Pupation Site Selection and Enemy Avoidance in the Introduced Pine Sawfy (*Diprion similis*)

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Abstract - Insects that pupate on the branches of trees and shrubs suffer mortality from both predators and parasitic wasps. Which natural enemy represents the greater threat and therefore the stronger selection force on pupation site selection depends upon the time of year, the relative abundance of predators versus parasitoids, and the availability of alternative prey or hosts. Predation by foraging birds and mammals is likely to occur most commonly in winter when leaves have fallen, cocoons are conspicuous, and higher quality prey are scarcer. Inaccessibility and crypsis of pupation sites may provide protection from visually hunting predators. Attacks by parasitic wasps, which take place only during warmer months, may not be as easily avoided by inaccessibility or crypsis. We studied the patterns and mortality risks of pupation site selection in *Diprion similis* (Hymenoptera: Diprionidae; Introduced Pine Sawły). Cocoons that were smaller than average and situated in relatively inaccessible sites (thinner branches, underside of branches) were less likely to be attacked by predators; background matching in terms of branch size proved not to improve survival. In contrast, the probability that a cocoon would be attacked by parasitic wasps (primarily *Monodontomerus dentipes*; Hymenoptera: Torymidae) was unaffected by location along branches, indicating that parasitoids are more difŁcult to escape through pupation site selection. Because Pine Sawłies were twice as likely to be killed by predators than by parasitoids during the cocoon stage, inaccessibility may be the most important factor for selecting pupation sites.

Introduction

Predators and parasites can regulate the population size of herbivorous insects, influence their population structure, and act as a selective force on the evolution of morphology, behavior, and life-history traits (Bernays and Graham 1988,

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few insect species in which it has been studied (e.g., Limacodidae [slug caterpillars]; Murph

shrubs (Wilson 1966). Cocoons remain Łrmly attached to the branches where they

we haphazardly selected a typical-sized individual of each species. All branches and twigs were cut from these 4 trees and laid end-to-end. The diameter of available

were much lower in 2016 than in 2000 or 2007. In 2007, we determined the density of trees and shrubs within 10 m x 10 m quadrats beneath White Pines to test whether Pine Sawłies selected deciduous versus coniferous plants randomly with respect to their availability as pupation sites. In the 2007 pilot study, a smaller sample of Pine Sawłies and their parasitoids were reared from cocoons after storing them in plastic bags kept indoors under conditions similar to our 2016 experiment.

Statistical analyses

We performed a series of binary logistic regressions to estimate the effects of all measured variables (categorical as well as continuous) on the success or failure of cocoons (IBM SPSS 2015). We did not include intact cocoons in the regression analysis because a portion of those were likely parasitized, with the parasitoid having not yet emerged. Microhabitat variables that were not normally distributed were log-transformed. Models were run on different combinations of variables, and we selected the łnal model based on the percentage of cases that were correctly classiłed and how much variation in success could be explained by the model using the Nagelkerke R^2 value. We dropped from the model variables with *P*-values greater than 0.05 or those that contributed little to the model (as indicated by no increase in Nagelkerke R^2 values). Rejected variables included the species and DBH of the tree or shrub on which the cocoon was found; distance of the pupation site from the trunk and from the branch tip; and diameter of the branch at its base. Using a simpler dataset from 2007, we repeated the same analyses. We performed additional statistical tests using SPSS and R (R Core Team 2015). Descriptive results are given as means ± 1 SD.

Results

Pine Sawły cocoons were found on 20 different shrub and tree species beneath White Pines. The seven most common species are listed in Table 1. Deciduous woody plants were preferred over conifers for pupation sites, based on quadrat surveys in 2007 ($\chi^2(1) = 6.99$, $P = 0.008$, $n = 520$ deciduous shrubs and trees and 142 conifers examined for cocoons). Within individual plants, observed pupation sites were a non-random subset of available sites with respect to branch diameter within 3 of the 4 plant species where cocoons were most commonly found, and marginally non-random for a fourth (Red Maple, Red Oak, White Pine: $P < 0.01$; Black Cherry; $P = 0.055$; Fig. 2). Larval Pine Sawłies preferred to spin their cocoons on the bottom of branches (32%) and in the forks of twigs (32%) ($n = 611$).

