

***Cleora scriptaria* larvae exhibit reduced growth on a diet of kawakawa leaves subjected to artificial wounding**

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Introduction

Under field conditions, the leaves of kawakawa (*Macropiper excelsum* (Forst. F. Miq: Piperaceae)) commonly exhibit a 'bullet holed' appearance due to feeding by larvae of *Cleora scriptaria* (Walker), a geometrid moth (Beever 1987). A patchy arrangement of caterpillar damage is often displayed in plant-insect systems where injury to foliage induces the production of repellent or anti-feedant compounds by the plant. However, previous laboratory experiments indicated that *C. scriptaria* does not avoid, or show reduced feeding rates, on wounded kawakawa leaves, although no assessment was made of whether the quality of damaged leaf tissue affected larval development (Hodge *et al.* 1998, 2000). We address this issue by examining the effects of prior leaf wounding on the survival and growth of *C. scriptaria* larvae reared under standardized laboratory conditions.

Methods

Young *Cleora scriptaria* larvae (~ 1.0 – 3.8 mg) were collected from kawakawa bushes at Okuti Valley on Banks Peninsula. Each larva was weighed and placed in a flat-bottomed glass tube (75 x 25mm diameter) capped with a polyurethane foam bung, and then maintained in a constant temperature room (20°C, 16:8 light:dark cycle).

Larvae were allocated a diet of either young leaves from undamaged nursery-reared kawakawa plants or young leaves that had been damaged 24 hours previously using a hole punch (ten holes of 4mm diameter). Every 24 hours the larvae were scored for mortality and each surviving larva was presented with one new leaf. On no occasion did any larva consume its total food allocation within the 24 hour period. Every five days the larvae were reweighed and then housed in a clean glass tube. The experiment was concluded after 15 days because some larvae were beginning to pupate.

The experiment was performed on two batches of larvae with ten replicates of each diet treatment on each occasion. The growth of the larvae during the experiments approximated an exponential curve, so the mean daily growth rate (r) of each larva was

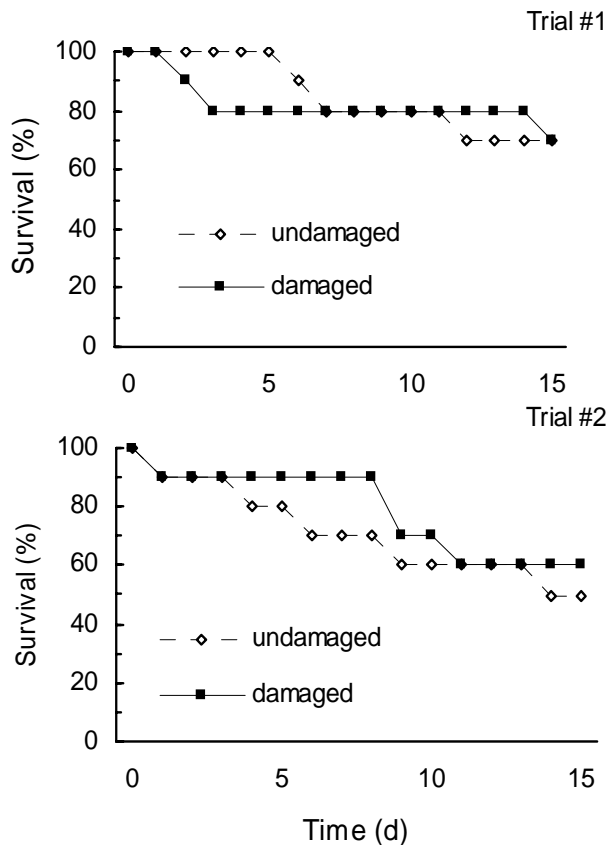


Figure 1. Survival of cohorts of *Cleora scriptaria* larvae reared on diets of damaged or undamaged kawakawa leaves.

estimated as:

$$W_t = W_0 \cdot r^t$$

where t is the duration of the experiment (in days), W_0 is the initial weight and W_t the weight at time t . The mean daily growth rate parameter, r , was used in statistical analysis, rather than final body weight, as it takes into account variation in the initial weights of the caterpillars.

Results

The trends in survival probability were similar for larvae allocated to the different leaf diets, with approximately 60% surviving until the end of the 15 day experiments (Fig. 1).

Only measurements from those larvae that survived the full 15 days were used in the analysis of caterpillar growth. A slight divergence in body weights between the two diet treatments was observed after ten days (Fig. 2). By 15 days there was a clear difference: the larvae reared on the wounded leaves were, on average, only 65% the size of those reared on the control leaves. A general linear model indicated there was a small, but significant, difference in the mean daily growth rates between the two trials ($F_{1,21} = 5.2, P < 0.04$; Fig. 3). However, the principal factor influencing caterpillar growth rate was leaf damage treatment ($F_{1,21} = 10.7, P < 0.005$).

