Cooperative Research Centre
for National Plant Biosecurity

Final Report

CRC40139

Optimising eradication strategies for exotic plant pathogen incursions on perennial crops (Phase 2)

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Table of contents

1. Executive Summary

This project has demonstrated that we can non-destructively eradicate a pathogen from a perennial plant crop. Eradication of pathogen incursions is very important for the protection of plant industries, managed gardens and natural environments. The consequence of an introduced pathogen becoming endemic can be serious, in some cases impacting on the national economy. A review of eradication methods (see Appendix) indicated that the current strategy for pathogen eradication relies on techniques for the treatment, removal and disposal of affected host plants. While there are many examples where these techniques have been successful, there are also many where they have not. Success relies on a sound understanding of the biology and epidemiology of the pathogen and its interaction with the host. Removal and disposal of infected plant material for eradication and containment of plant and soil inhabiting fungal, bacterial and viral pathogens were reviewed. Various techniques including burning, burying, pruning, composting, soil- and biofumigation, solarization, steam sterilization and biological vector control were considered. The review identified gaps in the literature and emphasized the insufficient detail of information available from past eradications. It was concluded that more effort is required to produce and publish scientific evidence to evaluate the success of various techniques. Suggestions for future research were also proposed. This report presents results of the research consequently initiated.

Eradicating exotic grapevine diseases using current strategies, which include complete removal of affected and suspected vines from the vineyard, can incur significant costs to the industry. An alternative strategy has been developed through collaboration between Australia and the USA. The strategy optimises eradication of a pathogen while minimising the economic cost of returning the crop to its previous quality and production levels. In Australia, black spot disease or anthracnose of grapevine (*Elsinoe ampelina*) was used as a model to evaluate a drastic pruning eradication strategy developed for the fungal disease black rot (*Guignardia bidwellii*), exotic in Australia but endemic in eastern USA. The protocol involved cutting off vines at the top of the trunk, removing debris from the ground beneath and between vines, mulching the vineyard floor, removing low water shoots during vine regrowth and applying a targeted fungicide program. The same protocol was evaluated in a black rot-infested vineyard in New York, USA. Following two seasons of conducive weather conditions, no disease was detected on treated vines, whereas leaf and fruit infections developed on the control vines. These results confirmed the efficacy of the non-destructive protocol for eradicating black rot from vineyards. It provides an alternative to razing a vineyard if there were an incursion of black rot in Australia and it will be included in the Viticulture Industry Biosecurity Plan. This strategy may have potential for use on other diseases of grapes and other perennial crops but will require validation in those systems through further research.

A partnership between CRCNPB and Better Border Biosecurity New Zealand (B3NZ) was formed based on concurrent research across the Tasman with similar objectives for optimising eradication responses. The B3NZ team, led by John Fletcher of Plant and Food Research NZ, investigated strategies for the eradication of two nematodes affecting tomato and potato as well as bacterial and fungal diseases of celery. Results are provided in documents included in the Appendix.

Two PhD projects were established as part of this project area. Outcomes of the project CRC60097 on the exotic plant disease, Fusarium wilt of banana, will be reported to CRCNPB separately. A second related project "Pathogen eradication using pistachio dieback as a model", not funded by CRCNPB, has provided important information on the survival of the bacterial pathogen *Xanthomonas translucens* pv. *pistaciae* (Xtp) in pistachio wood following burning and burial. Viable Xtp was detected for up to 28 months in buried wood, but not when placed on the soil surface. Field experiments showed that Xtp was eradicated by burning, so long as the wood was completely burnt. Controlled environment experiments revealed that durations of temperature required to kill Xtp were 50ºC for 180 minutes, 55ºC for 120 minutes and 60ºC for 15 minutes.

2. Aims and objectives

The current strategy for eradication of an EPP is based partly on the removal of whole affected plants, followed by burning and/or burial. However this strategy may incur significant costs to industry and the community when perennial species are involved. Alternative strategies need to be developed and validated that meet eradication goals while minimising economic and social impact.

