

Industry Biosecurity Plan for the Grains Industry Threat Specific Contingency Plan

Red Clover Vein Mosaic Virus

Prepared by Dr Angela Freeman
and Plant Health Australia

July 2008

Disclaimer:

The scientific and technical content of this document is current to the date published and all efforts were made to obtain relevant and published information on the pest. New information will be included as it becomes available, or when the document is reviewed. The material contained in this publication is produced for general information only. It is not intended as professional advice on any particular matter. No person should act or fail to act on the basis of any material contained in this publication without first obtaining specific, independent professional advice. Plant Health Australia and all persons acting for Plant Health Australia in preparing this publication, expressly disclaim all and any liability to any persons in respect of anything done by any such person in reliance, whether in whole or in part, on this publication. The views expressed in this publication are not necessarily those of Plant Health Australia.



1	Purpose of this Contingency Plan.....	4
2	Pest information/status.....	4
2.1	Pest Details	4
2.1.1	General information	4
2.1.2	Life cycle.....	4
2.1.3	Dispersal.....	4
2.2	Affected Hosts.....	5
2.2.1	Host range.....	5
2.2.2	Geographic distribution.....	5
2.2.3	Symptoms	5
2.3	Entry, establishment and spread.....	6
2.4	Diagnostic information	7
2.4.1	Diagnostic protocol	7
2.5	Response checklist.....	7
2.5.1	Checklist.....	7
2.6	Delimiting survey and epidemiology study	8
2.6.1	Sampling method	8
2.6.2	Epidemiological study	8
2.6.3	Models of spread potential	9
2.6.4	Pest Free Area (PFA) guidelines	9
2.7	Availability of control methods.....	9
2.7.1	General procedures for control.....	9
2.7.2	Control if small areas are affected	10
2.7.3	Control if large areas are affected.....	10
2.7.4	Cultural control	10
2.7.5	Host plant resistance	11
2.7.6	Chemical control	11
3	Course of Action – Eradication Methods.....	11
3.1	Destruction strategy.....	11
3.1.1	Destruction protocols	11
3.1.2	Decontamination protocols.....	11
3.1.3	Priorities.....	12
3.1.4	Plants, by-products and waste processing	12
3.1.5	Disposal issues	12
3.2	Quarantine and movement controls.....	13

3.2.1	Quarantine priorities.....	13
3.2.2	Movement control for people, plant material and machinery	13
3.3	Zoning.....	13
3.3.1	Destruction zone	13
3.3.2	Quarantine zone.....	13
3.3.3	Buffer zone	14
3.3.4	Restricted Area	14
3.3.5	Control Area	14
3.4	Decontamination and farm clean up	14
3.4.1	Decontamination procedures	14
3.4.2	Decontamination if disease is identified in a small area	14
3.4.3	Decontamination if disease is identified in large areas	15
3.4.4	General safety precautions.....	15
3.5	Surveillance and tracing	15
3.5.1	Surveillance.....	15
3.5.2	Survey regions	15
3.5.3	Post-eradication surveillance	16
4	References.....	17
5	Appendices	19
Appendix 1.	Standard diagnostic protocols	19
Appendix 2.	Experts, resources and facilities	19
Appendix 3.	Communications strategy	20
Appendix 4.	Market access impacts	20

1 Purpose of this Contingency Plan

This Contingency Plan provides background information on the pest biology and available control measures to assist with preparedness for an incursion into Australia of Red Clover Vein Mosaic Virus. It provides guidelines for steps to be undertaken and considered when developing a Response Plan to this pest. Any Response Plan developed using information in whole or in part from this Contingency Plan must follow procedures as set out in PLANTPLAN and be endorsed by the National Management Group prior to implementation.

2 Pest information/status

2.1 Pest Details

2.1.1 General information

Taxonomic position – Family: Flexiviridae

Red clover vein mosaic virus (RCVMV) (genus *Carlavirus*, family Flexiviridae) is predominately a virus of temperate pasture species, particularly clovers, but it also infects a number of temperate pulses including *Cicer arietinum* (chickpea), *Lathyrus odoratus* (sweet pea), *Lens culinaris* (lentil), *Pisum sativum* (field pea), and *Vicia faba* (faba bean, broad bean, tick bean). RCVMV is seedborne in *Trifolium pratense* (red clover), *Pisum sativum* and *Vicia faba*. It occurs in North America, South Africa, Europe, and India. Although it is exotic to Australia and causes serious disease and yield loss in a range of important pulse and pasture legume species grown in Australia it is not listed by AQIS as a quarantineable virus.

RCVMV has a wide host range among temperate pulses and annual and perennial legume pasture species. It is spread non-persistently by a range of aphid vectors, including the pea aphid (*Acyrtosiphon pisum*) and the green peach aphid (*Myzus persicae*). It is also seed transmitted in red clover, peas and faba beans. It survives between growing seasons of annual pulse hosts in perennial pasture legumes and in seed.

2.1.2 Life cycle

RCVMV is an obligate plant pathogen. It survives in perennial legume hosts such as clovers or possibly in infected red clover, pea or faba bean seed. Initial infection of a crop occurs when aphid vectors feed on the infected pastures and become infected with the virus and then feed on the crop. Secondary spread occurs within the crop during aphid vector feeding.

