3} = 59.6$; $P < 0.005$) and condensed tannins ($F_{1,3} = 23.4$; $P < 0.02$). Fertilizer had neither a direct nor interactive effect on phenolics or total foliar tannins (Parry et al.,1998). At the time near peak defoliation (July 3, 1997) relative growth rates of 4th instar hybrids are negatively correlated with total foliar phenolics ($R^2 = 0.808$). However, at 6–7 weeks post-defoliation (August 11, 1997) the growth of backcross larvae in the different plots was positively correlated with total foliar phenolics with the lowest phenolic levels in the defoliated plots (penultimate instar $R^2 = 0.567$; final instar $R^2 = 0.787$; Figure 5).

Discussion

Severe defoliation of hybrid poplars by gypsy moths negatively altered the suitability of regrowth leaves, especially for hybrid and backcross *Papilio* genotypes. This held true early in the season (July 3 just after peak plot defoliation) and at 6–7 weeks post-defoliation (mid-August). All of the pure *Papilio glaucus* larvae (Salicaceae-unadapted) died on all poplar treatment leaves. In contrast, essentially all of the Salicaceae-adapted *P. canadensis* larvae survived and showed little, if any, suppression of growth rates on leaves of any of the 4 defoliation/fertilizer treatment plots. Reduced survival and growth of hybrids in early July correlated negatively with total phenolic concentrations (suggesting that leaves from the defoliated plots may be poorer for this reason). However, this would not explain our observations that later in the season (mid-August) the hybrid backcrosses grew the best on the undefoliated (control) plot leaves which had the highest levels of total phenolics (Figure 5). Leaf water content of leaves did not vary among treatments from mid-July to mid-September. However, immediately after regrowth (July 3, 1997) the defoliated plot leaves had higher water contents (80–82%, compared to 70–72% in the control plots). It is interesting that the fastest larval growth of hybrids at this time was on the undefoliated plot leaves in spite of their lower water content. We suspect that differences in nitrogen content of leaves or specific phenolic glycosides such as tremulacin (Ayres et al., 1997) may largely explain the *Papilio* backcross larval growth rate suppression on the defoliated (with lower total leaf phenolics) versus the undefoliated controls, however we have not yet completed these analyses.

Acknowledgements

This project was supported by the NSF (LTER, BSR-8702332), (Ecosystems, DEB-9510044) and the REU Program, as well as the MAES, College of Agriculture and Natural Resources, College of Natural Sciences of Michigan State University. We thank Kevin Kosola for extensive help in the field plots. We also thank those helping with the gypsy moth defoliation, field plots, and lab studies: Mark Deering, Joshua Edwards, Carrie Fetzner, Piera Giroux, Heather Govenor, Gabe Ording, Christina Schoen, and Aram Stump. We are grateful for help with the chemical analyses from: Bruce Birr, Bill Mattson, Muralee Nair, and James Nitao.

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