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Plant viruses.

R. A.C. Jones

DEPARTMENT OF AGRICULTURE

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PLANT VIRUSES

R.A.C. JONES

PLANT VIROLOGIST

PLANT RESEARCH DIVISION

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1. CUCUMBER MOSAIC VIRUS IN LEGUMES

(a) Occurrence in West Australian lupin and subterranean clover breeding programmes, and in annual medic propagation plots

Very widespread infection of lupins in the West Australian lupin breeding programmes with seed-borne CMV resulting in stunted, chlorotic plants with bunched leaves was first noticed in July on the South Perth plots. Among the F_1 lupin progenies 102/336 belonging to one breeder were affected as were 59/95 of those of a second breeder. This infection was due to the inadvertent use of infected mother plants for crossing in 1985 and previously. In the historical lupin cultivar collection, 13/16 named cultivars of L. angustifolius contained infected plants. Infection with seed-borne CMV was also very widespread in crossing block lines and in the wild lupin species collection. A similar picture was revealed when later phases of the lupin breeding programmes were examined at Medina and Badgingarra, with very widespread infection being found throughout the material being grown. After the end of August, aphid activity resulted in a considerable amount of current season spread from seed-infected plants to healthy plants, causing bunching and chlorosis at shoot apices and decreased pod set. Extensive roguing out of symptom-bearing plants and use of insecticides was recommended for all three sites to decrease this spread.

Seed of the new lupin cultivar Wandoo (grown at Badgingarra in 1985) was found to be 3-31% infected with CMV. Crops grown for certified seed production in 1986 contained up to 50% of infected plants when inspected; seed-borne and current season spread types of symptoms were both present. It was found on inspection that where wide spacing occurred in the Wandoo crops the seed-infected plants continued to grow, act as sources of infection for neighbouring plants and produce some seed. However, with dense spacing they were shaded out and killed except near the edges of the paddocks where more open growing conditions permitted their survival and the development of infected patches around them due to current season spread.

Infection with CMV was very widespread in subterranean clover breeding and introduction plots at the University of Western Australia field station at Shenton Park and on the South Perth plots. Symptoms of stunting, leaflet downcurling, mottle and/or leaf pallor were present in about 20% of plots. Other plots had less severe symptoms (faint mottle). Cultivars which were severely affected included Northam, Esperance, Green Range, Enfield and Nangeela. Cultivars in which only mild symptoms were noted included Trikkala, Meteora, Dininnup, Dalkeith and Nungarin. In glasshouse inoculations with CMV, severe symptoms (stunting, leaflet downcurling and mottle) were reproduced in cultivar Daliak by a CMV isolate from cultivar Nungarin.

When 16 Medicago murex introduction plots at South Perth were examined four had plants with severe stunting, mottle and leaf deformation. These symptoms were caused by CMV. Similarly, the virus was found in samples of poorly-growing plants from three M. murex introduction plots at Medina.

(b) Rates of seed transmission in lupins and subterranean clovers, and development of an ELISA test for seeds

Lupins

In glasshouse growing on tests on suspect lupin seed lots from different sources, the following levels of CMV seed transmission were obtained:

Cultivar	Source and year of harvest	% seedlings infected
Wandoo (IA 886)	Badgingarra (home farm), 1985	25
" (IA 786)	Badgingarra (home farm), 1985	31
" (IA 686)	Badgingarra (new land), 1985	3
" (commercial)	Beverley, 1986	2
Illyarie (commercial)	Dongara, 1986	13
" (IB 385)	Chapman, 1984	3
" (seed crop)	Chapman, 1984	8
Blanco (Historical cultivar collection)	Medina, 1984	3
261 (Pedigree 3)	Badgingarra (new land), 1986	0.2

These results confirm that high levels of seed transmission of CMV can occur in narrow leafed lupins and suggest that both commercial seed stocks and stocks of seed belonging to the Department of Agriculture are likely to be carrying the virus.

In glasshouse growing on tests on seed samples from F_1 progenies of lupins from crosses made at South Perth in 1985, levels of seed transmission were as follows:-

F_1 lupin progeny	Seedlings infected/ Total No. of seedlings
85A024	6/30
85A122	7/16
85A123	10/10
85A153	4/12
85A163	0/9
85A217	1/9
85A219	1/11

These results confirm that infection with CMV is present in seeds at the very beginning of the lupin breeding programmes.

In an experiment to determine whether CMV infection was related to seed size, infected Wandoo (IA 686) seed was sieved differentially, the seed fractions obtained grown on in the glasshouse and the seedlings tested for infection:-

Sieve size		% seedlings infected
1.	Unsieved Control	34
2.	6.35 mm round hole	26
3.	5.95 mm round hole	28
4.	5.11 x 19 mm slot	31
5.	4.80 x 19 mm slot	35
6.	4.29 x 19 mm slot	62

These results suggest that sieving cannot be used to clean up CMV infected seed lots, though small seed has a greater likelihood of being infected and might still be worth screening out.

