

Responses of invertebrate herbivores to stinging trichomes of *Urtica dioica* and *Laportea canadensis*

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We investigated whether stinging trichomes of two plant species, *Urtica dioica* and *Laportea canadensis*, are effective defenses against four species of invertebrate herbivores (*Vanessa atalanta*, *Popillia japonica*, *Chortophaga viridifasciata* and *Anguispira alternata*). Feeding was compared on leaf pieces of contrasting stinging trichome density, in petri-dish feeding trials. In addition, a test of snail movement over hairy and shaved *L. canadensis* stems was carried out. In no case was there significant evidence that stinging trichomes deter or interfere with feeding by these herbivores. Factors of body size and feeding behavior allow them to feed with little interference from nettle stings. Stinging trichomes are known to be effective against mammalian herbivores, and are well-suited to deterrence of large grazers. We therefore hypothesize that stinging has evolved as a defense against mammalian herbivory.

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Plants bearing stinging trichomes are known as “nettles”, and occur in the families Urticaceae, Euphorbiaceae, Loasaceae, and Hydrophyllaceae (Thurston and Lersten 1969). Experiments have shown that stings of *Urtica dioica* deter feeding by mammals, which preferentially eat intraspecific variants with fewer trichomes (Pollard and Briggs 1984). In populations under intense mammalian grazing, plants have more stinging trichomes than in lightly grazed areas (Pollard 1986, Pullin and Gilbert 1989). These findings suggest that stinging could evolve as a defense mechanism under natural selection imposed by grazing mammals (Geltman 1992, Pollard 1992).

Although non-stinging hairs often reduce insect herbivory (Levin 1973, Stipanovic 1983), the role of stinging trichomes in defense against invertebrates is less clear. In *Wigandia urens* (Hydrophyllaceae), Cano-Santana and Oyama (1992a) found that leaves with stinging trichomes suffer as much or more insect damage as those without. *U. dioica* is a common food of snails and slugs

(Grime et al. 1968, 1970, Pallant 1969, Mason 1970, Wolda et al. 1971, Cates and Orians 1975), and attracts many insects (Davis 1973, 1983). Fiercely stinging Australian Urticaceae in the genus *Dendrocnide* (formerly included in *Laportea*) experience high levels of insect herbivory (Lowman and Box 1983, Lowman 1985, Southwood 1986, Hawkeswood 1991).

Stinging trichomes consist of an elongate cell, 1 to 8 mm long, atop a multicellular pedestal (Thurston 1974). Contact with a hair breaks off its brittle tip, leaving a beveled point that can penetrate mammalian skin, injecting an irritating fluid. The stinging chemicals include histamine, acetylcholine and serotonin in *U. dioica* (Emmelin and Feldberg 1947, Collier and Chesher 1956), but other compounds may also be important (Leung et al. 1986, Czarnetzki et al. 1990).

Demonstrating the defensive action of a substance or structure can be difficult, as can determining whether a defense has evolved under selection imposed by herbivory, i.e. as an adaptive defense (Pollard 1992). Food-

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plant preferences in the wild and comparisons between plant species in feeding trials are problematic in this regard, as the attribute in question is only one character among many that differ between species. Comparisons of herbivore preferences for intraspecific plant variants, differing in levels of the putative defense, provide a powerful and evolutionarily relevant means of assessing defensive function (Jones 1971, Pollard 1992).

We attempted to determine whether nettle stings defend against invertebrates, by investigating herbivore responses to variation in numbers of stinging trichomes on plant surfaces. Defenses can operate by affecting either feeding rates or feeding choices, i.e. by interference or deterrence. The interference hypothesis predicts negative correlation between number of stinging trichomes on a plant part and the amount of feeding on it. The deterrence hypothesis differs slightly, predicting that feeding depends on the relative stinging trichome density among available food items. We test both predictions, and also the hypothesis that movement of snails on nettle stems is hindered by stinging trichomes.

