

SHORT COMMUNICATION

Induced defences in kawakawa (*Macropiper excelsum*):
do caterpillars avoid previous leaf damage?S. Hodge, M. Barron¹ and S. D. Wratten*

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Abstract: This study examined whether two species of lepidopteran larvae (*Cleora scriptaria* and *Epiphyas postvittana*) were deterred from feeding on the leaves of kawakawa (*Macropiper excelsum*) after the leaves had been damaged in three different ways: by larval feeding, using a hole punch or a metal rasp. A hierarchy of choice experiments was performed in the laboratory, examining the feeding 'preference' of these insects between undamaged or previously damaged tissue within the same leaf, between different leaves and between different plants. On no occasion did larvae of either species show a significant preference for undamaged or damaged leaves. It is concluded that, on present evidence, kawakawa does not have a system of induced chemical defences which deters the feeding of these two insects.

Keywords: Kawakawa; *Macropiper excelsum*; *Cleora scriptaria*; *Epiphyas postvittana*; induced defences; herbivory.

Introduction

Kawakawa (*Macropiper excelsum* (Forst. f.) Miq.; Piperaceae) possesses a range of anti-insect compounds. Leaf material is toxic to the larvae of *Musca domestica* L. (Diptera: Muscidae) and deters feeding in some other insects (Russell and Lane, 1993). A number of bioactive compounds have been identified in kawakawa leaves, including juvadecene, myristicine and novel lignans related to the juvenile hormone mimic sesomolin (Russell and Fenimore, 1973; Nishida *et al.*, 1983; Brooker *et al.*, 1987).

In spite of this range of insect anti-feedant chemicals, kawakawa leaves often exhibit a 'bullet-hole' pattern, caused by the feeding of the larvae of *Cleora scriptaria* (Walker) (Lepidoptera: Geometridae) (Beever, 1987). This pattern of feeding is characteristic of systems of plant defence where damage induces the production of anti-feedant compounds (see Edwards *et al.*, 1985). The deterrent compounds increase in concentration as the herbivore continues to feed and the animal is eventually impelled to move away. This process produces a pattern of relatively regularly-spaced, discrete areas of feeding damage, and the pattern seen on kawakawa may be an example of this.

Hodge *et al.* (1998), however, found that kawakawa leaves subjected to herbivory or artificial damage were not rendered less acceptable to *C. scriptaria* larvae. In their experiments, larvae were not presented with any feeding choice and the conclusion was that damage did not reduce the palatability of the leaves. No information was obtained on whether the larvae would have fed on undamaged leaves given the freedom to do so.

The aim of this investigation was to assess the potency of any wound-induced defences in kawakawa by performing a series of choice experiments. To examine the generality of the response, the larvae of two lepidopteran species were examined; these were the primary insect herbivore on kawakawa, *C. scriptaria*, and the polyphagous lightbrown apple moth, *Epiphyas postvittana* (Walk.) (Lepidoptera: Tortricidae).

Methods

C. scriptaria larvae (II / III instar) were obtained by beating kawakawa branches in Okuti Valley, Banks Peninsula, New Zealand. These larvae were kept in plastic boxes (24 x 17 x 8 cm) in a controlled

environment room (20 ± 1 °C) with a 16:8 hour light:dark cycle and maintained on a diet of kawakawa leaves. *E. postvittana* larvae and their rearing diet were obtained from HortResearch, Mount Albert Research Centre, Auckland.

Kawakawa seedlings (20 to 50 cm in height) were obtained from the Department of Conservation nursery, Motukarara, Canterbury, and from Trees for Canterbury, Christchurch. The plants were maintained in an outside shade house, in polythene planter bags (PB 18; 18 cm x 18 cm x 32 cm) using a general bark/sand/compost potting mix.

Damaging the leaves

As the induced defences of some plants respond differently to damage caused by insect feeding compared to artificial damage (Karban and Myers, 1989), kawakawa leaves were damaged in three different ways. The first method used a metal punch to create holes (4 mm diameter) in the leaves. This removed discrete areas of leaf tissue, mimicking the pattern of natural damage. The second method involved crushing the leaf against a steel rasp, which created an array of punctures (c. 12 punctures per cm^2) in the leaf without removing leaf tissue. The third method used II / III instar *C. scriptaria* larvae contained in muslin bags tied around a leaf and allowing them to feed. This latter treatment assessed whether herbivory or general damage led to the induction of chemical defences. Twenty-four hours were allowed after damage (or, for the *C. scriptaria* damage, 24 hours after the larvae were introduced to the leaf) for the development of any responses to wounding (see Nelson *et al.*, 1983).