The objectives of this project were:

- Development and evaluation of drastic pruning as an alternative eradication strategy for grapevine black rot (high priority exotic) using grapevine black spot (endemic in Australia) as a model.
- Implementation of drastic pruning as a novel alternative eradication strategy through USA based trials where black rot (high priority exotic) is endemic.
- Development of rapid containment and control systems for soil-borne pathogens in partnership with the B3 program in New Zealand.
- Development of postgraduate study on *Fusarium* wilt 'tropical' race 4 of Bananas, which is localized in the Darwin area, providing an opportunity for research to build knowledge on its epidemiology and develop methods for containment and control.
- Development of postgraduate study on the efficacy of burning and burial for eradication of infected plant material using pistachio dieback as a model.

This report provides detailed findings of the core research on optimising pathogen eradication from grapevine in Sections 3.1-3.4. Outcomes of partnership research funded by Better Border Biosecurity New Zealand are provided in documents in the Appendix. The outcomes of the PhD project on Fusarium wilt of banana (CRC60097) will be reported separately to CRCNPB and the key findings of the PhD project on the effects of burning and burial on pistachio dieback are summarised in Section 3.6.

3. Key findings

3.1. Development and evaluation of drastic pruning protocol in Australia

Authors

M. Sosnowski (SARDI), R. Emmett (DPI Vic), W. Wilcox (Cornell University) and T. Wicks (SARDI)

Introduction

Eradication of exotic grapevine diseases can incur significant costs to growers and the industry using current strategies which include complete removal of affected and suspected vines. Alternative strategies need to be developed which optimise efficiency of the eradication process and minimise the economic cost of returning the crop to its previous quality and production levels (Sosnowski *et al.* 2009). Initially, the endemic disease of grapevine, black spot (*Elsinoe ampelina*), was used as a model to develop a drastic pruning eradication strategy for the exotic disease black rot (*Guignardia bidwellii*). These pathogens have similar biology and epidemiology, infecting fruit, leaves and shoots of grapevines producing similar symptoms on these parts of the vine (Magarey *et al.* 1993; Wilcox 2003).

Methods

In 2006, a trial was established in the Sunraysia district of Victoria to develop and assess a drastic pruning protocol. Using a split plot design, the trial comprised four table grape cultivars (Red Globe, Christmas Rose, Blush Seedless and Fantasy Seedless) as main plots (Figure 1). Sub-plots consisted of three vines which were either drastically pruned (as described below) or pruned to standard two-bud spurs as controls. Spacing between plots within rows was at least 7.3 m and between rows was 10.5 m.

Vines were inoculated in spring 2007 by spraying a suspension of *E. ampelina* conidia (1-3 x 10^6 spores/ml) onto new shoots with 2-4 unfolded leaves. Inoculations were conducted at three different times to cater for differences in phenology between the cultivars. Shoots were covered with polyethylene bags overnight to provide high humidity for spore germination and infection (Figure 2A). Vines were assessed in December 2007 by counting the number of inoculated canes with cankers on each vine.

In July 2008, vines were drastically pruned as follows. Vines were cut at the crown using a chainsaw and excised material from above the crown was removed (Figure 2B) and placed in an excavated area about 25 m from the trial plots. The vineyard floor around the treated vines was raked (Figure 2C) and the debris was placed in the excavated area to be burnt and buried (Figure 2D). Soil between vines was disc cultivated to bury any remaining debris. Trunks of the treated vines were drenched with lime sulphur (100 ml/L water; ai. 200g/L polysulphide sulphur) using a back pack sprayer.

To provide conditions conducive for disease development an irrigation system was installed in the canopy to mist for 24 h every 3-4 days from early September to late November 2008 as new shoots emerged (Figure 2E). Vines were assessed for recurrence of symptoms in December 2008. Healthy 1-year-old sentinel vines (cv. Thompson Seedless) in pots were placed strategically within and around the trial site during spring and early summer to detect any movement of the pathogen between plots or from external sources (Figure 2F). After periods of 2-3 weeks in the vineyard, the potted vines were placed in a glasshouse, drip irrigated, incubated at 22-28ºC and then inspected for symptoms four weeks later.