Materials and methods

Plant and herbivore species

Urtica dioica L. ssp. *dioica* (stinging nettle), native to Europe, is an introduced weed in parts of North America. Though uncommon in the south-east, it is established on the Biltmore Estate, Buncombe County, North Carolina (35°35'N, 82°27'W; probably its most southerly large population – Woodland et al. 1982), where our plants were collected. *Laportea canadensis* (L.) Wedd. (wood nettle) is native to moist areas in eastern North America. Plants for these studies were collected near Oil Camp Creek, Greenville Co., South Carolina (35°05'N, 82°36'W). In both species, stinging trichomes occur on all aerial parts of the plants except flowers and fruits.

Four herbivore species were tested, as follows:

Vanessa atalanta L., red admiral butterfly (Lepidoptera, Nymphalidae): Larvae were collected from *U. dioica* plants at Biltmore Estate. This species is native to

North America. Nettles, including *Urtica* and *Laportea*, are reported to be preferred food plants (we never observed it on *L. canadensis*).

Popillia japonica Newman, Japanese beetle (Coleoptera, Scarabaeidae): This polyphagous, introduced species was observed in the field feeding on both *U. dioica* and *L. canadensis*. However, insects for our experiments were caught in traps ("Bag-A-Bug," United Industries Corp., Jacksonville, FL, USA) in a vegetable garden with no nettles nearby, at Furman University, Greenville Co., SC (34°56'N, 82°26'W).

Chortophaga viridifasciata (De Geer), grasshopper (Orthoptera, Acrididae): Like most grasshoppers, this native species is reported to be a dietary generalist. It was never observed on nettles in our studies. Animals were captured with sweep nets in a field near Furman Univ.

Anguispira alternata (Say), terrestrial snail (Pulmonata, Stylommatophora): Most terrestrial snails are reported to be generalists. This common native species was collected on or near *Laportea canadensis* plants at the Oil Camp Creek site.

Herbivores were kept in aquaria with appropriate food (including nettle leaves for *V. atalanta*). Pre-trial food deprivation and experimental feeding periods are detailed in Table 1. If an individual animal was re-used, at least 24 h elapsed between trials.

Feeding trials

The experimental unit for feeding trials was a petri dish lined with moist filter paper (Whatman #1), containing three squares of leaf tissue, each approximately 2 cm × 2 cm, from a single plant species. The number of trichomes on the upper (adaxial) surface of each leaf square was counted. One herbivore was placed in the center of the dish. Before and after feeding, the area of each square was measured with a digital image analysis system ("Agvision", Decagon Devices, Pullman, WA); area consumed was determined by subtraction.

For *L. canadensis*, trichome densities were highest near the base of each leaf and declined toward the leaf

Table 1. Experimental protocols for feeding trials to examine the responses of invertebrate herbivores to stinging trichome variation in *Urtica* and *Laportea canadensis*.

Plant ¹	Herbivore ²	Pre-trial food deprivation (h)	Duration of trial (h)	Animal re-used?
U	V	0	0.25	yes
U	P	4	1.5	no
U	C	4	2.0	yes
U	A	24	1.5	yes
L	V	1	0.25	no
L	P	1	1.5	no
L	C	4	19.0	yes
L	A	24	17.0	yes

¹ Plants: U = *Urtica dioica*; L = *Laportea canadensis*.

² Herbivores: V = *Vanessa atalanta* (caterpillar); P = *Popillia japonica* (beetle); C = *Chortophaga viridifasciata* (grasshopper); A = *Anguispira alternata* (snail).

apex. The three leaf squares in each dish were taken from proximal, medial and distal portions of a single leaf. For *U. dioica*, the pattern of hair distribution was less predictable, but contrasting hair densities could be obtained among leaves on a single shoot. Thus, in each dish, the three leaf squares were assigned to relative stinging hair density categories designated as low, medium and high.

Data from each herbivore species on each plant species were analyzed separately, leading to eight sets of analyses. If there was no feeding on any of the three leaf squares, that replicate was discarded, on the basis that it indicated refusal to eat, unrelated to plant characteristics.

The interference hypothesis was tested by analyzing the correlation between hair density and area consumed from that square. The deterrence hypothesis was tested by comparing feeding on low, medium and high hair density categories using a one-way ANOVA with dishes as replicates. All analyses were performed using SPSS for Windows (release 6.0).