The frequency of successful eclosion (28%) was approximately equal to that of predation (31%), whereas parasitism (17%) was less common. One quarter of the cocoons that we found were intact ($n = 611$). There was high variability in most of the quantitative characteristics of the cocoonsø microhabitat (Table 1).

The best logistic regression model used microhabitat features to explain 22.1% of the variance in success of cocoons (Nagelkerke R^2; $\chi^2(9) = 81.63$, $P < 0.001$; Table 2). The model correctly classiłed 86.0% of unsuccessful outcomes but only 48.8% of successful eclosions (overall classiłcation rate = 72.3%). Signiłcant

predictors of success were small cocoon diameter, thin branches where cocoons were situated, and cocoons positioned on the underside of branches. No other variables significantly influenced success (Table 2). We repeated the logistic regression model to identify important risk factors separately for parasitism versus predation. Although the model focusing on parasitism was significant overall (Nagelkerke R^2 = 0.14; $^2(10) = 19.79$, $P = 0.031$), no individual variable was a significant predictor of success (all $P > 0.08$). In the model focusing on predation by vertebrates, cocoons

Table 1. Mean characteristics of Pine Sawfly cocoons and their pupation sites and frequency by plant species ($n = 611$).

Trait	Mean ± SD
Cocoon diameter (mm)	4.38 Ô 0.50
Branch diameter at pupation site (mm)	3.91 Ô 3.72
Branch diameter at branch base (mm)	10.13 Ô 9.96
Cocoon height above ground (m)	1.38 Ô 0.60
Cocoon mass (g)	0.03 Ô 0.02
Distance from pupation site to branch tip (cm)	21.36 Ô 20.16
Distance from pupation site to trunk (cm)	64.12 Ô 79.92

Plant species selected for pupation sites	No. cocoons (%)
Acer rubrum L. (Red Maple)	166 (27.2%)
Quercus rubra L. (Northern Red Oak)	127 (20.8%)
Prunus serotina Ehrh. (Black Cherry)	99 (16.2%)
Pinus strobus L. (Eastern White Pine)	42 (6.9%)
Fagus grandifolia Ehrh. (American Beech)	35 (5.7 %)
Ilex verticillata (L.) A. Gray (Winterberry Holly)	33 (5.4%)
Betula papyrifera Marshall (White Birch)	23 (3.8%)
Other species ($n = 13$)	86 (14.0%)

Table 2. Binary logistic regression estimating the effect of 6 variables on success (survival) or failure (mortality) of Pine Sawfly cocoons. The probability of success was higher when the cocoon was smaller, when the diameter of the branch at the pupation site was small, and when the cocoons were positioned on the bottom of branches (versus top, in a fork, or on the trunk; bottom = reference). No other variables included in the model significantly influenced success.

							95% C.I. for Exp(B)	
Variable	B	S.E.	Wald	df	*P*	Exp(B)	Lower	Upper
Pupation site diameter	-0.330	0.070	21.960	1	<0.001	0.719	0.626	0.825
Cocoon diameter	-0.623	0.222	7.840	1	0.005	0.536	0.347	0.830
Position (bottom)	-	-	12.411	4	0.015	-	-	-
Position (side)	0.237	0.529	0.201	1	0.654	1.268	0.450	3.573
Position (top)	-0.180	0.614	0.086	1	0.770	0.836	0.251	2.786
Position (fork)	-0.158	0.532	0.089	1	0.766	0.853	0.301	2.422
Position (trunk)	-0.742	0.528	1.980	1	0.159	0.476	0.169	1.339
Cocoon height	-0.083	0.057	2.111	1	0.146	0.920	0.823	1.029
Log(distance from trunk + 1)	-0.371	0.244	2.311	1	0.128	0.690	0.428	1.113
DBH of pine	0.005	0.006	0.620	1	0.431	1.005	0.992	1.018
Constant	4.035	1.128	12.785	1	<0.001	56.539		

