PASTURE AND LUCERNE PESTS AS VIRUS VECTORS

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ABSTRACT

Four weevil species, Argentine stem weevil, sitona weevil, *Niceana cervina*, and *Irenimus stolidus*, as well as the grey field slug and grass grub larvae were investigated as potential vectors of plant viruses. None of the invertebrates were shown to transmit any of the viruses tested. In addition, the influence of ryegrass mosaic virus and cocksfoot mottle virus on Argentine stem weevil feeding and oviposition was investigated. Argentine stem weevil feeding was not affected by the presence of ryegrass mosaic virus but was reduced by cocksfoot mottle virus. Neither virus appeared to influence oviposition during the study period. The feeding preferences of sitona weevils presented with alfalfa mosaic virus infected or uninfected lucerne were also investigated but no preference was observed.

Keywords: invertebrate pasture pests, plant viruses

INTRODUCTION

Smales *et al.* (1995) investigated the pasture pests Argentine stem weevil (*Listronotus bonariensis* Kuschel), porina (*Wiseana* sp) and the grey field slug (*Deroceras reticulatum* Muller) as potential vectors of ryegrass mosaic virus (RgMV) and cocksfoot mottle virus (CfMV) in laboratory tests. Argentine stem weevil was shown to transmit RgMV between infected and uninfected ryegrass (*Lolium perenne*) plants. No transmission of CfMV by any of these pests was demonstrated although virus particles were detected in the faeces of both the grey field slug and porina. RgMV particles were also found in slug faeces. Slugs have been implicated as vectors of plant viruses. Cook *et al.* (1989) found that the *D. reticulatum* could transmit white clover mosaic virus (WCIMV) to white clover plants. The mechanism of transmission is thought to be by contamination of the slug mouthparts in feeding and the retention of infectivity of the virus when the contaminated slugs are transferred to another plant. Another mechanism may be by passive transfer of WCIMV from the fecal pellets.

The findings of Smales *et al.* (1995) suggested that incidents of transmission by the pasture pests studied were low. However, when magnified up to field scale such virus transmission may add to the pest status of the invertebrates concerned. The current study further investigated Argentine stem weevil as a virus vector as well as two other widespread and common invertebrate pests, sitona weevil (*Sitona discoideus* Gyllenhal) and grass grub (*Costelytra zealandica* (White)) larvae. It also included two native weevils, *Irenimus stolidus* Broun and *Niceana cervina* (Broun) (Coleoptera: Curculionidae), whose biology is generally not well known, but which represent genera which are common in modified pasture environments.

A complex interaction often exists between virus, host and vector. Research has shown that insect vectors of plant viruses may be both positively and negatively affected by virus. Some viruses have been shown to increase mortality of the vector (Sehgal 1994) but other vectors are known to show increased growth or reproduction when fed on virus infected plants (Thresh 1974). Feeding preference tests with aphids have also shown that some aphids prefer to feed on virus infected plants over uninfected plants (Garran and Gibbs 1982). This study also investigated the influence of RgMV and CfMV on feeding and oviposition of Argentine stem weevil and the influence of alfalfa mosaic virus (AlMV) on feeding of sitona weevil.

METHODS

Virus transmission experiments

In all experiments the plants infected with viruses were grown in a glasshouse and the presence of virus confirmed by ELISA (enzyme linked immunosorbent assays), as described previously (Smales *et al.* 1995).

Argentine stem weevils collected from a ryegrass/white clover (*Trifolium repens* L.) pasture at Sutton, in the Strath Taieri, Otago, were held in cages in a laboratory at 20°C and with a 16:8 hour light:dark photoperiod for 1 month prior to the investigation. During this time Grasslands Manawa ryegrass (*L. perenne* x *multiflorum*) x *perenne* L.) was supplied as food. Eighty five weevils were then caged on CfMV infected cocksfoot plants for 4 days before being placed individually in polycarbonate tubes (80 mm high, dia. = 26 mm) fitted with gauze tops and containing uninfected maize (*Zea mays* L.) seedlings for 4 days. One hundred weevils were similarly caged on CfMV infected wheat plants before being caged on uninfected wheat seedlings.