A bioassay was conducted to determine if vine debris in the soil below vines was a source of inoculum for emerging shoots. Soil from the base of treated trunks was collected and organic debris was separated using a sieve (Figure 2G). The debris was soaked in water overnight and the water decanted and sprayed over emerging leaves on potted grapevines (cv. Thompson Seedless). The leaves were incubated overnight as described above (Figure 2H) and assessed for symptoms 12 days later.

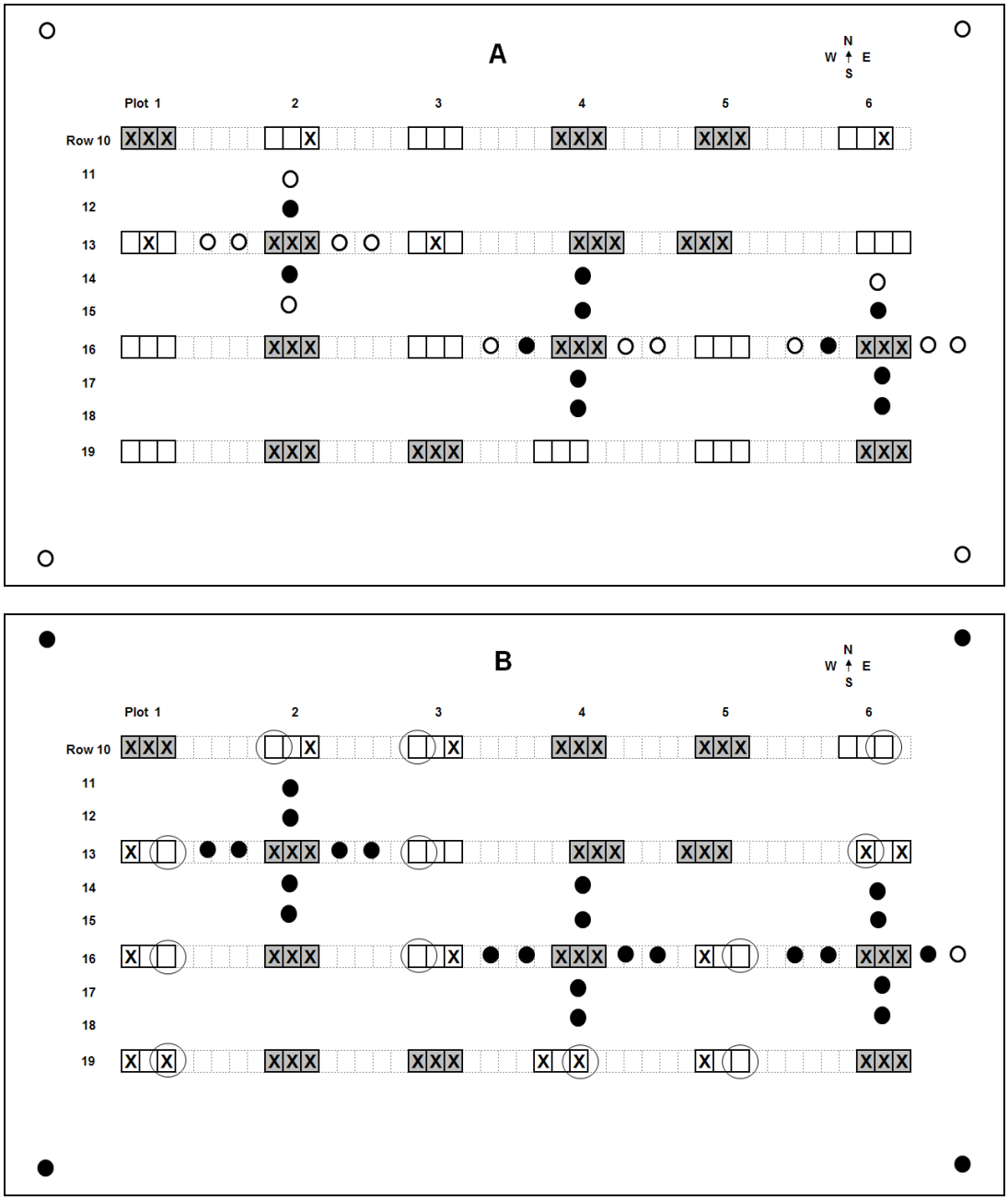

Figure 1. Black spot field trial located at the Irymple Research Centre near Mildura in Victoria showing results in A. 2008 and B. 2009. Plots of three vines (bold framed boxes) were randomly assigned as control (grey) or treated (white). Rows 11, 12, 14, 15, 17 and 18 were excavated and vines between plots (light-grey dashed boxes) cut and stumps killed with herbicide. Each remaining row comprised a different grape cultivar (Row 10, Red Globe; 13, Christmas Rose; 16, Blush Seedless; 19, Fantasy Seedless). Vines observed with black spot symptoms are indicated (X). Sentinel vines (○) were strategically positioned to monitor disease spread from control plots and those recorded with symptoms are indicated (●). Fungicide (mancozeb) was applied to foliage on one half of each treated plot in 2009 (circled). Figure not to scale.