Tests of herbivore movement

We tested whether stinging trichomes interfere with snail movement, using paired leafless stems of *L. canadensis*, of similar diameter and hairiness, cut to identical length and suspended horizontally. All stinging trichomes were removed from one stem in each pair, using forceps and razor blade. One *A. alternata* was placed at the end of each, and the time required to traverse the stem was noted. The procedure was repeated 10 times. Each snail was tested once on a shaved stem and once on a hairy stem.

Results

In the field, damage by invertebrate herbivores was much more conspicuous on *L. canadensis* than on *U. dioica*. Invertebrates feeding on *L. canadensis* included four species of Lepidoptera (larvae), two species of Hemiptera, one species of Coleoptera, and one snail. Species feeding on *U. dioica* included five Lepidoptera, six Coleoptera, five Hemiptera, three Homoptera and three Orthoptera.

In no cases were there statistically significant correlations between trichome densities and leaf area removal by herbivores (Table 2). The only correlation approaching statistical significance was weakly positive ($r=0.16$, $P=0.07$, two-tailed), relating hair density on *U. dioica* and feeding by *V. atalanta* larvae.

ANOVA revealed highly significant differences in hairiness among the low, medium and high trichome density categories (Fig. 1); all pairwise differences were significant based on Scheffé tests. Averaged across the whole experiment, *U. dioica* leaf pieces were signifi-

Table 2. Results of correlation analyses comparing feeding by invertebrate herbivores (measured as leaf area removed) with stinging hair densities (hairs cm^{-2} , adaxial surface) on nettle leaf pieces. Probabilities are two-tailed.

Plant ¹	Herbivore ²	<i>n</i>	<i>r</i>	<i>P</i>
U	V	129	0.160	0.071
U	P	81	-0.057	0.616
U	C	90	-0.135	0.206
U	A	60	-0.165	0.207
L	V	36	0.051	0.767
L	P	93	-0.007	0.945
L	C	48	0.135	0.352
L	A	15	0.281	0.310

¹ Plants: U = *Urtica dioica*; L = *Laportea canadensis*.

² Herbivores: V = *Vanessa atalanta* (caterpillar); P = *Popillia japonica* (beetle); C = *Chortophaga viridifasciata* (grasshopper); A = *Anguispira alternata* (snail).

cantly more hairy than those of *L. canadensis* ($F_{1,550}=333.4$, $P<0.0001$). There were no significant differences in herbivore preference or deterrence among the three trichome densities in each dish (Fig. 2; Table 3).

Removal of trichomes did not increase the speed with which snails traversed stems (135s with vs 179s without trichomes; paired comparison $t=2.09$, 9 d.f., $P=0.07$, two-tailed). On stems with trichomes, the correlation between hair density and time to traverse the stem was not statistically significant ($r=0.44$, 9 d.f., $P=0.20$, two-tailed).

Discussion

We found no evidence for a defensive role of stinging trichomes against invertebrate herbivores. Herbivore choice, feeding rate and movement were not significantly related to stinging hair density, over the range of trichome densities tested. This was not due to a lack of statistical power: in cases approaching statistical significance, trends in the data were opposite those predicted under a hypothesis of defense (e.g. *Vanessa* on *Urtica*

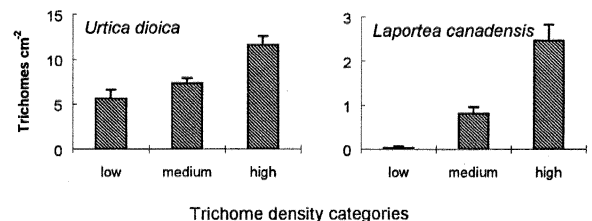


Fig. 1. Stinging trichome densities on upper (adaxial) surfaces of leaf pieces offered to herbivores grouped into three hair density classes per species. Data are averaged across all leaf pieces used in the experiments, for all four herbivores. Bars represent standard errors of means. $n = 120$ for *Urtica*; $n = 64$ for *Laportea*.