were signiŁcantly more likely to survive if they were small in diameter, on thin branches, and on the bottom or side of branches, as found in the overall model (Nagelkerke $R^2 = 0.25$; $^2(10) = 53.52$, $P < 0.001$). Although we could not measure
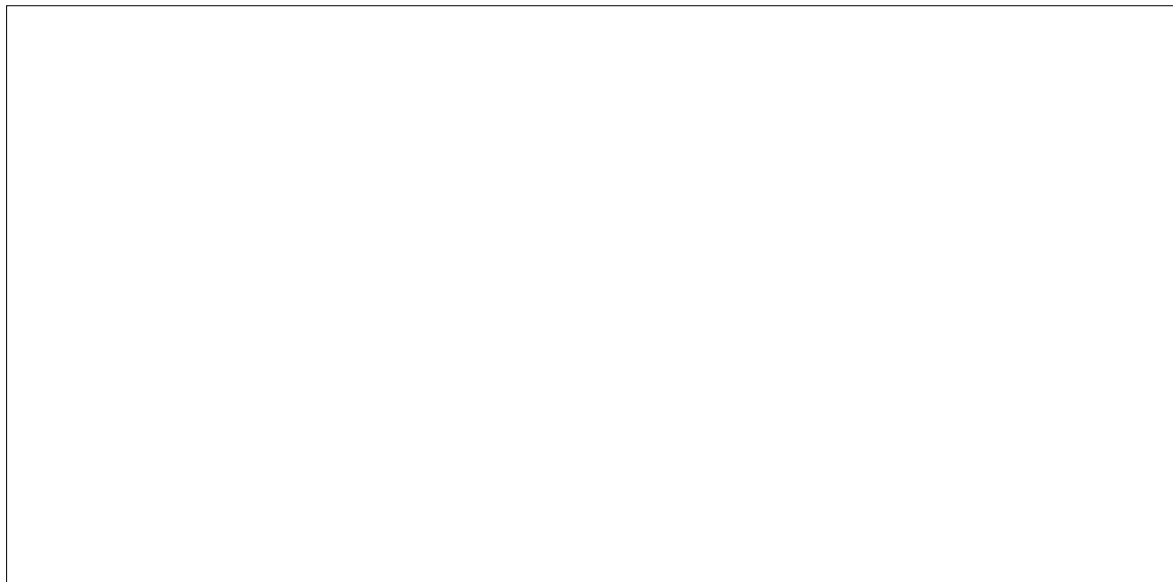
proved not to be significant. Although the model correctly classified 98.1% of successful cases (representing 79% of 138 cocoons), it misclassified 89.7% of unsuccessful cases (Nagelkerke $R^2 = 0.048$; $^2(5) = 4.28$, $P = 0.51$).

Cocoons from which Pine Sawflies eclosed were significantly smaller than cocoons that were parasitized ($P = 0.002$) or preyed upon ($P < 0.001$). There were no differences in size, however, as a function of the source of mortality ($P > 0.05$). Cocoons of female Pine Sawflies collected in 2007 were larger in diameter on average than those of males (4.8 ± 1.8 mm vs. 4.0 ± 2.2 mm; *t*-test: $P < 0.001$, $n = 5$ females and 5 males). Female mass was nearly triple that of males (F vs. M: 31 Õ 18 mg vs. 9 ± 4 mg; *t*-test: $P = 0.03$). These results suggest that rates of predation and parasitism may be higher for female than male Pine Sawflies.

