

Predation and avoidance of tough leaves by aquatic larvae of the moth *Parapoynx rugosalis* (Lepidoptera: Pyralidae)

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Abstract. 1. Aquatic larvae of the pyralid moth *Parapoynx rugosalis* Möscher repeatedly construct protective cases by cutting portions (discs) from leaves of the waterlily *Nymphaea ampla* and sandwich themselves between the disc and the underside of the host leaf. Construction of a new case requires leaving the old case, thus increasing exposure to predators and parasites.

2. In an experiment, larvae with protective cases experienced no mortality due to predation by fish, whereas larvae without cases experienced substantial predation.

3. In a series of choice tests, larvae preferentially selected young, tender leaves over old, tough leaves for construction of cases, and larvae spent significantly less time completing their shelters when cutting discs from young, tender leaves.

4. A partial explanation of why larvae select young, tender leaves for construction of their protective shelters may be that exposure time to predators during construction is minimized.

5. The same mechanism linking preferences for tender leaves to reduced exposure to predators during construction may also apply to other insect herbivores exhibiting leaf-rolling or case-building behaviour.

Key words. Leaf age, leaf toughness, herbivory, *Nymphaea ampla*, *Parapoynx rugosalis*, predation.

Introduction

Most herbivorous insects attack only certain age-classes of leaves (Raupp & Denno, 1983), generally preferring to feed on young leaves over mature ones (Cates, 1980; Coley, 1983). Several factors may account for the attractiveness of young foliage as a food source. First, mature leaves may be better defended, both chemically (e.g. higher concentrations of secondary compounds) or structurally (e.g. greater toughness because of greater lignification) (Feeny, 1970; but see Coley, 1983, Coley & Aide, 1991). Second, young leaves are generally more nutritious than older leaves (Scriber & Feeny, 1979; Mattson, 1980; Scriber & Slansky, 1981; Coley & Aide, 1991; Slansky,

1993), thereby accelerating growth rates of herbivores feeding on young foliage. Increased growth rates, in turn, have been thought to reduce mortality due to predation and parasites (Moran & Hamilton, 1980; Price *et al.*, 1980). Preference for younger leaves may therefore represent an adaptive response by herbivores to decreased developmental times and thus reduce exposure to natural enemies (Stamp & Bowers, 1990; Montllor & Bernays, 1993; but see Damman, 1987).

We suggest here an additional mechanism linking preferences for younger leaves to reduced exposure to natural enemies. In insect species that construct protective shelters from leaves of their host plant (e.g. leaf rollers or case builders), toughness of older leaves may greatly increase the time necessary to complete a shelter. Selecting young leaves will therefore minimize construction time and thus minimize exposure to predators and parasites. This problem is expected to be exacerbated (i) during early

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development because small larvae are less capable of manipulating tough leaves (Reavey, 1993); and (ii) in species that build several new shelters during development, thereby repeatedly exposing themselves to natural enemies.

To test the proposed link between leaf toughness and exposure to predators, we conducted a series of experiments with larvae of the case-building aquatic moth *Parapoynx rugosalis* Möscher (Lepidoptera, Pyralidae, Nymphulinae). As in most genera of the Nymphulinae, development from egg to adult proceeds entirely in a subaquatic environment (Packard, 1884; Welch, 1916; Berg, 1950; McGaha, 1954; Lange, 1956; Scoble, 1992). Larvae feed on aquatic plants and respire through gills or functional spiracles. Each larva cuts a single leaf disc from its host plants to serve as a protective case or 'tent'. A tent is typically attached to the lower surface of floating vegetation, with the larva sandwiched in between its tent and the leaf of the host plant. The size of a tent is dependent on larval size. As the larva grows, it will repeatedly cut a new, larger, disc and discard the old one. When cutting a new disc, a larva leaves its old case (Welch, 1916; personal observation), thus exposing itself to aquatic predators (Welch, 1916) and parasites (Resh & Jamieson, 1988).

To determine the role of leaf toughness in tent-making behaviour and its indirect effect on predator exposure, we first tested the hypothesis that tents function as a refuge from predators (Welch, 1916) by comparing predation on larvae with and without tents. In a second experiment we examined (i) the predicted positive relationship between leaf toughness and disc cutting time (and indirectly predator exposure), and (ii) the predicted negative relationship between larval size and disc cutting time. Lastly, we investigated the ability of larvae to discriminate between leaves that differ in toughness, predicting that larvae prefer to cut protective tents from those leaves that allow rapid tent construction and thus minimize predator exposure.

Methods

We studied larvae of the pyralid moth *Parapoynx rugosalis* Möscher from 1 to 4 February 1990 at Palo Verde marsh, Guanacaste, Costa Rica. The marsh is temporal and usually dries out in March, towards the end of the dry season. During most rainy seasons the marsh covers about 60 hectares, with a depth from 30 to 100 cm. Voucher specimens of *P. rugosalis* are deposited at the National Museum of Natural History, Smithsonian Institution, Washington D.C.