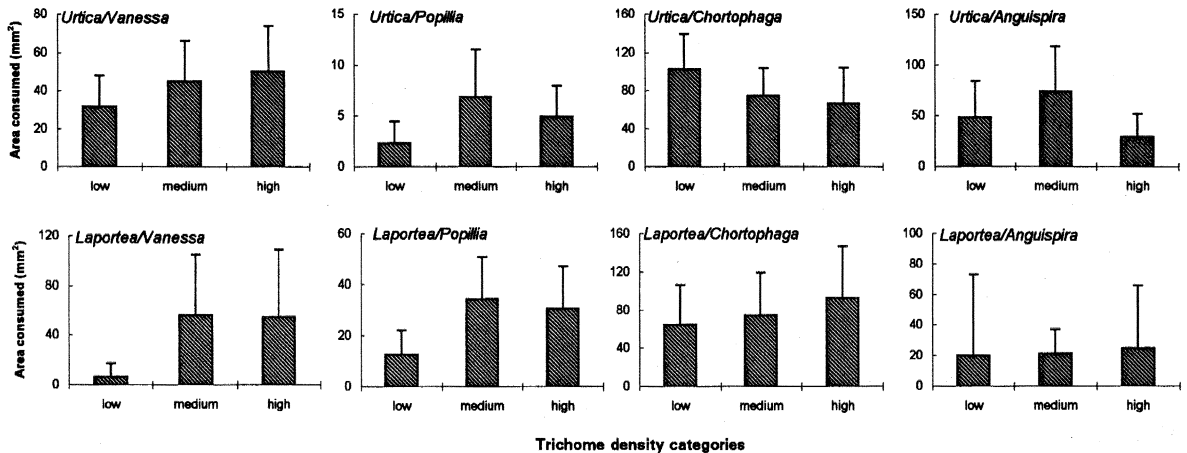


Fig. 2. Mean leaf area consumed by four species of herbivores feeding on two species of nettles, each subdivided into three stinging trichome density categories. Bars represent standard errors of means.

based on feeding rate, *Popillia* on *Laportea* based on preference, *Anguispira* on *Laportea* based on movement).

The phenotypic variation in trichome densities that we measured on North American *U. dioica* ssp. *dioica* was similar (based on upper leaf surfaces) to the range of genetic variation described within and among British populations and tested on mammalian herbivores by Pollard and Briggs (1982, 1984). Their studies also included a “stingless variant”, *U. dioica* var. *subinermis* (perhaps *U. galeopsifolia* – see Geltman 1992); nothing comparable to these stingless plants was tested in our present study. However, the results of Pollard and Briggs (1984) do show negative correlations between stinging hair density and mammalian grazing, even without the inclusion of the stingless plants.

Coevolution in plant-herbivore “arms races” can lead to situations where defense is undetectable because herbivores can resist or circumvent it (Rhoades 1979), par-

ticularly for specialist herbivores. In designing this study, we chose both native and introduced plants, and herbivores including native generalists (grasshoppers, snails), introduced generalists (Japanese beetles) and native specialists (red admiral larvae). To the extent that learning is important in circumventing a defense, we included herbivores that had not previously encountered nettles.

Cano-Santana and Oyama (1992b) studied feeding on *Wigandia urens* by Noctuid moth larvae. The herbivores were not given a choice between “bristly” and “smooth” leaves; however, the conclusion that stinging trichomes do not affect their behavior or performance is in agreement with our findings. Grime et al. (1970) studied snail movement on nettle stems, concluding that snails ascend stems of *U. dioica* because its “extraordinarily attractive interior” overcomes the deterrence of its “repulsive exterior”. Our findings agree that stinging trichomes are not an effective defense against snails. However, the as-

Table 3. Summaries of results of eight one-way ANOVA's, comparing feeding by invertebrate herbivores on nettle leaf pieces classed into three categories of stinging trichome density. In all analyses, d.f._{between} = 2.