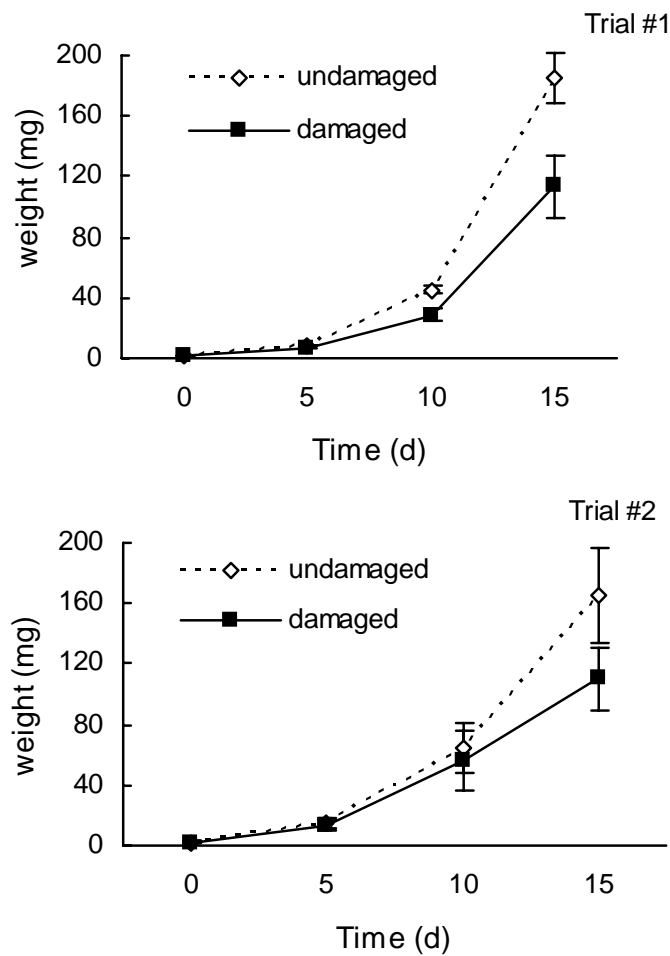


Figure 2. Growth of *Cleora scriptaria* larvae reared on diets of damaged or undamaged kawakawa leaves (mean \pm se).

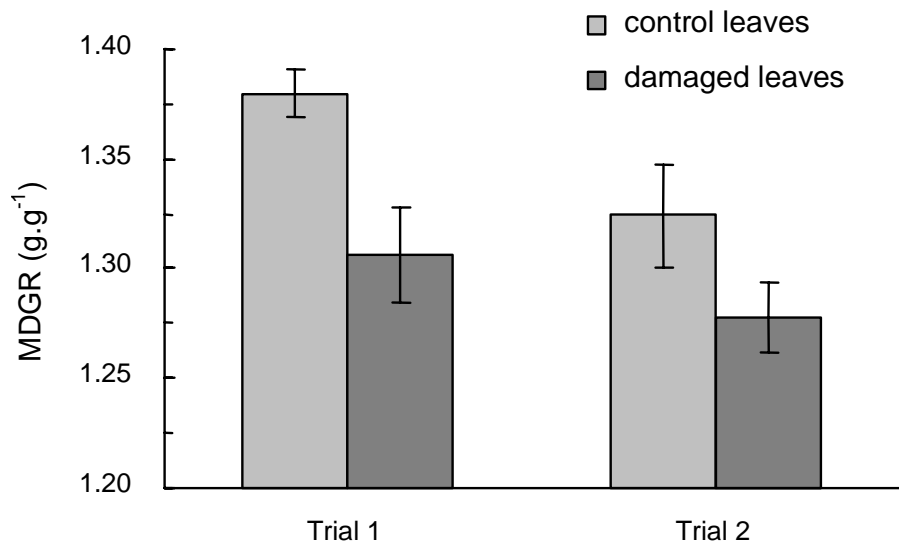


Figure 3. Mean daily growth rates of *Cleora scriptaria* larvae reared on diets of undamaged and damaged kawakawa leaves (mean \pm se).

Discussion

Much recent research has focussed on the identification of botanically-derived insecticides and repellents from members of the Piperaceae (*e.g.*, Scott *et al.* 2005) and kawakawa is known to contain a number of chemicals with anti-insect properties (Russell & Lane 1993). Briggs (1941) suggested that the compounds myristicin and elemicin might account for the insecticidal activity of kawakawa extracts and Nishida *et al.* (1983) described the presence of juvadicene, an insect juvenile hormone mimic that delayed moulting in the hemipteran bug, *Oncopeltus fasciatus*. The results of our bioassays indicate that mechanical wounding induces changes in some property of kawakawa leaves that reduces the growth of *C. scriptaria* larvae. Although the benefits of extending the length of the herbivorous larval instars of an insect's life cycle might seem somewhat paradoxical, induced inhibition of insect development is generally considered beneficial to the host plant. Decreasing larval body weight can result in the production of a smaller puparium, leading to smaller adults with lower fecundity. Therefore, retarding the development of juvenile Lepidoptera can lead to a reduction in overall population growth rate and decrease the number of generations of larvae produced in a given season. There is also potential for tritrophic effects to occur if the plant-induced delays in *C. scriptaria* development mean they remain susceptible to attack from their hymenopterous parasitoids and arboreal predators (see Devine *et al.* 2000, Hodge *et al.* 2001, Schnitzler *et al.* 2004).

At this time we cannot say whether the reduction in insect growth was due to (a) the production of compounds which actively delay insect development or (b) a reduction in the nutritional quality of the leaves caused by accumulation of compounds (phenolics, peroxidases and so on) that are part of the plant's chemical responses to mechanical wounding. More detailed chemical (or even proteomic / metabolomic) analysis of leaf tissue is required to name the compounds that are induced by artificial and/or caterpillar-mediated leaf wounding in kawakawa. These findings could then be used to identify potential chemical pathways that might result in the inhibition of *C. scriptaria* growth.

Acknowledgements

The authors wish to thank Trees for Canterbury, Christchurch, for supplying the kawakawa plants. This work was partly funded by British Ecological Society Small Ecological Project Grant 1504.

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