INVERTEBRATE PESTS OF PASTURE AS POTENTIAL PLANT VIRUS VECTORS

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ABSTRACT

Pasture invertebrates are being studied to see if they have a role in spreading ryegrass mosaic virus and cocksfoot mottle virus to ryegrass and cocksfoot. Argentine stem weevil (*Listronotus bonariensis*), the grey field slug (*Deroceras reticulatum*) and porina (*Wiseana* spp) were investigated as potential vectors of cocksfoot mottle virus and ryegrass mosaic virus. The invertebrates were allowed to feed on infected plant material before being placed individually in containers containing non infected plants. These plants were grown on before being assayed for the presence of virus by ELISA. Faeces from slugs and porina were also tested for the presence of virus. One Argentine stem weevil transmitted RgMV to ryegrass and an unknown virus to cocksfoot. The grey field slug did not transmit RgMV or CfMV to ryegrass or cocksfoot respectively. Porina did not transmit CfMV to cocksfoot. RgMV and CfMV particles were found in the faeces of slugs and CfMV was detected in porina faeces. A previously unrecorded virus was detected during the course of these experiments. A chance finding has implicated *Rhopalosiphum* sp. in the transmission of CfMV.

Keywords: cocksfoot mottle virus, ryegrass mosaic virus, pasture pests, transmission, invertebrate vector

INTRODUCTION

Ryegrass mosaic virus (RgMV) has been demonstrated in Europe (Mulligan 1960; Jones *et al.* 1977; Heard *et al.* 1974) and Australia (Eagling *et al.* 1992) to reduce the yield of perennial ryegrass (*Lolium perenne* L.). RgMV has been shown to detrimentally affect the growth and persistence of annual ryegrass (*Lolium multiflorum* Lam.) and perennial ryegrass in pastures (Guy 1993) and has been associated with reductions in yield of annual ryegrass of up to 27% (Wilkins and Hides 1976). Holmes (1979) also demonstrated a reduction in organic matter, carbohydrate content and digestibility of RgMV infected ryegrass.

Cocksfoot mottle virus (CfMV) was first detected in New Zealand at Taupo by Mohamed (1980) and subsequently found at Waitati, Orokonui, Waianakaura, and Gore (P.L. Guy, unpublished). CfMV has been associated with severe mottling of cocksfoot (*Dactylis glomerata* L.) grown for forage and seed production (Catherall 1970) and can cause plant death (Upstone 1969).

Both these viruses may have the potential to directly or indirectly jeopardize maximum pastoral productivity in New Zealand. The means by which they are spread in New Zealand has not been investigated. Transmission of plant viruses between hosts often requires an invertebrate vector, in many instances arthropods (Harris 1981). For transmission to occur, pathogen, vector, and host populations must overlap both spatially and temporally. They must also interact in a way that meets the requirements of virus acquisition, retention and inoculation (Harris 1981). This may be through virus particles adhering to body parts, mainly mouth parts and tarsi, or by virus particles passing through regurgitant or salivary excretion to susceptible plant cells. Most beetles exhibit reflexive bleeding at the articulations of the legs and

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mandibles and in some cases virus particles have been found in this haemolymph (Fulton *et al.* 1987). Several plant viruses are transmitted by leaf-feeding beetles (Fulton *et al.* 1987). Of the known beetle vector species, 11 are weevils (Coleoptera: Curculionidae) (Harris 1981).

RgMV is spread when sap from infected plants comes into contact with sap from uninfected plants. The eriophyid mite (*Abacarus hystrix* Nal.) is the only known vector of RgMV and is present in New Zealand. RgMV is also thought to be spread mechanically by livestock treading and haycutting machinery (Upstone 1969).

Oulema melanopa (L.) and *O. lichenis* (Voet.) (Coleoptera: Chrysomelidae) have been shown to transmit CfMV from infected to uninfected cocksfoot in the United Kingdom (A'Brook and Benigno 1972) but neither are known to be present in New Zealand (Foord 1993), and the vector here is unknown.

This study investigated the ability of Argentine stem weevil (*Listronotus bonariensis* Kuschel), the grey field slug (*Deroceras reticulatum* Muller) and porina (*Wiseana* spp) to transmit RgMV and CfMV from infected to uninfected plants in laboratory experiments. In the field these pasture pests move widely between plants to feed. They also feed in a way in which plant material can adhere to mouth or body parts and they may defaecate on food plants. This behaviour implicates them as possible vectors in virus transmission.

METHODS

Ryegrass plants infected with RgMV and cocksfoot plants infected with CfMV were obtained from the University of Otago Botany Department. Infections were confirmed by ELISA (Enzyme linked immunosorbent assays) before being used in the investigation.