2.1.3 Dispersal

RCVMV has been reported to be transmitted by the following aphids: *Acyrtosiphon pisum*, *Aphis fabae*, *Cavariella aegopodii*, *C. theobaldi*, *Myzocallis onomidis*, *Myzus persicae* and *Therioaphis maculate* (Weber and Hampton 1980, Edwardson and Christie 1991). Seed transmission has been reported in red clover (*Trifolium pratense*) (Matsulevich 1957, Sander 1959, Brunt et al. 1997, Edwardson and Christie 1991, Kraft et al. 1998), faba bean (*Vicia faba*) (Sänder 1959) and field pea (*Pisum sativum*) (Kraft et al. 1998) but is not common.

2.2 Affected Hosts

2.2.1 Host range

Chickpea, clover spp., African daisy, sweet pea, lentil, alfalfa, french bean, field pea, purple globe clover, kura clover, alsike clover, crimson clover, red clover, white clover, subterranean clover, arrow leaf clover, faba bean, common vetch.

2.2.2 Geographic distribution

Czechoslovakia, India, Italy, Lithuania, Netherlands, South Africa, United Kingdom, USA.

2.2.3 Symptoms

Red Clover Vein Mosaic Virus (RCVMV) generally causes vein mosaic, mosaic, streaking and stunting in various legumes (Varma 1970). RCVMV causes a characteristic chlorosis of leaf veins, veinlets and tissue immediately adjacent to the veins in red clover and reduces its yield by reducing the foliage growth, decreasing persistence and increasing susceptibility to root rots (Khan et al. 1978). Vein mosaics are a common symptom of RCVMV in many clover species (Sander 1959) although Gibbs et al. (1966) found that white clover plants were symptomless when infected with RCVMV unless also infected with Clover yellow vein virus (CYVV).

The first report of RCVMV in pulses was of a disease called Wisconsin pea stunt which was characterised by severe plant stunting, tight apical rosetting, leaves of reduced size, often wrinkled and folded upward showing marked vein clearing (Hagedorn and Walker 1949). Rubio-Huertos and Bos (1973) reported that in most pea cultivars, the first symptoms of systemic infection were usually systemic vein clearing and leaf curling although some cultivars developed necrotic stem streaking sometimes followed by irregular yellowing and premature plant death. Bos et al. (1972) isolated a new highly deviating strain of RCVMV which caused necrotic stem streaking in peas but found that although readily transmitted to 30 pea cultivars, it was latent in most cultivars.

RCVMV is reported to cause stunting in faba beans (Sanders 1959) and inoculated plants have been reported to show chlorotic lesions or general chlorosis, tip mottle and abscission (Gibbs et al. 1966). Common vetch (*Vicia sativa*) is reported to develop necrotic local lesions, which sometimes become systemic (Stuteville 1964; Varma 1970).

Larsen et al. (1996) reported a disease of chickpeas caused by RCVMV, with symptoms including severe stunting, mosaic, proliferation of axillary buds, malformation of leaves and branches and reduced flower and pod numbers. Larsen and Myers (1998) found lentil plants with mixed infections of RCVMV and Pea enation mosaic virus (PEMV), many of which developed symptoms typical of PEMV but others exhibited severe stunting, proliferation of axillary branches and general chlorosis or death. Glasshouse inoculations of peas, faba beans, chickpeas and lentils with this RCVMV isolate resulted in mild systemic mosaic symptoms in all species.

RCVMV decreases the yield of red clover by reducing foliage growth, decreasing persistence and increasing plant susceptibility to root rot organisms, particularly *Fusarium* spp. (Mushtaq et al. 1978). Studies in Wisconsin, USA, found that the losses and frequency of occurrence of RCVMV in red clover were variable but it was the most common virus in red clover and alsike clover and sweet clover (Hanson and Hagedorn 1961).

2.3 Entry, establishment and spread

Entry potential: High

RCVMV is seed-borne in red clover, faba bean and field pea (Matsulevich 1957, Sänder 1959, Kraft et al. 1998).

Establishment potential: High

The presence of vectors and hosts of this virus in Australia create an ideal environment for its establishment. RCVMV has been found extensively in surveys of forage legumes and clovers in the USA (eg. McLaughlin 1983, McLaughlin and Boykin 1988, McLaughlin and Ensign 1989, McLaughlin et al. 1992, Rahman 1993, Sherwood 1997) and also in the United Kingdom (Gibbs et al. 1966).

Spread potential: High

Four of the seven vectors reported to transmit the virus are present in Australia: *Acyrtosiphon pisum*, *Cavariella aegopodii*, *Myzus persicae* and *Therioaphis maculata*. The pea aphid (*Acyrtosiphon pisum*) is generally seen as the main vector and is common in temperate Australia where legumes are grown. The virus is also seedborne in three legume species (red clover, faba bean, field pea) (Edwardson and Christie 1991, Naumann 1993).

Economic impact: High

RCVMV has been reported in natural infections in 18 species in 6 genera of the Fabaceae (Edwardson and Christie 1991). Significant yield losses due to RCVMV infection have been reported for most of the economically important pulse hosts grown in Australia. Due to the abundance of aphid vectors in legume production areas in Australia, yield losses in legumes due to RCVMV are potentially high if the virus were to become established in Australia.