Plant ¹	Herbivore ²	MS _{between}	d.f. _{error}	MS _{error}	F	P
U	V	0.3940	126	0.4513	0.8730	0.420
U	P	0.0146	78	0.0076	1.9104	0.155
U	C	1.0687	87	0.8666	1.2333	0.296
U	A	1.0166	57	0.5714	1.7791	0.178
L	V	0.9614	33	0.4523	2.1257	0.135
L	P	0.4258	90	0.1607	2.6500	0.076
L	C	0.3283	45	0.7786	0.4216	0.659
L	A	0.0033	12	0.1044	0.0319	0.969

¹ Plants: U = *Urtica dioica*; L = *Laportea canadensis*.

² Herbivores: V = *Vanessa atalanta* (caterpillar); P = *Popillia japonica* (beetle); C = *Chortophaga viridifasciata* (grasshopper); A = *Anguispira alternata* (snail).

sumption that stings are inherently repulsive may be unwarranted: snail movement may simply be unaffected by these structures.

Small size, low body mass, and (for hard-bodied insects) a rigid exoskeleton appear to prevent penetration by stinging trichomes. For most terrestrial invertebrates, nettle trichomes are large enough to walk around or climb over (Hawkeswood 1991), in contrast to smaller, non-stinging trichomes that deter insects on other pubescent plants. We observed caterpillars chewing off nettle trichomes at the base, as has been previously reported (Davis 1983, Dillon et al. 1983, Pollard and Briggs 1984). Caterpillars also ingested whole trichomes, and intact trichomes were found in their feces; Grime et al. (1970) report similar findings for snails. The mucus of snails probably helps to lubricate their passage across a hairy surface, and was seen to keep the hairs appressed to the plant surface after the snail had passed.

Behaviors such as these are likely explanations for our findings, that herbivore choice and feeding rate are unaffected by intraspecific variation in stinging trichome density. This implies that nettle stings, unlike other forms of pubescence, do not effectively defend plants against invertebrates. Stinging trichomes do effectively deter mammals, and their mode of action, involving instantaneous pain before significant quantities of plant material are consumed, is well suited to large, destructive, generalist grazers (Pollard 1992). Therefore, it seems likely that stinging trichomes represent a defensive adaptation against vertebrate herbivores, especially mammals.

