THE BEHAVIOUR OF ADULT *SITONA LEPIDUS* GYLLENHÅL (COLEOPTERA: CURCULIONIDAE) IN RESPONSE TO WHITE CLOVER

S. HARDWICK and B. HARENS

AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton

ABSTRACT

Individual adult *Sitona lepidus* were given the choice between damaged or undamaged seedlings, mature plants of white clover, damp soil or a blank in laboratory experiments carried out in a simple olfactometer. Adult weevils displayed five different behavioural responses, two of which, 'head lifting' and 'antennal movement,' appeared to be associated with host location. In choice tests, 74–84% of adults chose a white clover plant over the blank or damp soil. When given a choice 72% of adults selected white clover seedlings over mature plants. Root and leaf damage did not affect response of *S. lepidus* to white clover seedlings.

Keywords: *Sitona lepidus*, behaviour, white clover, olfactory stimuli, leaf and root damage.

INTRODUCTION

Sitona lepidus Gyllenhål (Coleoptera: Curculionidae) was first recognised in New Zealand in 1996 (Barratt *et al.* 1996). Currently, *S. lepidus* is known to be present from south of Whangarei to just north of Lake Taupo in the North Island. While not being an obligate feeder on white clover (*Trifolium repens* L.), *S. lepidus* strongly favours this legume (Murray and Clements 1994; Hardwick 1998; Murray and Willoughby 1998). Adult *S. lepidus* feed on the foliage of host species (Murray and Clements 1994) while the soil dwelling larvae feed on roots and root nodules of the plant (Byers and Kendall 1982). *S. lepidus* therefore represents a potential threat to the profitability of pastoral farming in New Zealand as farmers rely on white clover for both its nitrogen fixing capability and high nutritional value (Ulyatt *et al.* 1977; Caradus *et al.* 1995).

Odour from host plants can be an important factor in determining an insect's behaviour and its ability to find a host. Within the *Sitona* genus, studies have shown host plant volatiles can influence adult locomotor behaviour (Landon *et al.* 1997) and increase the catch rate of pheromone traps (Blight *et al.* 1984). Olfactory cues provided by white clover might therefore be a factor involved in location of fresh host material by *S. lepidus*. This paper describes four experiments investigating the response of adult *S. lepidus* to olfactory stimuli. Choices offered were pots containing damaged or undamaged seedlings, mature plants of white clover, damp soil or a blank.

MATERIALS AND METHODS

Plant preparation

Single seedlings of white clover (cv. Grasslands Sustain) were established in soil in 10 ml cardboard pots. Seedlings used in all experiments were at the first trifoliate leaf stage. Mature white clover plants (cv. Grasslands Sustain) were established from 15 mm stolon cuttings planted into soil in 10 ml cardboard pots. Plants were used in experiments approximately seven days later.

Olfactometer design

The olfactometer used in each experiment consisted of an A4 acetate sheet rolled into a 36 mm diameter tube 295 mm long. Halfway (ca 145 mm) along the tube a 15 × 8 mm opening was cut to admit adult *S. lepidus*. This opening could be closed with a 10 × 20 mm flap of acetate sheet held in place by sticky tape. Both ends of the tube

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opened into chambers constructed out of frosted plastic pots (105 mm high, 115 mm diameter top and 87 mm base diameter) with removable lids. The tube protruded 5 mm into the test chamber through a 36 mm diameter hole cut into the side of the chamber 65 mm from the base. A corresponding 36 mm diameter hole was cut opposite to this entrance and covered with fine mesh to permit the flow of air through the apparatus while preventing the escape of *S. lepidus*.

Experimental design

In each of the four experiments, adult weevils were offered the choice between two chambers. Each chamber held one of two test materials or was blank, giving a total of three combinations (blank + material A, blank + material B, material A + material B). The test materials are listed in Tables 1–4.