Damage avoidance in detached leaves

These experiments examined whether larvae preferred intact areas of a leaf, compared with those parts which had suffered some previous damage. Leaves on five undamaged kawakawa plants were damaged on one side of the midrib. Five leaves on each plant were allocated to each of the three damage treatments described above. Twenty-four hours after leaf damage, the leaves were removed from the plant by cutting the base of the petiole with a scalpel. The leaves were placed individually on a moistened filter paper inside plastic Petri dishes (9 cm diameter). A single *C. scriptaria* larva was then placed in the centre of each leaf. The position of the larva (damaged or undamaged side of the leaf) was recorded 1 hour and 24 hours later. Any ambiguous larval positions, such as straddling the middle of the leaf or on the lid of the Petri dish, were not included in the data (similarly in the experiments described below).

The experiment was repeated using *E. postvittana* larvae but using only the treatments employing the hole punch and rasp to create leaf damage.

Damage avoidance between detached leaves

In the previous experiment, there was a possibility that damaging one side of the leaf may have induced defensive compounds to occur over the whole leaf, so the larvae were not actually presented with a feeding choice. This second experiment was carried out to assess whether larvae 'preferred' tissue from an undamaged leaf compared with that from a separate, previously damaged leaf. Leaves on five undamaged kawakawa plants were damaged by each of the three damage techniques outlined above. Five leaves were allocated to each damage treatment on each plant. Twenty-four hours after the leaf damage had been carried out the leaves were removed as described above. The leaves were then separated into two halves by cutting longitudinally through the midrib and one half was placed on moist filter paper in a Petri dish. A matching half, obtained from undamaged leaves on control plants, was then placed alongside each of the damaged leaf halves. A single *C. scriptaria* larva was then placed in the centre of each dish. The position of the larva (damaged or undamaged leaf half) was recorded 1 hour and 24 hours after introduction to the dish.

The experiment was repeated using *E. postvittana* larvae but using only the treatments employing the hole punch and rasp to create leaf damage.

Damage avoidance within a plant

In the previous experiment, there was a possibility that removing the leaves from the plant and bisecting them may have induced the production of any chemical defences, so masking the effects of experimental leaf damage. To counter that problem, an experiment was carried out which retained the damaged and undamaged leaves on the plant. Twenty-five kawakawa seedlings (approximately 20 cm high with 8 to 10 leaves) had alternate leaves damaged using a hole punch. The seedlings were placed into individual nylon mesh cages (1m x 1m x 1m), maintained at room temperature (c. 20 °C) and illuminated using two halogen bulbs (Phillips TLD 58W). One *C. scriptaria* larva was placed on the upper leaf 24 hours after the plants were damaged. The position of the larva was recorded (damaged or undamaged leaves) 1, 3, 6 and 24 hours after introduction to the plant, excluding any ambiguous larval positions.

Damage avoidance between plants

In the previous experiment, there was a possibility that damaging some leaves on a plant caused an induction

of defences in all leaves. To counter that problem, in this experiment the damaged and undamaged leaf treatments were confined to separate plants. Kawakawa seedlings were matched into pairs according to height. One plant had each of its leaves damaged by using a hole punch, with 4-6 holes per leaf depending on the size of the leaf. The other seedling was retained as a no-damage control. The pairs of seedlings were placed beside each other in a 1 m x 1 m x 1 m nylon mesh cage, maintained as above. Twenty-four hours later, a single *C. scriptaria* larva was placed on top of one of the seedlings; half the larvae were placed initially on the damaged plant and half on the controls. The plants were close enough so that their leaves overlapped, providing the larva with easy opportunities to cross between plants. The position of the larvae was then recorded (damaged or undamaged plants) 1, 3, 6 and 24 hours after introduction to the plants. Twenty-one replicates were used.

Results

The results of all the choice tests are summarized in Table 1. On no occasion was there a significant preference for the undamaged (or the damaged) leaf tissue by *C. scriptaria* or LBAM larvae (χ^2 with Yates' correction applied to account for 1 d.f.). Using a χ^2 test with Yates' correction for a single degree of freedom can be conservative, especially when the number of replicates is small. This can lead to a Type II statistical error, where the null hypothesis is erroneously accepted due to the low power of the test (Zar, 1984). However, the distribution of the larvae between the control and damaged leaves was generally fairly even, and when considering the distribution of all the larvae at 1 hour and at 24 hours there was no significant difference found from a predicted median of 50% of larvae on the control leaves (Wilcoxon test; $N=11$; $P>0.4$ for both 1 hr and 24 hrs).