For the ELISAs plant material was ground in a Waring blender with 2 mls of PBS-etc buffer and the resulting extract collected in Micronic tubes. The standard ELISA procedure (Agdia Indirect ELISA) was followed for RgMV detection. For CfMV a direct double antibody sandwich technique (Clarke and Adams 1977) was used. For both RgMV and CfMV ELISAs the colour intensity of the resultant solution was assessed using a Metertech 960 ELISA microtitre plate reader at A405 nm.

Uninfected Grasslands Manawa ryegrass and Grasslands Wana cocksfoot plants were obtained by sowing single seeds into cells 27 x 27 x 45 mm deep, filled with commercial seed raising mix. These plants were grown in a greenhouse at 20 ± 2 °C for approximately 6 weeks before being used in the experiment. When presented to the invertebrates the root ball of each plant was wrapped in moist filter paper which kept the plant in good condition.

Argentine stem weevils were collected by vacuum sampling from a ryegrass/white clover (*Trifolium repens* L) pasture at Kyeburn, Otago, in March 1995. These were maintained in a controlled environment laboratory (20 ± 2 °C) with a 16:8 L:D photoperiod. Between weevil collection and the beginning of the bioassay the weevils were supplied with uninfected Grasslands Manawa ryegrass. After 3 weeks 71 weevils were starved for 24 h before being placed in a cage (185 x 165 x 70 mm deep) for 5 days with a ryegrass plant infected with RgMV. A control group of five weevils was supplied with uninfected ryegrass for the same time. A further 85 weevils were caged with a cocksfoot plant infected with CfMV and a control group of five weevils with uninfected cocksfoot both for 4 days. At the end of these periods the weevils were removed and caged individually in 75 ml polycarbonate specimen tubes fitted with gauze lids. Each tube contained an uninfected ryegrass or cocksfoot plant, according to the treatment. The weevils were left to feed for 48 h after which time they were removed and the plants potted up into 50 x 50 x 75 mm deep plastic pots and grown on for 2 weeks in the greenhouse before ELISAs were performed on the plant foliage.

Grey field slugs (mean extended length 38 mm (SEM= 0.7)) were collected from cabbages in an organic market garden at Mosgiel in late March 1995 and held in plastic cages containing cabbage leaves, in a dark cabinet at 10 °C for 7 days. For both the RgMV and CfMV bioassays slugs were individually placed in each of 55 petri dishes (9 cm dia.) lined with moist filter paper and containing a 35 mm length of virus infected

grass tiller. An additional five petri dishes, for each virus, containing uninfected tillers were used as controls. After 4 days the slugs were removed and placed in a fresh petri dish containing uninfected ryegrass or cocksfoot plants for 48 h. The plants were potted up and grown on as above.

Porina larvae, (mean length 26 mm (SEM=1.8)) collected from a ryegrass/white clover pasture in South Otago, were held without food at 2 °C for 4 days. Thirty eight larvae were then placed individually in petri dishes as above and provided with 35 mm lengths of CfMV infected cocksfoot leaf. An additional five dishes containing uninfected grass served as controls. After 4 days at 10 °C in a dark cabinet the larvae that had fed on the virus-infected leaves were placed in petri dishes containing uninfected cocksfoot plants for 48 h. These plants were grown on as for the Argentine stem weevil and slug bioassays.

The faeces of slugs fed virus infected leaves of ryegrass or cocksfoot, and the faeces of porina fed infected cocksfoot were tested for the presence of virus. This was done by placing 30 slugs in petri dishes as above with infected ryegrass, 19 slugs with infected cocksfoot and 21 porina larvae with infected cocksfoot for 10 days. Control groups of five slugs and five porina larvae were fed on uninfected leaves. The filter paper was changed on the fifth day and on day ten 0.1 g of faeces, for slugs, and 0.035 g for porina, from each dish was ground using a mortar and pestle in 2 mls of PBS-etc buffer and tested for the presence of virus by ELISA.

The susceptibility of ryegrass and cocksfoot to mechanical inoculation with RgMV and CfMV respectively was tested. Ryegrass and cocksfoot seedlings were dusted with carborundum powder and then rubbed with sap collected from RgMV infected ryegrass diluted 1:2 with tap water and sap from CfMV infected cocksfoot diluted ten fold respectively. Plants were tested for virus infection, 3 weeks after treatment by ELISA.

Cocksfoot plants infected with CfMV and uninfected cocksfoot plants growing together in a mesh covered container were unintentionally subjected to an aphid (*Rhopalosiphum* sp.) outbreak. The "uninfected" plants were subsequently tested by ELISA.

RESULTS

None of the ELISAs carried out on material from the control bioassays gave a positive test (Table 1). Argentine stem weevil produced one positive result for RgMV but only negative results for CfMV. The grey field slug gave negative results for the feeding experiments but both viruses were detected in the faeces. Porina also gave negative results for plant material but almost half of the faecal samples contained CfMV. The mechanical transmission tests gave 18% infection of ryegrass with RgMV and 44% infection of cocksfoot with CfMV.