Pine Sawflies that spun large cocoons tended to choose pupation sites of larger diameter (Spearman Rank test: $r_s = 0.26$, $P < 0.001$). Nonetheless, cocoons and pupation sites were not closely matched in size (Fig. 3). Branch diameters at pupation sites averaged 0.47 mm smaller than cocoon diameters. In accordance with the results of the logistic regression, pupation site diameter differed among fates of cocoons (Kruskal-Wallis tests: $P < 0.001$). Cocoons that had been preyed upon
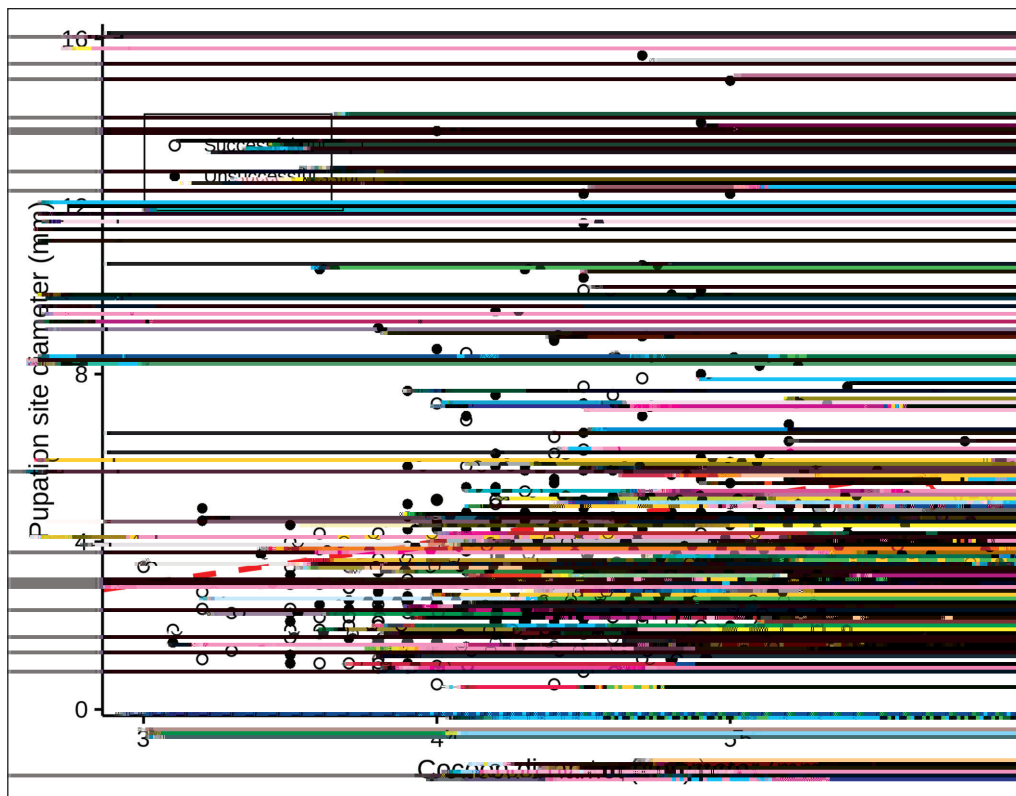
were found on branches of larger diameter than parasitized or successful cocoons (adjusted pairwise comparisons: both $P < 0.001$). Parasitized cocoons were also found at signi�Łcantly larger-diameter sites than cocoons from which a Pine Sawﬂy had successfully emerged ($P = 0.004$). Of the cocoons that were smaller in diameter than their pupation site (i.e., above the line of equality in Fig. 3), 80.5 % failed ($n = 118$), compared to 57.4% of cocoons that were larger in diameter than their pupation site ($n = 345$; $x^2(1) = 19.24$, $P < 0.001$).

determine the cause of death of the remaining larvae and pupae, although 14 were

diameters. Cocoons tucked in the forks of branches often seemed to the human eye to be particularly cryptic, yet that location was associated with the highest predation rates. Birds and small mammals may develop a search image for such locations or Łnd it easier to attack the tough cocoons in a well-anchored fork of a branch. Our study did not record mortality of cocoons that might have been removed entirely from branches.

Although we observed variation in population density of Pine Sawflies between years, sources of mortality in 3 different years remained generally consistent. Parasitoids are a major source of mortality for Pine Sawflies and may regulate their population sizes (cf. Herz and Heitland 1999). However, the selection of pupation sites evident in this study, especially the preference for small diameter branches near the tip, appear to be shaped mainly by predation by birds and possibly small mammals.

Unlike Murphy and Lill (2010), we found that small cocoons were more successful than large ones. Smaller cocoons may be difŁcult to attack or may not be worth the energetic investment of pecking or biting through them to get at the pupa. Alternatively, the larger cocoons of female Pine Sawłies may make them a bigger or more attractive target for natural enemies, resulting in sex-speciŁc mortality (cf. Ercit 2014). Depending upon the intensity of predation and the reproductive value of targeted individuals, sex-selective predation could have a disproportionate effect on population dynamics (see Hoy et al. 2015) and potentially favor sex-speciŁc strategies in pupation site selection in Pine Sawłies.

Acknowledgments