Khan and Singh (1997a) conducted field trials on peas and found that in moderately diseased pea plants, pea stunt induced by RCVMV caused 70, 75, 88, 77 and 88% reduction respectively in the number of flowers, pods, grains, percentage normal grains and grain weight per plant. The reduction in these yield components increased progressively with increasing disease severity and reached 100% in the case of severely infected plants. Khan and Singh (1997b) found that with moderately infected pea plants, pea stunt disease, incited by RCVMV caused 43, 51, 42, and 36% reduction respectively in shoot length, shoot volume, number of branches per plant and size of internodes.

Larsen and Miklas (2000) evaluated the effects of RCVMV on biomass, seed yield and seed quality in chickpea under field and greenhouse conditions. Test plants were inoculated with the virus at pre-bloom (PE), bloom (B), and post-bloom (PO) stages. Biomass was significantly ($P=0.05$) reduced in PE and B plants but not in PO plants. Mean dry weights were 136.7 and 31.4 g for B and PE treatments, respectively, compared to 289.2g in the healthy treatment. Seed collected from infected plants resulted in yield losses of 61.5% and 49.9% for B and PO, respectively. Plants inoculated at the PE stage resulted in losses of 100%. Seed quality, as evaluated by seed size, was markedly reduced in all infected treatments. Quality decreased proportionally with earliness of infection. Only 2.3% of premium size 26 seed was obtained in the PO treatment compared to 9.8% in healthy. Seed size 24 consisted of 18.9, 52.7, and 78.7% of the total yield from B, PO, and healthy, respectively.

RCVMV decreases the yield of red clover by reducing foliage growth, decreasing persistence and increasing susceptibility to root rots, particularly *Fusarium* spp. (Khan et al. 1978). The losses caused by RCVMV in red clover in Wisconsin, USA, were found to be variable but RCVMV was the most common virus in red clover, alsike clover and sweet clover (Hanson and Hagedorn 1961). Alconero et al. (1986) commented that it was well documented that viruses such as RCVMV and others reduce the persistence and vigour of perennial red clover and white clover.

Environmental impact: Negligible

There is no potential for PEMV to degrade the environment or otherwise alter the ecosystems by affecting species composition or reducing the longevity or competitiveness of wild hosts. It has no effect on human or animal health.

Overall risk: Medium

2.4 Diagnostic information

2.4.1 Diagnostic protocol

Please refer to the National Diagnostic Protocol for Red clover vein mosaic carlavirus held by Plant Health Australia and Biosecurity Victoria.

2.5 Response checklist

2.5.1 Checklist

Guidelines for Response Checklists are still to be endorsed. The following checklist provides a summary of generic requirements to be identified and implemented within a Response Plan:

- Destruction methods for plant material, soil and disposable items
- Disposal procedures
- Quarantine restrictions and movement controls
- Decontamination and farm cleanup procedures
- Diagnostic protocols and laboratories
- Trace-back and trace-forward procedures
- Protocols for delimiting, intensive and ongoing surveillance
- Zoning
- Reporting and communication strategy

Additional information is provided by Merriman and McKirdy (2005) in the Technical Guidelines for Development of Pest Specific Response Plans and by Freeman (2007) in National Diagnostic Protocol for Red Clover Vein Mosaic Virus.

2.6 Delimiting survey and epidemiology study

Delimiting surveys should comprise local surveys around the area of initial detection concentrating on areas of poor growth. Surveys for viruses in pulses are conducted routinely every year in Victoria and regularly in other states and overseas surveys are regularly published. The normal procedure is to collect symptomatic plants and test them to confirm the presence of the virus, then to collect 100-200 random samples per crop for testing to enable an estimate to be made of the within crop virus incidence. Surrounding crops would then be surveyed. The extent of the survey beyond the initial infected crop should be guided by the test results from surrounding crops.

2.6.1 Sampling method

RCVMV is seedborne and non-persistently transmitted by aphids. If it is assumed that the area of initial detection is the first site of introduction of the virus, it would follow that the virus has been introduced in seed. In this case the initial infected plants would be randomly distributed throughout the area where the seed was sown. Therefore the first step would be to determine the area sown with the seed. RCVMV is seedborne in faba beans, field peas and red clover. If the seed is faba bean or field pea, both annual crops, it could be assumed that the introduction was in the current season (although it is possible that the infection went unnoticed in a crop in a previous year and has been carried over in seed). If the virus is detected in red clover, which is a perennial crop, then the introduction would have occurred in the year that the pasture was sown. Initial surveying should be of this crop or pasture to establish the incidence of the virus. A representative selection of symptomatic plants from the field should first be collected and tested to establish the presence of RCVMV. One hundred plants collected randomly, preferably in a W pattern which covers the whole field, should then be tested for RCVMV to establish the percentage of infected plants. RCVMV is non-persistently transmitted by a range of aphids. This type of spread involves acquisition and transmission of the virus over a short period of time, sometime seconds, and the aphid loses its ability to transmit the virus within about an hour. The virus is picked up on the external mouth parts of the aphid and is usually lost in the first couple of subsequent probes. These factors mean that spread is usually over short distances; therefore the next area to survey would be the closest crops or pastures of host plants. Due to the short distance over which the aphids are likely to transmit the virus, host crops should be systematically surveyed using the above technique (100 samples per crop) moving out from the infected crop (delimiting survey). The extent of the surveying and distance from the infected crop will depend on whether further infected crops are found. The spread of a virus by non-persistent aphid transmission would be slower than for an airborne pathogen but faster than a root pathogen.