TABLE 1: Incidences of virus transmission by Argentine stem weevil, grey field slug, porina and mechanical means, and the incidence of virus in slug and porina faeces.

	Number infected with virus/number of tests		Number infected with virus/number of tests	
	Ryegrass with RgMV Test	Control	Cocksfoot with CfMV Test	Control
Plants				
Argentine stem weevil	1 /71	0 /5	0 /85	0 /5
grey field slug	0 /55	0 /5	0 /55	0 /5
porina	-	-	0 /38	0 /5
mechanical	12 /65	-	29 /66	-
Faeces				
slug	1 /30	0 /3	7/19	0 /3
porina	-	-	10/21	0 /3

During the course of experiments one Argentine stem weevil did transmit an as yet unidentified virus to cocksfoot after feeding on a CfMV infected plant obtained from the field. This unknown virus produced visual symptoms similar to CfMV but gave a negative result for the ELISA and was not serologically related to CfMV or eight other isometric viruses from pasture grasses. Under the electron microscope it was seen to be an isometric particle about 30 nm in diameter.

A large percentage of the non-infected plants exposed to aphid attack developed symptoms of CfMV infection. When tested by ELISA, 23 out of the 32 uninfected plants (72%) were shown to contain the virus.

DISCUSSION

Argentine stem weevil is New Zealand's most important pasture pest and is estimated to cost \$78-251 million annually in terms of yield losses, pasture renovation costs and costs associated with reduced animal health brought about by changes in pasture quality (Prestidge *et al.* 1991). Argentine stem weevil is widespread throughout New Zealand and the grasses attacked include perennial and Italian ryegrass and cocksfoot. The grey field slug and porina are also serious pasture pests in New Zealand. The possibility that these agricultural pests may be acting as vectors for the spread of RgMV and CfMV has not been considered until recently.

In this investigation Argentine stem weevil was shown to be capable of transmitting RgMV from infected to uninfected ryegrass plants. Previously the only recorded vector was the mite *Abacarus hystrix* (Slykhuis 1972). RgMV is a potyvirus and until now there are no records of any beetle vectors transmitting any virus within this group (Fulton *et al.* 1987). Although only a low level of transmission of RgMV by Argentine stem weevil was demonstrated, further investigations are warranted to determine the importance of this finding. The mechanism of virus transmission is unknown. The virus may have been transmitted via feeding wounds or as contaminants on the mouthparts or tarsi. Alternatively, as in the case of some chrysomelid beetles, virus could be transmitted in regurgitant fluid during feeding (Dale 1953). There is no documentation of Argentine stem weevil regurgitating during feeding.

CfMV is a sobemovirus. Other viruses in this group are known to be transmitted by beetles and it was thought possible that Argentine stem weevil may be able to transmit CfMV. This was not demonstrated.

Neither the grey field slug nor porina were shown to transmit RgMV or CfMV from infected to uninfected ryegrass or cocksfoot plants respectively. Both RgMV and CfMV were, however, detected in the faeces of slugs and CfMV was detected in porina faeces. This shows that the virus can pass undegraded through the gut of slugs and porina suggesting that transmission through faecal matter via feeding wounds may be a potential mechanism for transmission. This also warrants further investigation.

It is possible that reduced feeding by the test invertebrates as a result of being in an artificial environment may have contributed to low or nil transmission rates but reasonable amounts of feeding appeared to have occurred in most instances. Another explanation for low rates of transmission may be that the plants were resistant to invertebrate transmission of viruses. Gergerich *et al.* (1991) suggested that plant hosts susceptible to mechanical transmission of a virus may be resistant to transmission by natural beetle feeding. Fulton *et al.* (1987) also suggested that the efficiency of transmission of viruses is controlled by the host plant. The lack of virus transmission by Argentine stem weevil may also be due to the virus itself because for some viruses the efficiency of transmission is consistently low (Fulton *et al.* 1987).

One Argentine stem weevil did transmit an unknown virus to cocksfoot. This along with the positive transmission in one instance of RgMV are the first recordings of an Argentine stem weevil transmitting viruses. Experiments are currently aimed at determination of the strain and serology of the new virus.

The chance result that aphids may be acting as vectors for CfMV is the first record of CfMV transmission by aphids. However other members of this virus group can be transmitted by aphids. Further investigations of aphid transmission are in progress.

Although Argentine stem weevil was demonstrated in one instance to transmit RgMV the investigations have not demonstrated that Argentine stem weevil, the grey field slug or porina are efficient vectors of RgMV and CfMV in the laboratory. However the results indicate that further research is warranted in all cases. Aphids were observed to transmit CfMV but the significance of this requires further investigation.

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