A SURVEY OF RYEGRASS MOSAIC VIRUS AND ENDOPHYTE IN OTAGO AND SOUTHLAND

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ABSTRACT

Twenty four ryegrass /white clover pastures throughout Otago and Southland were surveyed for the presence of ryegrass mosaic virus (RgMV), *Acremonium lolii* and eriophyid mites. Densities of Argentine stem weevil were measured at eight sites. RgMV was found to be widespread and was recorded from 15 locations for the first time. Infection of up to 48% of ryegrass tillers by RgMV was measured. *A. lolii* was also found to be widespread with levels of up to 94% recorded. Mites were found at six sites. No correlation of RgMV incidence with *A. lolii*, presence of mites or Argentine stem weevil densities was detected. A comparison of *A. lolii* detection by ELISA and epidermal staining was carried out. The ELISA technique appeared to have greater sensitivity.

Key words: *Acremonium lolii*, ryegrass mosaic virus, Argentine stem weevil, ELISA

INTRODUCTION

Ryegrass mosaic virus (RgMV) is a member of the potyvirus group of plant viruses. Ryegrasses are the most important hosts but other grasses such as cocksfoot (*Dactylis glomerata* L.), fescue (*Festuca* spp.) and annual meadow grass (*Poa annua* L.) can be infected (Clements *et al.* 1978). RgMV has a world wide distribution and is considered the most serious and widespread sap-transmissible virus infecting Gramineae in Britain (Clements *et al.* 1978) and Australia (Eagling *et al.* 1992). Its effects on pasture production in New Zealand have not been determined.

The virus causes pale green streaks in the leaf lamina and in severe cases, the leaf may show yellow flecking which is followed by necrosis and death of the tiller (Clements *et al.* 1978). Overseas studies indicate that RgMV infection can reduce dry matter production of Italian ryegrass (*Lolium multiflorum* Lam.) by up to 27% (Wilkins and Hides 1976), and by up to 50% in perennial ryegrass (Eagling *et al.* 1992). Holmes (1979) also demonstrated a reduction in organic matter, carbohydrate content and digestibility of RgMV infected ryegrass. RgMV was first detected in New Zealand at a site near Dunedin in 1992 (Guy 1993a). It is not known how long the virus has been present in New Zealand and its full distribution is not known.

Eriophid mites are known to transmit at least six plant viruses including RgMV (Mulligan 1960). *Abacarus hystrix* Nal. (Acarina: Eriophyidae) is the mite responsible for most RgMV transmission (Slykhuus 1972). *A. hystrix* is widely distributed in the northern hemisphere and Australia (Frost 1992) and occurs in South Africa (Salm *et al.* 1994). It has also been recorded in New Zealand (Guy 1993a) but its distribution is unknown. Argentine stem weevil (*Listronotus bonariensis* Kuschel) (Coleoptera: Curculionidae) has been shown to transmit RgMV but appears to be an inefficient vector (Smales *et al.* 1995). RgMV can also be transmitted by farm machinery and livestock (Catherall 1987).

The endophyte *Acremonium lolii* (Latch, Christenson and Samuels) in perennial ryegrass (*Lolium perenne* L.) is recognized as having a major impact on pasture performance in New Zealand. The relationship between plant and endophytic fungus

is considered mutualistic with the host grass benefiting from increased plant growth (van Heeswijk and McDonald 1992) and resistance to Argentine stem weevil (Goldson and Penman 1979; Prestidge *et al.* 1982). The presence of endophyte may also confer a degree of protection to plants from virus diseases. Latch *et al.* (1985) found that the main aphid vector of barley yellow dwarf virus (BYDV), *Rhopalosiphum padi* L. (Hemiptera: Aphididae) avoided fescue plants (*Festuca arundinacea* Schreber) infected with the endophyte *Acremonium coenophialum* (Morgan, Jones, and Gams). However the presence of *A. lolii* has no effect on the incidence of BYDV in perennial ryegrass (Guy 1993b). It is not known whether the presence of *A. lolii* confers resistance to ryegrass against infection by RgMV.

This investigation aimed to provide data to determine the distribution of RgMV in the southern South Island and relate this to the presence of its known mite vector and Argentine stem weevil. It also investigated the relationship between incidence of RgMV and *A. lolii* infection. ELISAs (Enzyme Linked Immunosorbent Assays) were used to determine *A. lolii* incidence in the survey and this technique was compared with the more commonly used epidermal staining technique.

METHODS

Survey of pastures

Fifty ryegrass tillers were collected from each of 25 pasture sites throughout Otago and Southland (Table 1) between September and November 1995. Tillers were collected by walking in a broad diagonal zigzag across the paddock, avoiding paddock margins, and hand picking one tiller every 20 paces. The whole grass tiller including the base was taken, placed in a plastic bag and stored at 4°C until a standard ELISA (Agdia Indirect ELISA) was carried out to detect RgMV.

A part of each sample prepared for RgMV detection was used to determine the presence of *A. lolii* by a double antibody sandwich (DAS) ELISA specific to *A. lolii* (Clark and Adams 1977) with some modifications (Ian Garthwaite, AgResearch, pers comm.).