A second possibility is that the initial site where the virus is detected is not the original introduction site or year. In this case, infected seed may have been introduced in a previous year and/or site and the virus carried through to the current year in infected seed. Therefore it is important to undertake both a trace-back and trace-forward of the seed source and test seed or crops from this source. Alternatively, the virus may have been spread to this site by non-persistent aphid transmission. In this case the survey approach outlined above would be used in conjunction with seed trace-back and trace-forward.

2.6.2 Epidemiological study

The degree of spread of the virus is dependent on the amount of virus initially present in the seed, the quantity of seed sown, the susceptibility of the plants and the aphid population involved in its subsequent spread.

If seed is available, a seed test should be undertaken on a minimum of 400 seeds, but preferably on 1000 seeds by germinating the seed and using the standard diagnostic protocol to determine the percentage of seed infection. The field survey (100 plants per crop) will give an estimate of the percentage of infected plants in the field. If the amount of virus in the crop is greater than in the seed, it would suggest that aphid vectors have been spreading the virus. This would indicate the need to extend the survey out from the initial crop to surrounding crops. The extent of a delimiting survey will depend on test results.

Seed trace-back and trace-forward will determine how many seedlots and crops or pastures will need to be tested. If seed has been sown at several sites, delimiting crop/pasture surveys should be conducted as described at each site.

It is critical to monitor the crops or pastures for aphids and undertake aphid eradication.

It is also important to look at vehicle and machinery movements in and out of the infected area as RCVMV is a carlavirus which can be spread mechanically.

2.6.3 Models of spread potential

No modelling data are available.

Spread may occur in the following ways:

- Movement of infected seed.
- Non-persistent aphid transmission between plants over a short distance and short time frame.
- Mechanical transmission through movement of vehicles and machinery.
- In clover pastures, movement and feeding of animals can spread RCVMV.

2.6.4 Pest Free Area (PFA) guidelines

Points to consider are:

- Design of a statistical delimiting field survey for RCVMV based on virus-testing of host (See 2.6.1 for points to consider in the design).
- Plant sampling should be based on 100 random samples per crop.
- Assessment of plants requires appropriate diagnostic tests such as ELISA, TBIA or PCR as plants may be symptomless or have symptoms which may be confused with RCVMV (nutrition, another virus, etc).
- Seed sampling should be based on a minimum of 400 randomly selected seeds, but preferably 1000 seeds should be tested. The author has been unable to find figures for the level of seed transmission in red clover, faba beans or field peas.
- Seed testing should be undertaken on germinated seedlings.

2.7 Availability of control methods

2.7.1 General procedures for control

- Keep traffic out of affected areas and minimize movement in adjacent areas. Adopt best-practice farm hygiene procedures to retard the spread of the pathogen between fields and adjacent farms. This is important as RCVMV is a carlavirus and they can be spread mechanically (eg vehicle and machinery movement, stock movement).

- If clover pastures are infected, remove stock.
- Ensure that seed production does not take place on affected farms and do not use red clover, faba bean and field pea seed from affected areas to plant new crops as RCVMV is seed borne in these plant species.
- Control/eradication of aphids, particularly aphids colonising the crop, as they are the main means of spread of the virus.
- After surveys etc are completed, destruction of the infected crop or pasture and any infected seed is the most effective control.
- Ongoing surveillance of infected paddocks, as red clover produces large quantities of seed.

2.7.2 Control if small areas are affected

As above.

2.7.3 Control if large areas are affected

A large area may be affected if a large source of seed has been widely distributed and in this case, seed movement may be difficult to track. If the disease has gone unnoticed for a number of years and has been spread by an unknown amount through seed, aphid transmission and mechanical means, control may also be difficult. Implementation of large area controls will depend on the ability to determine the source and track/trace the spread. It will also depend on whether the source is red clover seed (perennial pasture) or faba bean or field pea seed (annual crops). If RCVMV is only found in annual pulse crops, it may be possible to eradicate the virus by surveying, eradicating aphids and ensuring that seed from infected crops is sold for consumption and not resown. Alternatively the crop could be destroyed before seed set. As much of the pulse production area is under continuous cropping with no livestock phase or pasture, these steps, followed by some monitoring may be adequate for eradication. Ongoing monitoring would be required to eliminate self sown plants either with a selective herbicide or stock grazing. If RCVMV is found in perennial red clover pastures, it has the potential to spread to a number of other pasture legumes (lucerne, clovers) even though it is not seedborne in these species. It is likely to be much harder to control or eradicate RCVMV from irrigated or dryland dairy production areas due to large areas of continuous perennial legume pasture, suitable conditions for multiplication of aphid vectors and movement of stock and machinery.