In each experiment there were 20 replicates of each combination giving 60 olfactometers in total. The olfactometers were arranged in a randomised block design. Weevils used in each experiment were field collected 2–3 h prior to the start of the experiment. After release into the olfactometer the behaviour of each weevil was observed until either it entered one of the test chambers or until six min had elapsed. If the weevil had not made a choice within six min its position was further assessed 30 min after release. If weevils had not made a choice after 30 min they were discounted from the analysis. During the initial six min observation any distinctive behaviour displayed by the weevil was noted along with its movements along the release tube. All four experiments were repeated twice. The data from the initial and repeat experiment were pooled for the final analysis, as an exact test for difference in proportionality of choice between repeat experiments showed there was no significant difference between sets of results. The pooled data were analysed using a binomial two tailed test.

FIGURE 1: Three examples of behaviour exhibited by adult *S. lepidus* after release into an olfactometer with one blank chamber and one chamber containing a white clover seedling. The diamond represents the release point and lines show the paths of weevils introduced into the olfactometer. AM = antennal movements while walking; HL = head lifting behaviour.

RESULTS

There were five distinctive behavioural responses shown by *S. lepidus* adults after release: 1) standing still, 2) leg grooming, 3) antennal grooming, 4) head lifting and 5) antennal movement while moving. Head lifting consisted of the adult standing still, elevating its head and fully extending its antennae. Antennal movement was also frequently observed after full antennal extension had occurred during head lifting. Fig. 1 shows three typical patterns of *S. lepidus* movement down the tube towards the chamber of choice. After release weevils appeared to make a choice using antennal movement only (Fig. 1A), head lifting only (Fig. 1B) or by using a combination of both behaviours (Fig. 1C). It was also common to observe an increase in the frequency of antennal movements and head lifting as a weevil neared a test chamber containing a host plant. In only 22 out of 480 tests was a choice apparently made by an adult with no observable head lifting or antennal movement.

In Experiment 1 a similar proportion of weevils entered the chamber containing the pot of soil (54%) and the blank (46%) when offered a choice between the two (Table 1). However, when adults were offered a choice between the chambers containing the blank or a pot of soil and a chamber containing a seedling the majority of adults (78–84%) moved to chambers containing the seedling (Table 1).

In Experiment 2 a higher proportion of adult weevils selected a chamber containing a white clover seedling with an undamaged (79%) or damaged (75%) leaflet than selected the blank chamber (Table 2). There was no significant difference in the proportion of weevils selecting test chambers containing either white clover seedlings with undamaged (55%) or damaged (45%) leaf tissue when they were offered a choice between them. (Table 2).

TABLE 1: Experiment 1. Number of weevils in blank chamber or chambers containing a pot with moist soil or a white clover seedling in moist soil 30 minutes after release.

Combination	Blank	Seedling	Soil only
Blank-seedling	8	29*** ¹	
Blank-soil	18		21ns
Seedling-soil		33***	6

¹ns = $P > 0.05$, *** = $P < 0.001$

TABLE 2: Experiment 2. Number of weevils in blank chamber or chambers containing an undamaged white clover seedling or a white clover seedling with half a leaflet removed 30 minutes after release.

Combination	Blank	Undamaged seedling	Leaf-damaged seedling
Blank-undamaged	8	31*** ¹	
Blank-damaged	10		30**
Undamaged-damaged		21	17ns

¹ns = $P > 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

In Experiment 3 a significantly greater proportion of weevils selected chambers containing undamaged (87%) and root damaged (80%) white clover over blank chambers (Table 3). As with the leaflet damage in Experiment 2, white clover seedlings with a damaged root system were no more attractive to adult *S. lepidus* than undamaged seedlings (Table 3).

When adult *S. lepidus* were presented the choice between either a chamber containing mature or seedling white clover and a blank chamber the weevils selected the chamber containing white clover material in approximately 83% of tests (Table 4). When presented the choice between chambers containing mature white clover plant

material or chambers containing white clover seedlings, a greater proportion of weevils (73%) chose chambers containing the seedlings (Table 4).

TABLE 3: Experiment 3. Number of weevils in blank chamber or chambers containing an undamaged or root damaged white clover seedling weevils 30 minutes after release.