Grey field slugs, collected from the field, were held in a dark incubator at 10 °C and fed healthy wheat (*Triticum aestivum* L.) seedlings for 7 days. CfMV infected wheat leaves were then fed to 15 slugs, and CfMV infected cocksfoot to 18 slugs, for 5 days. Replicates of three slugs were then caged in 2 litre opaque plastic containers containing uninfected wheat plants for 3 days. The wheat plants were replaced with fresh plants every 24 hours.

Sitona weevil, collected from a lucerne (*Medicago sativa* L.) stand at Sutton, were maintained on healthy lucerne plants under the same conditions as Argentine stem weevil for 10 days before being starved for 24 hours and supplied with AIMV infected lucerne for 5 days. Fourteen replicates of ten weevils each, and three additional replicates of 25 weevils each, were caged on healthy lucerne plants in opaque containers (as described above), for 48 hours.

I. stolidus and *N. cervina* were also collected from Sutton and held as for Argentine stem weevil except that they were supplied with lucerne as food in addition to ryegrass. They were starved for 24 hours before three groups of *I. stolidus* (approximately 35 weevils/group) and five groups of 50 *N. cervina* were caged on white clover mosaic virus (WCIMV) infected subterranean clover (*T. subterraneum* L.) plants for 5 days. Replicates of either two *I. stolidus* or four *N. cervina* were then placed in tubes (as described above), containing healthy subterranean clover seedlings, for 48 hours.

Grass grub second and third instar larvae were collected from pasture at Ophir, Central Otago. Five larvae were immediately placed in each of 10 pots growing cocksfoot infected with CfMV. After 7 days the larvae were removed and in groups of two placed in pots containing uninfected cocksfoot where they were allowed to feed for 4 days. A further 48 larvae were placed in pots growing WCIMV infected subterranean clover, for 8 days before being placed for five days, in groups of two, in 24 pots containing healthy subterranean clover.

At the end of each exposure period, the invertebrates were removed and the plants grown on in a glasshouse for 4-6 weeks before being assayed for the presence of the respective viruses by ELISA.

Feeding choice and oviposition experiments

L. bonariensis were collected from a ryegrass/white clover pasture at Invermay, near Mosgiel, and held in cages (as above) for 1 month. During this time half of the weevils were fed healthy six week old Grasslands Manawa ryegrass, and the other half healthy Grasslands Wana cocksfoot (*Dactylis glomerata* L.). The weevils were starved for 24 hours immediately prior to being used in the investigation.

The experiments were conducted in 95 mm dia. petri dishes lined with moist filter paper. In each petri-dish 40 mm long sections of either RgMV infected and uninfected ryegrass, or CfMV infected and uninfected cocksfoot, were placed on opposite sides of the dish. Ten weevils were placed in the center of the dish which was then covered to exclude light and promote feeding (Barratt *et al.* 1995). After 8 hours the amount of feeding on each leaf segment was measured. The experiments with RgMV were repeated twice and those with CfMV three times. There were ten replicates on each occasion. Fresh weevils were used on each occasion.

S. discoideus collected from Sutton, were held as above and fed healthy lucerne for 1 month. Weevils were then starved for 24 hours and placed in petri dishes containing one trifoliate leaf of uninfected lucerne and one infected with AIMV. The dishes were covered and inspected as for Argentine stem weevil. The experiment was repeated twice.

For the oviposition experiment, 30 Argentine stem weevils were placed in each of 20 clear plastic cages (160 x 170 x 70 mm deep) fitted with a gauze lid. The cages were assigned to one of four treatments in which weevils were maintained on; 1) uninfected ryegrass, 2) RgMV infected ryegrass, 3) uninfected cocksfoot or 4) CfMV infected cocksfoot. All plants were 6 weeks old when the experiment commenced. Plants were replaced every 3 to 4 days for a month and examined for eggs when the plants were removed from the cages. Any dead weevils found during food changes were removed from the cages and stored in 70 % alcohol until dissected, along with all weevils surviving to the end of the experiment, to determine their sex.