2.7.4 Cultural control

Cultural control is possible by removal of stock from infected pastures, eradication of aphid vectors with insecticides and destruction of seed and infected crops or pastures. Crops and pastures may be destroyed by intensive animal feeding followed by removal of animals to non-host grass pasture. Use of herbicides to kill crops or pastures before seed set or ploughing in of the plant matter before seed set is also effective and may be necessary after animal grazing. After crop or pasture destruction it is important to prevent self-sown plants growing from seed as these are potentially infected with RCVMV. Therefore, follow up monitoring and further spraying or ploughing may be required. It is recommended that a non-host, preferably cereal or grass is then planted as this enables ongoing spraying for self-sowns with a selective herbicide. Clovers produce large quantities of seed, therefore if it is thought that infected clover pasture has set seed then surveillance and testing of self-sowns would be required.

2.7.5 Host plant resistance

Use of resistant varieties of red clover is the normal control practice for RCVMV in the USA (Hanson and Hagedorn 1961; Stuteville and Hanson 1964; Smith et al. 1973; Khan et al. 1978) but it is not known whether local varieties have RCVMV resistance. It is not known whether or not resistant faba beans or field peas are available overseas.

2.7.6 Chemical control

The virus cannot be controlled by the use of chemicals on the crop or pasture. However, the crop itself can be destroyed with herbicides. The aphid vector can be managed to a certain extent with insecticides. Spraying is most effective for killing colonising aphids, thus preventing secondary spread of the virus within a crop but is less effective at preventing flying aphids introducing virus to a crop.

3 Course of Action – Eradication Methods

3.1 Destruction strategy

3.1.1 Destruction protocols

- Disposable equipment, infected plant material or soil should be disposed of by autoclaving, high temperature incineration or deep burial. Any equipment removed from the site for disposal should be double-bagged.
- Herbicides could be used to destroy the infected crops or pastures.
- Infected crops or pastures could be ploughed in.
- Insecticides could be used to destroy the aphid vector.
- Farm machinery used in destruction processes needs to be thoroughly washed, preferably using a detergent such as Decon 90

3.1.2 Decontamination protocols

Machinery, equipment, vehicles in contact with infected plant material or soil or present within the Quarantine Area, should be washed to remove soil and plant material using high pressure water or scrubbing with products such as a detergent such as decon 90, a farm degreaser or a 1% bleach solution in a designated wash down area.

General guidelines for wash down sites and areas are as follows:

- Located away from crops or sensitive vegetation.
- Readily accessible with clear signage.
- Access to fresh water and power.
- Site, including entry and exit points, should be mud free site (e.g. gravel, concrete or rubber matting).
- Gently sloped to drain effluent away.
- Effluent must not enter water courses or water bodies.

- Allow adequate space to move larger vehicles.
- Away from hazards such as powerlines.
- Waste water, soil or plant residues should be contained (see PLANTPLAN Appendix 18).
- Disposable overalls and rubber boots should be worn when handling infected soil or plant material in the field. Boots, clothes and shoes in contact with infected soil or plant material should be disinfected at the site or double-bagged to remove for cleaning.
- If hands have been in contact with plant material they should be washed.
- Decon 90 (Enviroequip) is a suitable detergent for using to decontaminate equipment or personnel.
- All chemicals used according to label.

3.1.3 Priorities

Specific priorities for eradication or decontamination

- Confirm the presence of the pathogen.
- Prevent movement of vehicles and equipment through affected areas.
- Remove stock to a non-host grass pasture.
- Control aphid vectors to prevent further spread.
- Inform all groups in the Industry.
- Determine extent of infection through survey and seed trace-back and trace-forward.
- Destroy crop or pasture and any seed

3.1.4 Plants, by-products and waste processing

- Seed harvested from infected plants and any infected soil or plant material removed from the paddock should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial (in a non-cropping area).
- Once plant material has been killed with herbicide it is not possible for aphids to transmit the virus.
- However, as the virus can be mechanically transmitted, killed crops should be ploughed in.

3.1.5 Disposal issues

Once introduced and established, RCVMV can survive in soil in red clover seed. Therefore, if the virus is found in red clover, it is important to establish whether or not the infection occurred in the current season (ie no seed set) or in a previous year, which would mean that infected seed may be present in the soil. Particular care must be taken to minimize the transfer of infected soil or seed from the quarantine area.

No particular issues with resistance of vector or disease to chemicals or physical treatments are known to exist.

3.2 Quarantine and movement controls

3.2.1 Quarantine priorities

Aphid vectors have wings and can move long distances, but the non-persistent transmission of RCVMV is only for a short period after acquisition (less than one hour) and generally only over short distances.

3.2.2 Movement control for people, plant material and machinery

Movement of people, vehicle and machinery, to and from affected farms, must be controlled to ensure that infected soil or plant debris is not moved off-farm on clothing, footwear, vehicles or machinery.

Examples of movement controls include:

- Signage to indicate quarantine area and/or restricted movement in these zones.
- Fenced, barricaded or locked entry to quarantine areas.
- Movement of equipment, machinery, plant material or soil by permit only.
- Clothing and footwear worn at the infected site should either be double-bagged prior to removal for decontamination or should not leave the farm until thoroughly disinfected, washed and cleaned.
- Seed from the affected site should not be used for planting new crops, feeding stock or for human consumption.
- Hay must not be removed from the site.

3.3 Zoning

The size of each quarantine area will be determined by a number of factors, including the location of the incursion, climatic conditions and the proximity of the infected property to other infected properties.