Combination	Blank	Undamaged seedling	Root-damaged seedling
Blank-undamaged	5	34*** ¹	
Blank-damaged	8		32***
Undamaged-damaged		19	18ns

¹ns = P>0.05, *** = P<0.001

TABLE 4: Experiment 4. Number of weevils in blank chamber or chambers containing a seedling or a mature white clover plant 30 minutes after release.

Combination	Blank	Seedling	Mature plant
Blank-seedling	9		31*** ¹
Blank-mature	5	35***	
Mature-seedling		29	11**

¹** = P<0.01, *** P<0.001

DISCUSSION

Differences in host preference may be due to differences in plant structure and/or chemistry (Crawley 1983). Plant age affects the concentrations of secondary substances which may act as phagostimulants, attractants or repellents (Crawley 1983). Previously it has been proposed that antifeedants are involved in host selection by *Sitona* spp. (Mowat and Shakeel 1989; Barratt and Byers 1992). However, Murray and Clements (1994) suggested that host selection/differentiation by *S. lepidus* in the field may take place using either tactile or olfactory stimuli, as 'tasting bites' appeared to be consistently absent from some legume species in field situations. In this study it was impossible for the adults to touch the host before making a choice. Therefore, it would appear that olfactory cues originating from host plants played an important role in the selection of the potential host. However, while the current laboratory studies demonstrate that olfactory cues are important in host location and selection, it is unlikely that a single cue is involved. A more likely scenario would be that a combination of olfactory, gustatory, tactile and visual stimuli are involved in host selection.

Of the five observed behaviours displayed by adult *S. lepidus* standing still and leg grooming did not appear to be involved in detecting olfactory stimuli. The importance of antennal grooming was difficult to assess and cannot be discounted from playing a role in the detection of olfactory cues. However, head lifting and rapid antennal movement appeared to be important responses to possible olfactory stimuli. Landon *et al.* (1997) reported that *S. lineatus* responded to host odour both by displaying distinctive antennal movements and by moving towards the source of the host odour.

Adult *S. lepidus* in this study appeared to be able to use olfactory cues to discriminate between white clover plants of differing maturity and white clover seedlings are preferred to mature plants. This observation supports those from field trials carried in the springs of 1998 and 1999 (P. Addison and S. Hardwick, unpubl. data) where white clover seedlings appeared to be more attractive to and were attacked more heavily by *S. lepidus* adults than were mature white clover plants. As white clover is particularly vulnerable to attack by *Sitona* spp. during its establishment phase (Murray and Clements 1994; Hardwick 1998), the ability of *S. lepidus* adults to select the seedlings of white clover in preference to mature plants may have important

implications for both the long term sustainability of existing white clover/ryegrass swards and the ability to re-establish white clover into both established and new swards in areas where *S. lepidus* is present. If, as results suggest, adult *S. lepidus* can locate, feed on, and kill isolated seedlings within a population of white clover plants, one path of plant population regeneration is effectively reduced. This could lead to either a gradual collapse or greater fluctuations in the overall white clover population. Furthermore, the re-establishment of white clover in pasture through methods such as undersowing may become particularly difficult unless the seedlings are protected (e.g. through use of an insecticidal seed coating) or adult *S. lepidus* are controlled (e.g. through the use of adulticide treatments) during seedling establishment.

In conclusion, the four experiments outlined in this paper showed that adult *S. lepidus* responded to olfactory cues from white clover plants. Two of the five different behaviours observed, head lifting and antennal movement while walking, appeared to be involved in the detection of host plant (white clover) odours. The infliction of damage to either the roots or leaves of white clover seedlings made plants no more or less attractive to *S. lepidus*. However, the experiments did show that white clover seedlings were more attractive to adult *S. lepidus* than mature white clover plants. The relationship between the relative attractiveness of mature and seedling white clover to adult *S. lepidus* warrants further research due to the importance of sustaining white clover production in New Zealand pastures.

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