Immediately prior to the preparation of samples for the ELISAs, the adaxial surface of 20 tillers, randomly selected from each pasture, were examined under a binocular microscope for the presence of eriophyid mites.

Densities of adult Argentine stem weevil were measured at eight sites by digging 150 x 150 cm x 30 cm deep turfs from the pastures and heat extracting weevils from these in Tugren funnels (Proffitt *et al.* 1993).

Data were analysed by plotting one variable against the other and exploring the relationships.

Comparison of *A. lolii* detection by ELISA and epidermal staining

Two tillers from each of 219 ryegrass plants collected from five ryegrass / white clover (*Trifolium repens* L.) dominated pastures were examined for the presence of *A. lolii*. A 2-3 cell thick epidermal strip taken from the base of the leaf sheath of one tiller from each pair was stained with lactophenol cotton blue. This was examined for the presence of *A. lolii* hyphae under a microscope. The second tiller from each pair was tested using ELISA methods described above.

RESULTS AND DISCUSSION

Survey of pastures

The results showed that RgMV is widespread in ryegrass dominated pastures throughout Otago and Southland (Table 1). Of the pastures sampled, 60% contained RgMV with the highest level recorded being 48% of tillers infected (Table 1). This is the highest level recorded to date in the South Island but a level of 56% has been reported from Kaikohe, Northland (D. Webster, pers. comm.). The results of this survey are new records for RgMV.

A. lolii was also found to be widespread (Table 1). The only pasture where *A. lolii* was absent was at Invermay. In this case the paddock had been sown with endophyte free Grasslands Manawa ryegrass (*L. (perenne x multiflorum) x L. perenne*). The other Invermay site was also a Grasslands Manawa pasture and the 2% of tillers with *A. lolii*

here were probably from volunteer perennial ryegrass plants. All other sites were perennial ryegrass based and the levels of infection ranged from 12-94% (Table 1). This result supports the findings of Ferguson *et al.* (1994) who also showed widespread occurrence of *A. lolii* and similar levels of infection throughout Otago and Southland. No negative relationship was found between the levels of RgMV and *A. lolii* in the pastures. Excluding tillers from the Invermay sites, 78% of tillers infected with RgMV were also infected by *A. lolii*. These results suggest that, unless infection by RgMV occurs in seedlings before *A. lolii* expresses itself, the presence of *A. lolii* in a ryegrass plant does not confer resistance against RgMV infection. The high numbers of tillers infected with both organisms could indicate that there may be a positive relationship between RgMV and *A. lolii*. However, the results from the Invermay sites show infection by RgMV in the absence of *A. lolii*, and the majority of tillers at the other sites containing endophyte were not infected by RgMV.

Eriophyid mites were detected at only six of the sites sampled (Table 1). Identification of these mites to species level is difficult and it was not possible to confirm identification as *A. hystrix*. The most common eriophyid mite in southern South Island pastures is *Aculodes mckenziei* (Guy and Gould 1996) which has not as yet been shown to transmit RgMV. These two mites are difficult to distinguish from each other. This study failed to establish any link between the presence of eriophyid mites and RgMV.

TABLE 1: Incidence of RgMV and *Acremonium lolii*, densities of adult Argentine stem weevil (ASW) and eriophyid mites in Otago and Southland ryegrass pastures.

Location	RgMV (%)	<i>A. lolii</i> (%)	ASW/m ²	Mites/20 tillers
Ikiwai	30	70	-	0
Ophir 1	28	44	11	6
Ophir 2	4	79	38	2
Ophir 3	12	29	7	16
Ophir 4	20	94	16	5
Kyeburn	4	68	47	0
Redbank	13	43	-	7
Palmerston	0	30	-	0
Sutton 1	20	-	-	0
Sutton 2	27	70	56	0
Invermay 1	25	2	34	0
Invermay 2	48	0	9	0
Raes Junction	2	92	-	0
Puketi	0	41	-	0
Milton	0	30	-	36
Balfour	7	27	-	0
Waipahi	2	55	-	0
Riversdale	0	12	-	0
Mossburn	0	15	-	0
Otautau	29	63	-	0
Balclutha	0	14	-	0
Woodlands	0	70	-	0
Owaka	0	63	-	0
Tokanui	0	39	-	0
Stewart Island	0	89	-	0

Argentine stem weevil was found at all sites sampled (Table 1) but no correlation was found between weevil densities and levels of infection by RgMV. Although Smales *et al.* (1995) showed Argentine stem weevil was capable of transmitting

RgMV, it appeared to be an inefficient vector and the current result is not, therefore, unexpected.

Comparison of *A. lolii* detection by ELISA and epidermal staining

Of the 219 tests carried out comparing the two methods of *A. lolii* detection, the techniques agreed on 64 % of the samples. The ELISA detected *A. lolii* on 26 % of tests where it was not detected by epidermal staining while the reverse applied on only five occasions. Seventeen tests gave unclear ELISA results due to high background interference. Assuming that endophyte occurs in all tillers in the plant, it appears that the ELISA is a more sensitive method than epidermal staining. This is partly because it is quite conceivable that fungal hyphae may be absent from the epidermal sample even though it is present in the plant.

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