The data from the feeding and oviposition experiments were subjected to analyses of variance. The number of females per cage in the oviposition experiment were used as covariates in the analyses.

RESULTS AND DISCUSSION

This study failed to show transmission of CfMV by Argentine stem weevil or slugs from infected wheat plants to either wheat or maize. Both plants are attacked by these invertebrates in the field and CfMV has been found to naturally occur in wheat (Serjeant 1967). Although CfMV has been shown to occur in the roots of infected cocksfoot plants (Smales unpublished), grass grub larvae were not shown to transmit it from infected to uninfected plants. The present study failed to identify any new vectors of CfMV. The known vectors, *Oulema melanopa* (L.) and *O. lichenis* (Voet.) (Coleoptera : Chrysomelidae) (Serjeant 1967; A'Brook and Benigno 1972), are not present in New Zealand (Foord 1993). The agent responsible for the spread of this virus in New Zealand remains unknown. Smales *et al.* (1995) did however demonstrate high levels of mechanical transmission and it may be that invertebrates play a minor, if any, role in transmission and that cutting of pasture and stock grazing are more important (Upstone 1969; Catherall 1970).

Neither sitona nor the two native weevils tested, *I. stolidus* and *N. cervina* were shown to transmit AIMV or WCIMV respectively. This suggests that although they feed in a way in which plant material can adhere to mouth or body parts, defaecate on food plants and move widely between plants to feed, these weevils are not suitable vectors for the viruses tested.

The amount of feeding and oviposition (Table 1) by Argentine stem weevil was not significantly different on uninfected ryegrass and infected ryegrass. There was, however, significantly more feeding on uninfected cocksfoot plants than on CfMV infected plants (Table 1). Very few eggs were laid on cocksfoot regardless of infection by CfMV although the results suggest that the presence of CfMV may influence oviposition. Argentine stem weevil has been shown to discriminate between ryegrass tillers on the basis of nitrogen levels (Hunt and Gaynor 1982; Hunt *et al.* 1988) and the presence or absence of the endophyte *Acremonium lolii* (Barker 1989). Both of these factors influence feeding and oviposition and discrimination appears to be partly based on taste (Barker 1989). It is possible these weevils could detect the presence of virus in food plants and respond to it. This appears likely to be the case when CfMV is present in cocksfoot. The presence of RgMV did not appear to influence either feeding or oviposition in this investigation. Overall, there were significantly more eggs laid on both uninfected and infected ryegrass than on cocksfoot (Table 1) ($P < 0.05$). This agrees with Goldson (1982) who found that Grasslands Manawa ryegrass was preferred over an undescribed cocksfoot cultivar for oviposition but contrasts with Barker (1989) who showed both Grasslands Apanui cocksfoot and Grasslands Manawa ryegrass to be favourable plants for adult weevil feeding and oviposition. Neither of the viruses affected weevil survival over the period of the investigation.

TABLE 1: Amount of leaf area consumed (mm²) by weevils on healthy and CfMV, RgMV and AIMV infected plants and number of eggs laid by Argentine stem weevil on uninfected plants and virus infected ryegrass and cocksfoot.

	Ryegrass		Cocksfoot		Lucerne	
	Uninfected	RgMV	Uninfected	CfMV	Uninfected	AIMV
Feeding						
Expt 1	2.3	2.0	1.7	0.7	1.2	1.5
Expt 2	1.6	1.6	0.9	1.1	0.7	0.5
Expt 3			2.8	2.1		
SED		0.2		0.2		0.25
Oviposition						
Eggs/cage	34	22	6	0		
SED		8		-		

There was no significant difference in the amount of feeding by *S. discoideus* on lucerne not infected with AIMV and AIMV infected lucerne (Table 1) nor did AIMV have any observed effect on weevil survival.

CONCLUSION

The vectors of CfMV and WCIMV in New Zealand remain unknown. Argentine stem weevil appears to be able to detect CfMV and is influenced by its presence but fails to detect or is not influenced by the presence of RgMV. Similarly AIMV had no observed influence on sitona weevil.

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