Subterranean clovers

In glasshouse growing on tests on seed lots of subterranean clover harvested in 1985, the following seed transmission rates were obtained:-

Cultivar	Source	% seedlings infected
Green Range	Shenton Park	8
Enfield	South Perth	8
Esperance	"	1
Meteora	"	1
Nungarin	"	1
Northam	"	0.6

Thus, seed stocks from Shenton Park and South Perth are likely to be carrying CMV.

ELISA rapid seed test

In preliminary work to develop a reliable labour-saving laboratory procedure for indexing for CMV in lupin seed lots, preliminary results with a modified ELISA suggest that one infected lupin seed can still be detected when grouped with 49 healthy seeds and ground to a powder in a mill for testing. Further work is needed, however, to overcome high non specific background reactions which develop with extracts of seeds. At this level of grouping (1/50), only 20 actual grouped samples would be needed to index seed lots containing 1,000 seeds for virus presence, and results can be obtained within two days. By contrast, growing on seedlings in the glasshouse and testing grouped leaf samples by ELISA or by sap transmission to test plants takes up to 5 weeks for results to be obtained and is far more labour intensive especially when test plants are used. ELISA can also be developed for routine testing of medic and clover seeds for presence of CMV. (ELISA work on lupins done by T. Baker.)

(c) Weed hosts

Weed species growing on the South Perth plots were tested repeatedly for CMV during August-October. For each species at least 26 plants were tested as grouped samples. The results were as follows:

Most important hosts for CMV

Fumitory (Fumaria officinalis)
Stagger weed (Stachys arvensis)
King Island melilot (Melilotus indica)

CMV detected in two grouped samples

Flatweed (Hypochaeris glabra)
Wild radish (Raphanus raphanistrum)
Stonecrop (Crassula sp.)
Subterranean clover (Trifolium subterraneum)

CMV detected in one grouped sample

Capeweed (Arctotheca calendula)
Cornspurry (Spergula arvensis)
Hop clover (Trifolium campestre)
Hare's-foot clover (Trifolium arvense)
Lesser snapdragon (Misopates orontium)
Hyssop loosestrife (Lythrum hyssopifolia)
Monopsis simplex

No CMV detected

Evening primrose (Oenothera stricta)
Fleabane (Conyza bonariensis)
Four O'clock (Oxalis latifolia)
Paterson's curse (Echium plantagineum)
Pimpernel (Anagallis arvensis)
Sowthistle (Sonchus asper)
Blackberry nightshade (Solanum nigrum)
Common vetch (Vicia sativa)
Purple vetch (Vicia benghalensis)
Yellow seradella (Ornithopus compressus)

These findings have important implications regarding control of weeds in plot areas. Far more effective control than has been the case in 1986 would be required to combat this potent reservoir of infection with CMV. In particular, it would seem advisable to remove plants of fumitory, stagger weed and king island melilot as these are the most significant weed sources of CMV, but removal of the other weed species found infected would also be necessary. In addition, volunteer lupins should be controlled (c. 25% were CMV infected on the South Perth plots).

The role of clover pastures as reservoirs for infection of lupins with CMV could be important and requires further study.

(d) Recommendations for control

Based mostly on the results and observations described above, the following strategy could be applied in seeking to decrease or prevent CMV spread in lupins:-

- Avoid use of CMV infected seed stocks for planting.
- Use high seed rates (e.g. greater than 60 kg/hectare) to generate dense stands thereby shading out plants infected via the seed.
- Control weed hosts.
- Use wide cereal barriers to protect paddocks surrounded by clover or medic pastures from incoming viruliferous aphids.
- Protect small plots from incoming aphids using reflective mulch (or narrow cereal barriers).
- Use a prophylactic regular insecticide spraying regime on small plots to prevent aphid build up.
- Spray paddocks with insecticides if aphid build up becomes apparent (regular sprays too expensive).
- Regular inspection of small plots and important seed crops to determine if symptoms of CMV are present.
- Wherever possible, rogue out plants with symptoms especially with small plots.
- Strict application of appropriate tolerances for certification of seed crops.

2. ALFALFA MOSAIC VIRUS IN ANNUAL MEDICS

(a) Occurrence in medic introduction and propagation plots

Alfalfa mosaic virus (AMV) was widespread in medic plots at South Perth, 5/16 plots tested were found to be infected showing symptoms of mottle in young leaves (and later possibly early plant senescence). At Medina, 5/11 plots sampled were found infected, and the one plot sampled at Busselton was also infected. Some of the infected lines were of M. murex and some of M. polymorpha. Important propagation lines found infected included M. polymorpha cv. Circle Valley, N3816 and N4816, and M. murex CD 64.11.1.