3.3.1 Destruction zone

The entire crop (pulse) or pasture (clover) should be destroyed after the level of infection has been established. The delimiting survey will determine whether or not neighbouring host crops are infected and need to be destroyed. ie - the Destruction Zone may be defined as contiguous areas associated with the source of infection (i.e. the entire trial, paddock or farm if spread could have occurred in seed prior to the infection being identified).

The aphid vectors only spread the virus over short distances but if perennial red clover is the host and has been established for more than one season, the spread may have been greater due to sequential spread.

3.3.2 Quarantine zone

The Quarantine Zone is defined as the area where voluntary or compulsory restraints are in place for the affected property(ies). These restraints may include restrictions or movement control for removal of plants, people, soil or contaminated equipment from an infected property.

3.3.3 Buffer zone

A Buffer Zone may or may not be required depending on the incident. It is defined as the area in which the pest does not occur but where movement controls or restrictions for plant material, people, soil or equipment from this area are still deemed necessary. The Buffer Zone may enclose an infested area (and is therefore part of the Control Area) or may be adjacent to an infested area.

3.3.4 Restricted Area

The Restricted Area is defined as the zone immediately around the infected premises and suspected infected premises. The Restricted Area is established following initial surveys that confirm the presence of the pest. The Restricted Area will be subject to intense surveillance and movement control with movement out of the Restricted Area to be prohibited and movement into the Restricted Area to occur by permit only. Multiple Restricted Areas may be required within a Control Area.

3.3.5 Control Area

The Control Area is defined as all areas affected within the incursion. The Control Area comprises the Restricted Area, all infected premises and all suspected infected premises and will be defined as the minimum area necessary to prevent spread of the pest from the Quarantine Zone. The Control Area will also be used to regulate movement of all susceptible plant species to allow trace back, trace forward and epidemiological studies to be completed.

3.4 Decontamination and farm clean up

Decontaminant practices are aimed at eliminating the pathogen thus preventing its spread to other areas.

3.4.1 Decontamination procedures

General guidelines for decontamination and clean up

- Refer to PLANTPLAN (Plant Health Australia 2008) for further information on disinfection and decontamination.
- Keep traffic out of affected area and minimize it in adjacent areas. Adopt best-practice farm hygiene procedures to retard the spread of the pest and potentially infected plant material between fields and adjacent farms.
- RCMV can be mechanically transmitted by plant injury, vehicles or stock movement and feeding.
- Machinery, equipment, vehicles in contact with infected plant material or soil or present within the Quarantine Area, should be washed to remove soil and plant material using high pressure water or scrubbing with products such as Decon 90 detergent, a farm degreaser or a 1% bleach solution in a designated wash down area as described in 3.1.2.
- Plant material should be destroyed using herbicide.

3.4.2 Decontamination if disease is identified in a small area

Destruction of plant material by herbicide as described. If host is perennial red clover and infection cannot be definitely identified as occurring in the current year (ie if clover pasture was sown in a

previous year and trace back of seed to a sample for testing was not possible) then it is possible that infection occurred in a previous year and infected seed may be present in the soil. In this case decontamination cannot be guaranteed. The infected area would need to be monitored for a number of years for self sown clover which should be tested for RCVMV and then destroyed.

3.4.3 Decontamination if disease is identified in large areas

The process described for small areas may be feasible for larger areas if alternate non-hosts are planted in these paddocks and self-sown clover is sprayed out each year. Sampling and testing of larger areas for RCVMV would be feasible depending on the actual area affected.

3.4.4 General safety precautions

For any chemicals used in the decontamination, use as per label and follow all safety procedures listed within each MSDS.

3.5 Surveillance and tracing

3.5.1 Surveillance

Detection and delimiting surveys are required to delimit the extent of the outbreak, ensuring areas free of the pathogen retain market access and appropriate quarantine zones are established.

Initial surveillance priorities include the following:

- Survey of all host crops and host growing properties in the pest quarantine area;
- Survey of all properties identified in trace-back and trace-forward analysis as being at risk;
- Survey of all host growing properties that are reliant on trade with interstate or international markets which are sensitive to red clover vein mosaic carlavirus presence.

3.5.2 Survey regions

Establish survey regions around the surveillance priorities identified above. These regions will be generated based on the zoning requirements (section 3.3), and prioritised based on their potential likelihood to currently have or receive an incursion of this pest. Surveillance activities within these regions will either: allow for the area to be declared pest free and maintain market access requirements; or establish the impact and spread of the incursion to allow for effective control and containment measures to be carried out.

Steps outlined below form a basis for a survey plan. Although categorised in stages, some stages may be undertaken concurrently based on available skill sets and resources.

Phase 1:

Identify properties that fall within the buffer zone around the infested premise.

Complete preliminary surveillance to determine ownership, property details, production dynamics and tracings information (this may be an ongoing action).

Phase 2:

Preliminary survey of host crops in properties in buffer zone establishing points of pest detection.

Phase 3:

Surveillance of an intensive nature, to support control and containment activities around points of pest detection.