All larvae used in the experiments were collected at Palo Verde marsh from larvae of the waterlily *Nymphaea ampla* (Nymphaeaceae). Larvae were kept on their leaves in plastic buckets until the beginning of the experiments (within 24 h after collection). Each larva was used for only one experiment. The general experimental procedure consisted of placing a single larva in a covered petri dish with water and pieces of leaves of *N. ampla*. To select

leaves of different toughness, we categorized leaves into three size classes (small = <10 cm; medium = 10–20 cm; large = 20–30 cm length along midrib) and used leaf size as an initial predictor of leaf toughness. In *N. ampla*, leaf toughness increases with leaf length (Spearman $r = 0.80$, $P < 0.0001$, $n = 50$) and thus presumably with leaf age. Toughness of each experimental leaf was recorded after experimentation by averaging three measurements (from the blade, not the midrib) taken with a penetrometer to the nearest 5 g/8 mm².

Tent construction and predation. The protective function of tents was tested by exposing larvae with and without tents to predatory fish. Ten larvae on small leaves of *N. ampla* were placed into each of three round plastic bowls (24 cm diameter, 8 cm high). In each bowl, five larvae were allowed to remain enclosed in their tents which were attached to the underside of leaves, while the remaining five larvae were extricated from their tents and placed on the underside of the same leaves. Three guppies (*Poecilia gillii*) and one shiner (*Astynax fasciatus*) from a pond near Palo Verde marsh (about 1 km distance) were then added to each of the bowls. Survival rates of larvae with and without tents in each of the three bowls were scored after 24 h and compared by paired *t*-test analysis after arc-sine transformation of survival rates.

Leaf toughness and disc cutting behaviour. To determine if leaf toughness affected (i) the propensity of larvae to cut discs, and (ii) the time needed to cut discs, we placed individual larvae in petri dishes with one section (10–15 cm²) of a leaf cut from either a small leaf (<10 cm length), medium leaf (10–20 cm length) or large leaf (20–30 cm length). We first matched twenty-four quadruplets of larvae for size. Within each quadruplet, two were randomly allocated to the small leaf condition, and one each to the medium and large leaf conditions. Each larva was placed singly into its petri dish; thus the number of replicates in the small leaf condition ($n = 48$) was twice of that in each of the other two conditions ($n = 24$). Larvae were observed continuously for 4 h between 7 and 11 a.m. We recorded whether larvae successfully cut a tent, and, if so, the time needed by a larva to complete the cut. After testing, we measured the toughness of all experimental leaf sections as described above, as well as larva size. The size of larvae (width of head capsule) was measured to the nearest 0.1 mm using a microscope fitted with an ocular micrometer. In a multiple regression analysis, cutting time was regressed on the two independent variables of leaf toughness and larval size. Cutting time was transformed logarithmically to eliminate heteroscedasticity.

Leaf toughness and larval preferences. We conducted leaf choice experiments to determine if larvae preferred tender leaves. Individual larvae were placed in petri dishes with a choice of three 10 cm² sections cut from large, medium and small leaves. 24 h later we recorded the leaf type from which larvae had cut discs, and measured leaf toughness on all experimental sections following the procedure outlined above. The number of sections from small, medium and large leaves used by larvae for tent-making was compared using chi-square analysis, the null

hypothesis being that the tendency to cut discs is not affected by leaf toughness.

Results and Discussion

Tent construction and predation

Predation differed significantly between protected larvae with tents versus unprotected larvae without tents. All of the protected larvae survived the 24 h of exposure to predatory fish while ten of the fifteen unprotected larvae were preyed upon (two-tailed paired $t = 17.5$, $df = 2$, $P < 0.005$). Of the five surviving larvae originally without a protective tent, three escaped predation because they had managed to construct tents. Possession of a tent therefore reduces larval mortality due to predatory fish.

Leaf toughness and disc cutting behaviour

A significantly greater proportion of larvae completed discs within 4 h when given a leaf section cut from a small leaf than from a medium-sized or large leaf (chi-square = 11.1, $df = 2$, $P < 0.005$). Specifically, 58.3%, 20.8% and 8.3% of the larvae completed discs on small, medium and large leaves, corresponding to leaf toughnesses of 55.8 ± 25.19 , 94.7 ± 28.06 and 128.9 ± 43.25 g/mm², respectively. Therefore time to completion of tent construction is associated negatively with leaf toughness. In some cases, larvae attempted cutting a disc from medium or large leaves, but gave up midway, left the partial cut, and moved to a different area of the leaf section. Such partial cuts are not uncommon on leaves of *N. ampla* at Palo Verde marsh.