(b) Rates of seed transmission

In glasshouse growing on tests on seed lots of annual medics harvested in 1985, the following seed transmission rates were obtained for AMV:-

Medicago species	Seed line	Source	% seedlings infected
M. polymorpha	cv. Circle Valley	South Perth	49
M. murex	CD 64.11.1	Busselton	14

3. BEAN YELLOW MOSAIC VIRUS: probable role of clovers as the principal reservoir of infection for lupins

Bean yellow mosaic virus (BYMV) seemed to be the cause of patches of stunted, mottled plants common in subterranean clover paddocks north of Pingelly. It was also the virus most commonly found in samples submitted for diagnosis from subterranean clover trials in different localities in Western Australia.

When lupin crops were examined in different localities, BYMV infection was evident scattered throughout the crop causing death of plants. However, it was always commonest in the plants near the edges of paddocks killing up to 50% of them. Consistently, the affected paddocks were surrounded by subterranean clover pastures. The virus is not seed transmitted in narrow-leafed lupin but is probably seed-transmitted at a low level in subterranean clovers. It is likely that subterranean clovers are the main source of BYMV for spread into the lupin paddocks. What role native legumes may play as reservoirs for infection of lupins is unknown. Cereal barriers around lupin crops would seem worth trying for controlling BYMV spread into paddocks surrounded by pastures.

4. LUPIN PHYLLODY DISORDER

At the end of the growing season in 1986 many lupin paddocks showed plants which remained green after the others had dried off. Bunched leaves remained at the growing points but other leaves were mostly shed. Flower parts on affected plants were partially or entirely converted to leaves (= phyllody), a classic symptom of mycoplasma infection. Pod set was greatly decreased or absent. Incidence was usually less than 1% scattered through the crop. This was the case at five sites in the Geraldton region, one near Merredin and one at Lake Grace. However, in the Esperance region, two paddocks were 40-50% infected, one 25% infected and another 15% affected with plants with the disorder showing up in patches. Severe losses in grain yield resulted at the Esperance locations.

Dienes stain tests on stem sections (done by L. Price) revealed the typical reaction for mycoplasma infection in samples from the affected paddocks. Sections are being cut for observation in the electron microscope to confirm the probable mycoplasmal nature of the phyllody disorder.

5. CARNATION VIRUS OUTBREAK

An outbreak of a complex of two viruses - carnation necrotic fleck and carnation mottle - occurring together in the same plants caused considerable concern to flower growers in late 1986. Symptoms were leaf tip burn and mottling, decreased flower size and brittle stems. A third virus - carnation latent - was sometimes also present. Infection with the complex was found most commonly in the cultivar Alice. Carnation necrotic fleck virus, the most damaging of the three viruses, had not previously been reported in Western Australia.

The disease complex was being spread by propagation of cuttings from infected plants. Diseased plants had been distributed widely by one propagator to growers in the State. At some sites spread had then occurred to other carnation cultivars. It was recommended that all infected plants should be destroyed by growers to eliminate sources of infection and that aphids should be carefully controlled on nearby plants using insecticides as carnation necrotic fleck virus is readily spread by aphids.

6. ROUTINE VIRUS DIAGNOSIS

More than 250 samples were received for virus diagnosis during the period July 1 to December 31, these were mostly tested by sap transmission and/or by electron microscopy; dienes stain was used for suspect mycoplasmal diseases. Of these samples, 153 belonged to a wide spectrum of horticultural and agricultural plants but the rest were of lupins received as leaves or seeds for testing for presence of cucumber mosaic or bean yellow mosaic viruses. Samples received included the following:

Flowers and ornamentals - carnation, crysanthemum, acacia, cactus, iris, tulip, daffodil, camellia, philodendron, poppy, orchids, kangaroo paw, lily, dahlia, eucalyptus, rose.

Vegetables - globe artichoke, pumpkin, cabbage, cauliflower, rhubarb, tomato, cucumber, capsicum, potato.

Fruits - peaches, grapevine, tamarillo, pepino, cherry, watermelon, passionfruit, babaco, chestnut, cape gooseberry, cashew.

Agricultural crops and pastures - oats, wheat, barley, triticale, lupins, subterranean clover, white clover, rose clover, shaftal clover, annual medics, peas.

Apart from carnation necrotic fleck virus, all the viruses found that were fully identified, had been reported previously in Western Australia. However, probable mycoplasma-like diseases were found for the first time in the State in poppy, lupin, acacia and Eucalyptus platypus. These were detected using dienes stain (done by L. Price).