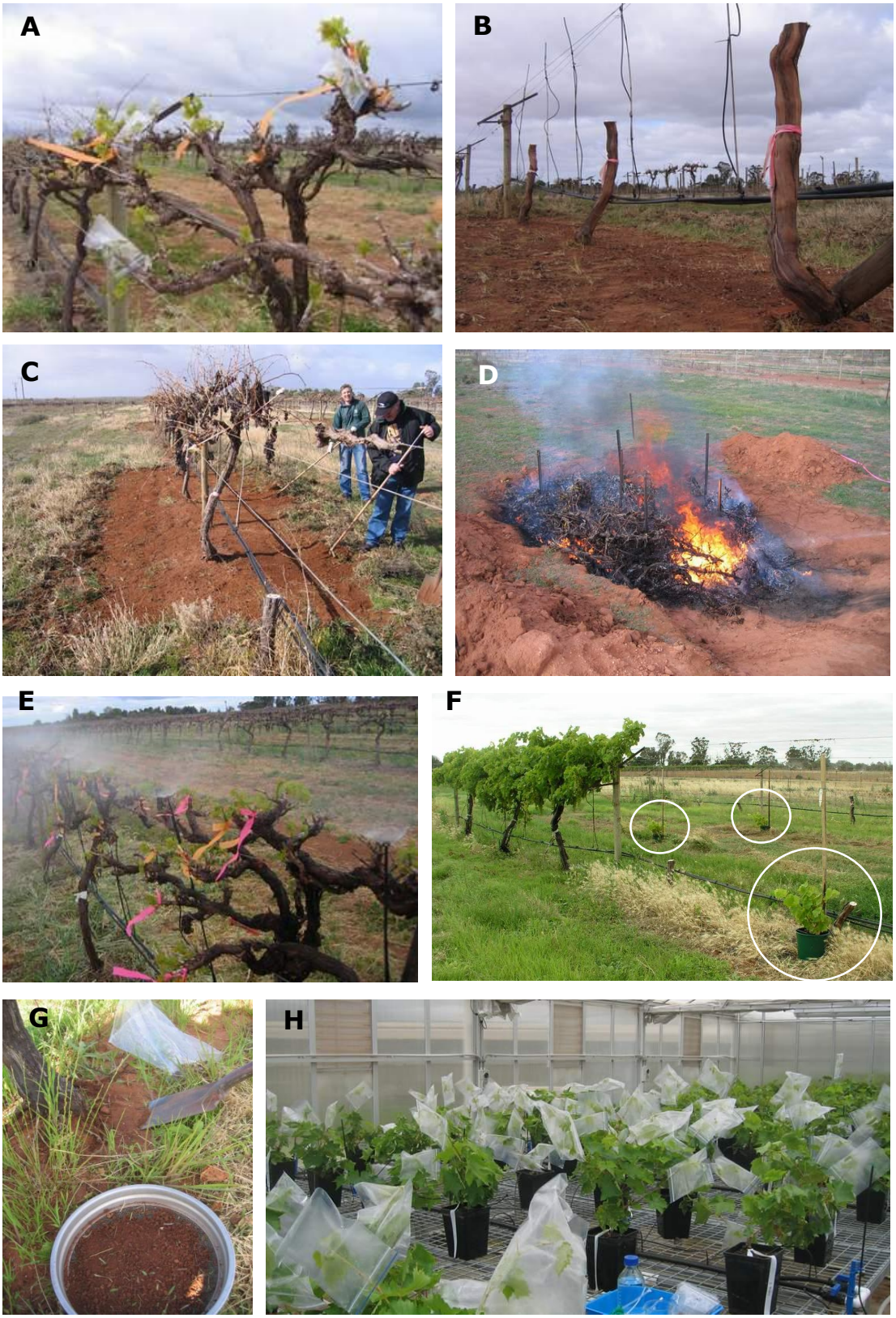

Figure 2. Drastic pruning trial at Mildura in the Sunraysia district of Victoria. A. Inoculated shoots covered with polyethylene bags overnight to provide high humidity, B. Drastically pruned vines, with normally pruned control vines in the background, C. Raking the ground beneath vines, D. Burning excised plant material in a pit, E. Canopy misters ensured optimal conditions for infection, F. Sentinel vines (circled) strategically placed around a control plot, G. Sieving plant debris from soil beneath vines and H. Incubating inoculated leaves in the bioassay.

During July 2009, vines in control plots were pruned to two-bud spurs as in the previous year. The longest canes extending from the crown of vines in treated plots were trained up to the trellis wire to form two new cordons (Figure 3A) and all other shoots were removed from trunks. Using a tractor mounted applicator, triticale hay mulch was spread as a thick mat approximately 20 cm high and 150 cm wide either side of trunks along the length of all plots (Figure 3B). During spring and summer any