In a multiple regression analysis, both leaf toughness and larval size were significant predictors of the time necessary to complete a cut (adjusted multiple $R^2 = 0.38$, $F = 10.68$, $df = 2/30$, $P < 0.0001$; Fig. 1). As predicted, leaf toughness showed a positive relationship (partial $t = 4.01$, $P = 0.0004$), whereas larval size showed a negative relationship (partial $t = -2.66$, $P = 0.012$) with cutting time. Thus, protective shelters are completed faster from tender leaves and by larger larvae.

Leaf toughness and larval preference

Twenty-nine of fifty larvae presented with choices of leaf sections from small, medium and large leaves completed cutting a disc for tent construction within 24 h. Of these twenty-nine larvae, twenty-three (79.3%) chose to cut discs from the small leaf sections, while three larvae each chose to cut discs from medium (10.3%) and large (10.3%) leaf sections. Thus, larvae preferred to cut discs from small leaves over medium and large leaves (chi-square = 27.6, $df = 2$, $P < 0.001$). This preference for sections from small leaves corresponds to larval preferences for leaves of least toughness. The average toughness of the leaves presented in the choice test was 42.7 ± 23.14 ,

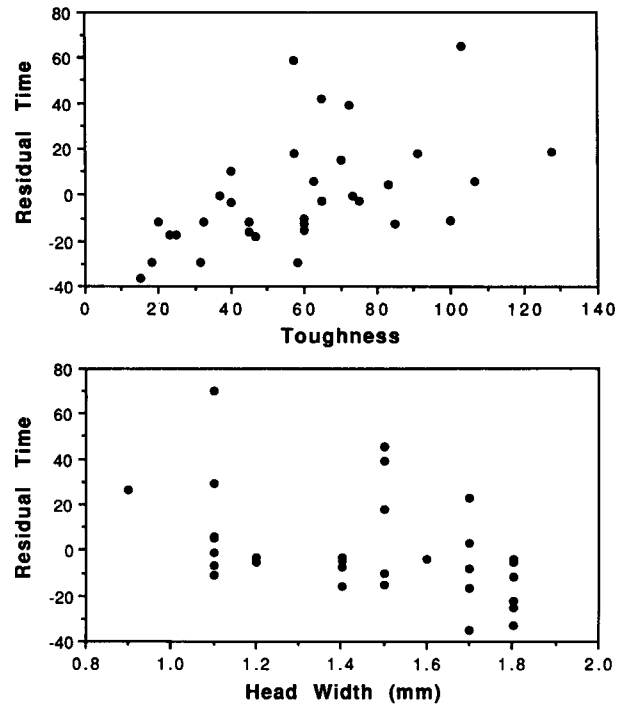


Fig. 1. Partial correlations between the time (seconds) required by larvae to complete cutting a tent-disc as a function of leaf toughness (g/mm²) and larval head width (mm). Residual time = time residuals after controlling for the covariates of leaf toughness (bottom) and larval head width (top) in a multiple regression. Both leaf toughness (partial $t = 4.01$, $P = 0.0004$) and larval head width (partial $t = -2.66$, $P = 0.012$) were significant predictors of cutting time.

78.2 ± 24.01 and 110.6 ± 49.03 g/mm² for the sections from small, medium and large leaves, respectively. Larval preference for tender leaves is further substantiated by the fact that, of the twenty-nine larvae that completed tent construction, twenty-six larvae selected the leaf section of least toughness (independent of the original size of the leaf from which the section was cut); the remaining three larvae selected the section with second least toughness. Larvae are therefore able to discriminate among leaves differing in toughness and preferentially select tender leaves for tent construction. Interestingly, larvae of *P. rugosalis* are found predominantly on small leaves at Palo Verde marsh (personal observation). Predominance on small leaves may be due to larval choice as they move between leaves, as suggested by the experiments, or due to female preferences for small leaves as oviposition sites.

Conclusion

Larvae of *P. rugosalis* preferred tender leaves over tough leaves for case-building. We argue that a partial explanation for this preference may be the decrease in the time larvae expose themselves to predators during tent construction, because protective shelters can be con-

structed faster from tender leaves. In a series of experiments, the use of more tender (and thus presumably younger) leaves enabled larvae to complete construction of protective shelters in significantly shorter time than when using tougher, older leaves (Fig. 1). This resulted in faster acquisition of protective shelters against aquatic predators. In a laboratory experiment, larvae experienced no predation when encased in protective shelters, but substantial predation when without shelters. Use of tender leaves and rapid construction of a protective tent is imperative whenever larvae discard old cases and expose themselves to predators while cutting a new case (Welch, 1916). Small larvae appear particularly vulnerable at such times, because they require significantly longer time for case making than larger larvae. A similar mechanism linking preferences for tender leaves to reduced predation may also apply to other insect herbivores exhibiting leaf rolling or case-building behaviour.

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