Table 1. Distribution of *C. scriptaria* and *E. postvittana* larvae between damaged and undamaged kawakawa leaves, expressed as total numbers of individuals.

Comparison made	Species of larvae tested	Damage technique	Time	Control	Damaged	χ^2	<i>P</i>		
Within a leaf	<i>C. scriptaria</i>	Hole punch	1 hr	10	11	0	1.00		
			24 hrs	7	14	1.71	>0.10		
		File	1 hr	9	9	0	1.00		
			24 hrs	10	10	0	1.00		
		Cleora	1 hr	13	10	0.17	>0.50		
			24 hrs	13	11	0.04	>0.80		
		Between split leaves		Hole punch	1 hr	10	13	0.17	>0.50
					24 hrs	13	17	0.30	>0.50
File	1 hr			9	7	0.06	>0.75		
	24 hrs			15	11	0.35	>0.50		
Cleora	1 hr			8	15	1.56	>0.20		
	24 hrs			11	10	0	1.00		
Within a plant				Hole punch	1 hr	11	10	0	1.00
					3 hrs	14	8	1.14	>0.20
		6 hrs	18		9	2.37	>0.10		
		24 hrs	15		10	0.64	>0.30		
Between plants		Hole punch	1 hr	12	9	0.19	>0.50		
			3 hrs	12	9	0.19	>0.50		
			6 hrs	11	10	0	1.00		
			24 hrs	10	11	0	1.00		
Within a leaf	<i>E. postvittana</i>	Hole punch	1 hr	5	10	1.07	>0.25		
			24 hrs	4	11	2.40	>0.10		
		File	1 hr	4	12	3.06	>0.05		
			24 hrs	10	6	0.56	>0.30		
Between split leaves		Hole punch	1 hr	4	11	2.40	>0.10		
			24 hrs	9	10	0.00	1.00		
		File	1 hr	12	6	1.39	>0.20		
			24 hrs	13	6	1.89	>0.10		

Discussion

There have been a number of critiques of experiments examining induced defences, on the grounds that no account is taken of possible induction between leaves, or between stems (e.g., Fowler and Lawton, 1985; Neuvonen and Haukioja, 1985). Damaging one leaf may induce the production of defensive compounds in another, undamaged leaf, so that there are no real experimental 'controls' (see Wratten *et al.*, 1984). This has been accounted for in the present study by using a hierarchy of experimental systems; these examined a larva's response to damage within a leaf, between leaves and between plants.

The feeding position of *C. scriptaria* larvae was not affected by previous damage to kawakawa leaves. Although these larvae are nocturnal in the field, in the laboratory they fed throughout the day and were almost always found close to the most recent feeding damage. It is probable, therefore, that feeding position of the larvae - rather than just 'resting' position - was measured. The larvae showed no avoidance of damaged leaves and have previously been shown to feed at a similar rate on damaged leaves as they do on undamaged leaves (Hodge *et al.*, 1998).

Kawakawa is the primary host of *C. scriptaria* and it can be speculated that this herbivore has evolved some tolerance of the constitutive defences of the plant (Levin, 1976). Because of the close association between the two species, it is also plausible that *C. scriptaria* has evolved a tolerance of any induced compounds that kawakawa produces. However, the polyphagous larvae of the LBAM also showed no avoidance of damaged leaves, supporting the hypothesis that kawakawa does not have any major system of induced defences. Although induced defences are widespread they are by no means a general rule (Chapin *et al.*, 1985; Fowler and Lawton, 1985) and it appears that kawakawa lacks such a system, possibly relying on its suite of constitutive chemical defences to deter most phytophagous insects.

From these results, it seems likely that the characteristic patchy feeding pattern of *C. scriptaria* is not produced by a larval response to induced defences. Observations in the laboratory suggest the larvae feed for short periods and then rest, often by lying along a petiole or dangling by a silken thread. It can be speculated that feeding may be interrupted to allow the larvae to digest the host-plant tissue (Reynolds, 1990) or to detoxify (or sequester) toxins (Slansky, 1992). Alternatively, the feeding pattern may result from behaviour evolved to facilitate predator avoidance (Heinrich, 1979). *C. scriptaria* larvae are cryptically coloured and exhibit many of the associated 'anti-predator' behaviours (e.g., forage on underside of leaves, feed at night, etc.; Heinrich, 1979;

though see Mauricio and Bowers, 1990). These hypotheses concerning the mechanism behind the feeding pattern of *C. scriptaria* larvae require further investigation.

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