shoots emerging from the trunk up to 30 cm from the ground were removed every 1-2 weeks. Misters were used to provide conditions conducive for infection in vine canopies as described for spring 2008. Half of each of the three-vine plots was sprayed to runoff with the fungicide Mancozeb (2g/L water; ai. 750 g/kg mancozeb) using a back-pack sprayer on 18 October and on 1, 14 and 29 November 2009. Sentinel vines were used as described above to detect any movement of the pathogen between plots or from external sources.

All vines were assessed for black spot symptoms in December 2009 by randomly selecting 20 shoots per vine and recording the number of shoots with leaf lesions and or cane cankers. For treated plots, the middle vine was left as a buffer between fungicide sprayed and unsprayed vines, so only shoots on the end vines were assessed. The percentage of shoots with black spot symptoms was calculated for each vine.

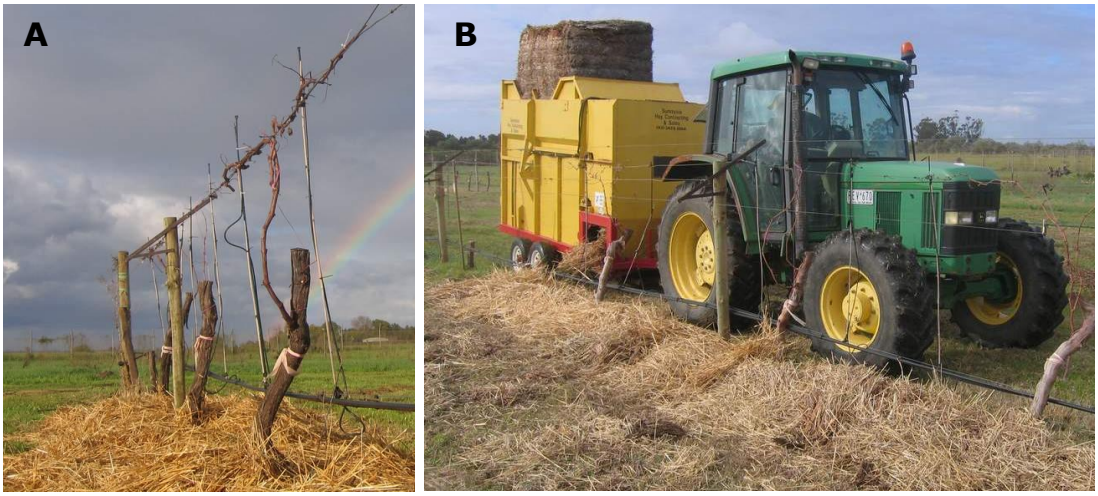

Figure 3. A. Vines subjected to eradication treatment with new canes trained up to cordon wire and B. Applying triticale mulch under vines.