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References

- Cano-Santana, Z. and Oyama, K. 1992a. Variation in leaf trichomes and nutrients of *Wigandia urens* (Hydrophyllaceae) and its implications for herbivory. – *Oecologia* 92: 405–409.
- and Oyama, K. 1992b. Leaf trichomes, quality of feed and efficiency of feeding and growth in *Lophoceramica pyrrha*. – *Southwest. Entomol.* 17: 333–339.
- Cates, R. G. and Orians, G. H. 1975. Successional status and the palatability of plants to generalized herbivores. – *Ecology* 56: 410–418.
- Czarnetzki, B. M., Thiele, T. and Rosenback, T. 1990. Immunoreactive leukotrienes in nettle plants (*Urtica urens*). – *Int. Arch. Allergy Appl. Immunol.* 91: 43–46.
- Collier, H. O. J. and Chesher, G. B. 1956. Identification of 5-hydroxytryptamine in the sting of the nettle (*Urtica dioica*). – *Br. J. Pharmacol.* 11: 186–189.
- Davis, B. N. K. 1973. The Hemiptera and Coleoptera of stinging nettle (*Urtica dioica* L.) in East Anglia. – *J. Appl. Ecol.* 10: 213–237.
- 1983. Insects on nettles. – Cambridge Univ. Press, Cambridge.
- Dillon, P. M., Lowrie, S. and McKey, D. 1983. Disarming the “evil woman”: Petiole constriction by a sphingid larva circumvents mechanical defenses of its host plant, *Cnidioscolus urens* (Euphorbiaceae). – *Biotropica* 15: 112–116.
- Emmelin, N. and Feldberg, W. 1947. The mechanism of the sting of the common nettle (*Urtica urens*). – *J. Physiol.* 106: 440–445.
- Geltman, D. V. 1992. *Urtica galeopsifolia* Wierzb. ex Opiz (Urticaceae) in Wicken Fen (E. England). – *Watsonia* 19: 127–129.
- Grime, J. P., MacPherson-Stewart, S. F. and Dearman, R. S. 1968. An investigation of leaf palatability using the snail *Cepaea nemoralis*. – *J. Ecol.* 56: 405–420.
- , Blythe, G. M. and Thornton, J. D. 1970. Food selection by the snail *Cepaea nemoralis* L. – In: Animal populations in relation to their food resources. British Ecological Society Symposium no. 10, pp. 73–99.
- Hawkeswood, T. J. 1991. Observations on a *Diphucephala* species (Coleoptera: Scarabaeidae) and its feeding relationships with the giant stinging tree, *Dendrocnide excelsa* (Wedd.) Chew (Urticaceae) in Queensland, Australia. – *Entomologist* 110: 170–177.
- Jones, D. A. 1971. Chemical defense mechanisms and genetic polymorphisms. – *Science* 173: 945.
- Leung, T. W. C., Williams, D. H., Barna, J. C. J., Foti, S. and Oelrichs, P. B. 1986. Structural studies on the peptide moroidin from *Laportea moroides*. – *Tetrahedron* 42: 3333–3348.
- Levin, D. A. 1973. The role of trichomes in plant defense. – *Q. Rev. Biol.* 48: 3–15.
- Lowman, M. D. 1985. Temporal and spatial variability in insect grazing of the canopies of five Australian rainforest tree species. – *Aust. J. Ecol.* 10: 7–24.
- and Box, J. D. 1983. Variation in leaf toughness and phenolic content among five species of Australian rain forest trees. – *Aust. J. Ecol.* 8: 17–25.
- Mason, C. F. 1970. Food, feeding rates, and assimilation in woodland snails. – *Oecologia* 4: 358–373.
- Pallant, D. 1969. The food of the grey field slug *Agriolimax reticulatus* (Müller) in woodland. – *J. Anim. Ecol.* 38: 391–397.
- Pollard, A. J. 1986. Variation in *Cnidioscolus texanus* in relation to herbivory. – *Oecologia* 70: 411–413.
- 1992. The importance of deterrence: responses of grazing animals to plant variation. – In: Fritz, R. S. and Simms, E. L. (eds), Plant resistance to herbivores and pathogens. Univ. of Chicago Press, Chicago, pp. 216–239.
- and Briggs, D. 1982. Genecological studies of *Urtica dioica* L. I. The nature of intraspecific variation in *U. dioica*. – *New Phytol.* 92: 453–470.
- and Briggs, D. 1984. Genecological studies of *Urtica dioica* L. III. Stinging hairs and plant-herbivore interactions. – *New Phytol.* 97: 507–522.
- Pullin, A. S. and Gilbert, J. E. 1989. The stinging nettle, *Urtica dioica*, increases trichome density after herbivore and mechanical damage. – *Oikos* 54: 275–280.
- Rhoades, D. F. 1979. Evolution of plant chemical defenses against herbivory. – In: Rosenthal, G. A. and Janzen, D. H. (eds), Herbivores: their interaction with secondary plant metabolites. Academic Press, New York, pp. 3–54.
- Southwood, T. R. E. 1986. Plant surfaces and insects: an overview. – In: Juniper, B. and Southwood, R. (eds), Insects and the plant surface. Edward Arnold, London, pp. 1–22.
- Stipanovic, R. D. 1983. Function and chemistry of plant trichomes in insect resistance. – In: Hedin, P. A. (ed.), Mechanisms of plant resistance to insects (American Chemical Society Symposium Series 208). American Chemical Society, Washington, pp. 69–100.
- Thurston, E. L. 1974. Morphology, fine structure, and ontogeny of the stinging emergence of *Urtica dioica*. – *Am. J. Bot.* 61: 809–817.
- and Lersten, N. R. 1969. The morphology and toxicology of plant stinging hairs. – *Bot. Rev.* 35: 393–412.
- Wolda, H., Zweep, A. and Schuitema, K. A. 1971. The role of

food in the dynamics of populations of the landsnail *Cepaea nemoralis*. – *Oecologia* 7: 361–381.
Woodland, D. W., Bassett, W., Crompton, C. and Forget, S.

1982. Biosystematics of the perennial North American taxa of *Urtica*. 2. Taxonomy. – *Syst. Bot.* 7: 282–290.