Phase 4:

Surveillance of contact premises. A contact premise is a property containing susceptible host plants, which are known to have been in direct or indirect contact with an infested premises or infected plants. Contact premises may be determined through tracking movement of materials from the property that may provide a viable pathway for spread of the disease. Pathways to be considered are:

- Items of equipment and machinery which have been shared between properties including bins, containers, irrigation lines, vehicles and equipment;
- The producer and retailer of infected material if this is suspected to be the source of the outbreak;
- Labour and other personnel that have moved from infected, contact and suspect premises to unaffected properties (other growers, tradesmen, visitors, salesmen, crop scouts, harvesters and possibly beekeepers);
- Movement of plant material and soil from controlled and restricted areas; and
- Storm and rain events and the direction of prevailing winds that result in wind-driven spread of the virus and aphid during these weather events.

Phase 5:

Surveillance of nurseries, backyards and native and weed hosts of RCVMV.

Phase 6:

Agreed area freedom maintenance, post control and containment.

3.5.3 Post-eradication surveillance

Surveys comprising plant sampling for RCVMV to be undertaken for a minimum of 12 months after eradication has been achieved. If RCVMV has been found in an annual pulse host, self-sowns should be eliminated in 12 months. If the virus was detected during the growing season it is not likely that there would be any seed set. If perennial red clover is the host and it is suspected that infected plants have set seed, then surveillance surveys may have to be continued for a number of years. Alternate non-host crops or pastures should be grown on the site, self-sown red clover plants sampled (100 per paddock as per the initial survey) and then all self-sown plants should be sprayed out with herbicide. This procedure should not be onerous or expensive. If, for example, the farmer was growing a wheat crop, spraying for broad-leaf weeds would be normal practice. What may need to be discussed is the timing of sprays and sampling.

4 References

- Aftab M, Freeman A, Bretag T. 2006. Seed health testing in pulses. DPI-Victoria, Agricultural Notes AG0000.
- Alconero R, Fiori B, Sherring W (1986). Relationships of virus infections to field performance of six clover species. *Plant Disease* 70: 119-121.
- Bos L (1973). Pea streak virus. CMI/AAB Descriptions of Plant Viruses. No. 112.
- Bos L, Maat DZ, Markov M (1972). A biologically highly deviating strain of red clover vein mosaic virus, usually latent in pea (*Pisum sativum*), and its differentiation from pea streak virus. *Netherlands Journal of Plant Pathology* 78: 125-152.
- Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ (Eds) Version: 16th January 1997. Plant viruses online: Descriptions and lists from the VIDE database. <http://biology.anu.edu.au/Groups/MES/vide/> (1996 onwards).
- Edwardson JR, Christie RG (1991). Handbook of viruses infecting legumes. (CRC Press, Inc.:Florida).
- Freeman A., Aftab M., McQueen V. and Davidson J. 2005. The occurrence of common viruses in pulse crops in south eastern Australia. In: Proceedings of the 15th Biennial Australasian Plant Pathology Society Conference, Geelong, Sept., 2005, p339.
- Freeman A (2007) National Diagnostic Protocol for Red clover vein mosaic carlavirus, Plant Health Australia.
- Gibbs AJ, Varma A, Woods RD (1966). Viruses occurring in white clover (*Trifolium repens* L.) from permanent pastures in Britain. *Annals of Applied Biology* 58: 231-240.
- Hagedorn DJ, Walker JC (1949). Wisconsin pea stunt, a newly described disease. *Journal of Agricultural Research* 78: 617-626.
- Hanson EW, Hagedorn DJ (1961). Viruses of red clover in Wisconsin. *Agronomy journal* 53: 63-67.
- Khan MA, Maxwell DP, Smith RR (1978). Inheritance of resistance to red clover vein mosaic virus in red clover. *Phytopathology* 68: 1084-1086.
- Khan AT, Singh RN (1997a). Effect of pea stunt disease on flowering, podding, grain setting and yield. *Indian Phytopathology*. 50: 282-284.
- Khan AT, Singh RN (1997b) Effect of pea stunt disease on shoot development in field pea. *Indian Phytopathology*. 50: 285-289.
- Kraft JM, Larsen RC, Inglis DA (1998). Diseases of pea. In, *The pathology of food and pasture legumes* (Eds DJ Allen, JM Lenne) (CABI & IRCISAT: New York) pp. 325-370.
- Larsen RC, Kaiser WJ, Wyatt SD (1996). First report of a virus disease of chickpea caused by a strain of red clover vein mosaic carlavirus. 80: 709.
- Larsen RC, Wyatt SD, Druffel K (1997). Partial nucleotide sequencing of red clover vein mosaic carlavirus (RCVMV) 3' terminus. *Phytopathology* 87: S56.
- Larsen RC, Myers JR (1998). First report of red clover vein mosaic carlavirus naturally infecting lentil. *Plant Disease*. 82: 1064.
- Larsen RC, Miklas PN (2000). Effect of red clover vein mosaic carlavirus infection on seed production and biomass yield in chickpea. American Phytopathological Society 2000 Pacific Division Meeting Abstracts (Joint with Canadian Phytopathological Society) June 18-21, 2000 - Victoria, BC, Canada.