Results

Assessment of the vines in December 2007 showed that 5-12 inoculated shoots on each vine had typical black spot leaf and berry lesions or stem cankers (Figure 4A-C). This indicated that infection was sufficient to simulate an exotic pathogen incursion distributed evenly across the trial plots.

In December 2008, following eradication treatment, shoots had emerged from the trunks of drastically pruned vines (Figure 4B). Symptoms were recorded on all control vines and on 4 of 36 treated vines (Figure 1A). On treated vines, diseased shoots grew from the trunk within 20 cm of the ground (Figure 4C). The bioassay indicated that symptoms were most likely caused by inoculum produced from vine debris remaining on the vineyard floor directly beneath low shoots (Figure 5). Assessment of the sentinel vines revealed that no spread of disease had occurred between plots or from external sources (Figure 1A).

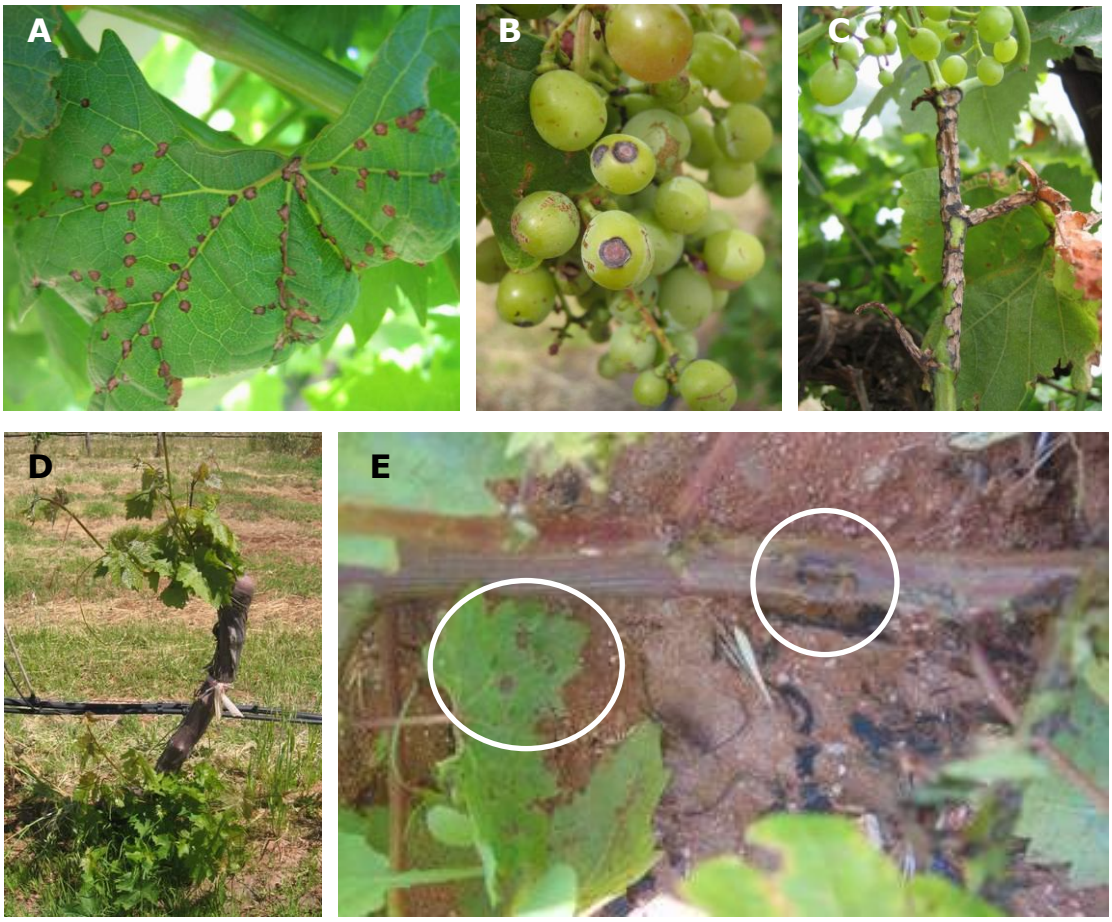

Figure 4. Black spot symptoms on leaves (A), berries (B) and stems (C), D. shoots arising from trunks of drastically pruned vines and C. leaf and stem symptoms (circled) on shoots growing close to the soil surface.