- Matsulevich BP (1957). The effects of clover mosaic on the productivity of red clover (Translated title). *Agrobiologiya* 2: 75-79.
- McLaughlin MR (1983). Viruses infecting forage legumes in Tennessee. *Plant Disease* 67: 490-492.
- McLaughlin MR, Ensign RD (1989). Viruses detected in forage legumes in Idaho. *Plant Disease* 73: 906-909.
- McLaughlin MR, Pederson GA, Evans RR, Ivy RL (1992). Virus diseases and stand decline in a white clover pasture. *Plant Disease* 76: 158-162.
- McLaughlin MR, Boykin DL (1988). Virus diseases of seven species of forage legumes in the south eastern United States. *Plant Disease*. 72: 539-542.
- Khan MA, Maxwell DP, Smith RR (1978). Inheritance of resistance to red clover vein mosaic virus in red clover. *Phytopathology* 68: 1084-1086.
- Merriman P, McKirdy S (2005) Technical Guidelines for the Development of Pest Specific Response Plans, Plant Health Australia.
- Naumann I (1993). CSIRO Handbook of Australian Insect Names (6th edn).CSIRO Division of Entomology.
- PLANTPLAN (2008) Australian Emergency Plant Pest Response Plan, Appendix 3: Sampling procedures and protocols for transport, diagnosis and confirmation of EPPs – Plant Health Australia.
- PLANTPLAN (2008) Australian Emergency Plant Pest Response Plan, Appendix 18: Disinfection and decontamination – Plant Health Australia.
- Rahman F, Peaden RN (1993). Incidence of viruses on alfalfa in Western North America. *Plant Disease* 77:160-162.
- Rubio-Huertos M, Bos L (1973). Light and electron microscopy of red clover vein mosaic virus in pea (*Pisum sativum*). *Netherlands Journal of Plant Pathology* 79: 84-103
- Sander E (1959). Biological properties of red clover vein mosaic virus. *Phytopathology* 49: 749-754.
- Sherwood RT (1997). Viruses of white clover in pastures of Pennsylvania, New York and Vermont. *Plant Disease*, St Paul, Minn., American Phytopathological Society. 81: 817-820.
- Smith RR, Maxwell DP, Hanson EW, Smith WK (1973). Registration of Arlington red clover. *Crop Science* 13: 771.
- Stuteville DL (1964). Virus diseases of red clover. *University of Wisconsin Dissitation Abstracts* 25: 4, 2162.
- Stuteville DI, Hanson EW (1964). Resistance to viruses in red clover. *Crop Science* 4: 631-635.
- Varma A (1970). Red clover vein mosaic virus. *CMI/AAB Descriptions of Plant Viruses*. No. 22.
- Weber KA, Hampton RO (1980). Transmission of two purified carlaviruses by the pea aphid. *Phytopathology* 70: 631-633.

5 Appendices

Appendix 1. Standard diagnostic protocols

For a range of specifically designed procedures for the emergency response to a pest incursion refer to Plant Health Australia's PLANTPLAN.

Appendix 2. Experts, resources and facilities

The following table lists the experts who can be contacted for professional diagnostics and advisory services in the case of an incursion.

Expert	State	Details
Angela Freeman	Vic	DPI Victoria Horsham Centre Natimuk Rd Horsham VIC 3400 Ph: (03) 5362 2111; Fax: (03) 5362 2187
Brendon Rodoni	Vic	DPI Victoria Knoxfield Centre 621 Burwood Highway Knoxfield VIC 3684 Ph: (03) 9210 9222; Fax: (03) 9800 3521
Roger Jones		Dept of Agriculture and Food, Western Australia 3 Baron-Hay Court South Perth WA 6151 Ph: (08) 9368 3721; Fax: (08) 9474 2658

The following table lists the facilities available for diagnostic services in Australia.

Facility	State	Details
DPI Victoria Knoxfield Centre	Vic	621 Burwood Highway Knoxfield VIC 3684 Ph: (03) 9210 9222; Fax: (03) 9800 3521
DPI Victoria Horsham Centre	Vic	Natimuk Rd Horsham VIC 3400 Ph: (03) 5362 2111; Fax: (03) 5362 2187
DPI New South Wales Elizabeth Macarthur Agricultural Institute	NSW	Woodbridge Road Menangle NSW 2568 PMB 8 Camden NSW 2570 Ph: (02) 4640 6327; Fax: (02) 4640 6428
DPI New South Wales Tamworth Agricultural Institute	NSW	4 Marsden Park Road Calala NSW 2340 Ph: (02) 6763 1100; Fax: (02) 6763 1222
DPI New South Wales Wagga Wagga Agricultural Institute	NSW	PMB Wagga Wagga NSW 2650 Ph: (02) 6938 1999; Fax: (02) 6938 1809
SARDI Plant Research Centre - Waite Main Building, Waite Research Precinct	SA	Hartley Grove Urrbrae 5064 South Australia Ph: (08) 8303 9400; Fax: (08) 8303 9403
Grow Help Australia	QLD	Entomology Building 80 Meiers Road Indooroopilly QLD 4068 Ph: (07) 3896 9668; Fax: (07) 3896 9446
Department of Agriculture and Food, Western Australia (AGWEST) Plant Laboratories	WA	3 Baron-Hay Court South Perth WA 6151 Ph: (08) 9368 3721; Fax: (08) 9474 2658

Appendix 3. Communications strategy

A general Communications Strategy is provided in PLANTPLAN

Appendix 4. Market access impacts

Details from AQIS – PHYTO checked for restrictions (June 2008).

For the import of broadbean and vetch seed for sowing New Zealand requires the specification that RCVMV is not known to occur in Australia