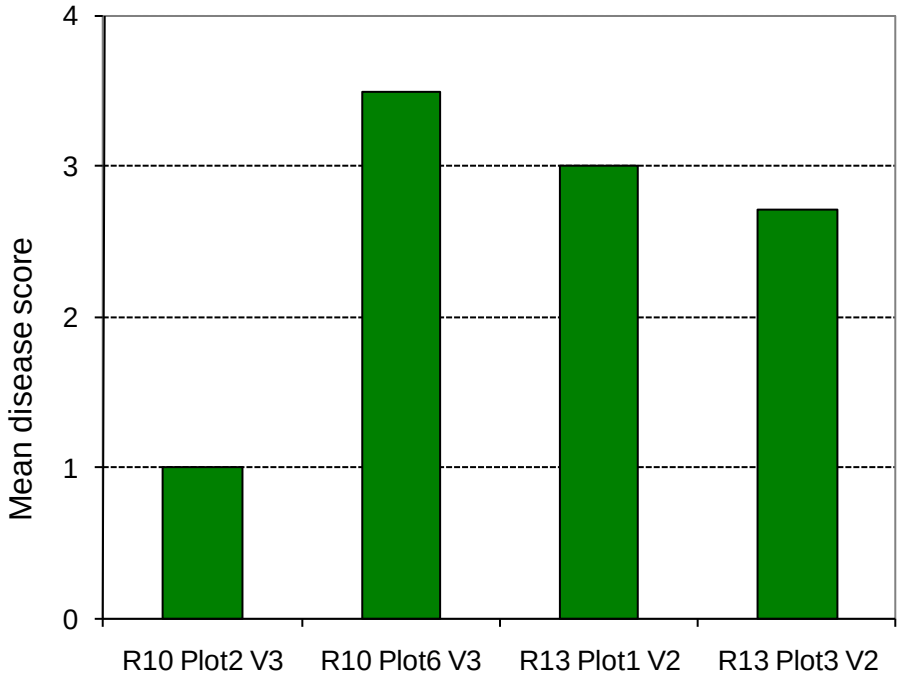

Figure 5. Mean disease score on leaves of potted vines inoculated with debris sieved from soil beneath treated vines with black spot symptoms in a greenhouse bioassay.

In December 2009, a final disease assessment of all vines was conducted. All shoots on unsprayed control vines were severely infested with black spot. Three of the 12 vines in the eradication treatment sprayed with fungicide had diseased shoots (Figure 1B), although the shoots were less severely infected compared to the control vines. Nine of the twelve unsprayed vines had diseased shoots. All but one sentinel vine was recorded with symptoms (Figure 1B) revealing significant spread between control and treated vines, which is most likely to have occurred during the period between 21 and 30 November, when 65 mm of rain fell and maximum wind gusts of 102 km/h were experienced (Bureau of Meteorology), conditions likely to be conducive for spore production and spread.

Conclusions

As a result of this trial, the original proposed eradication protocol was modified to include removal of lower shoots when regrowth occurs on vine trunks and the application of straw mulch onto the vineyard floor. The revised protocol was applied in the second year of the eradication trial in Australia but the trial was compromised by inclement weather and the treated vines becoming infected with inoculum from the control vines. In a real eradication program, infected control vines would not be present and the eradication treatment is likely to have been successful.

The use of drastic pruning as an eradication strategy was shown to have potential for black rot and validation of the protocol was initiated in an infected vineyard in New York USA, where the disease is endemic.

Acknowledgements

We thank Kathy Clarke (DPI Vic), David Sosnowski and Adrian Loschiavo (SARDI) for technical assistance and Chris Dyson (SARDI) for statistical support.

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Sosnowski MR, Fletcher JD, Daly AM, Rodoni BC and Viljanen-Rollinson SLH (2009) Techniques for the treatment, removal and disposal of host material during programmes for plant pathogen eradication. *Plant Pathology* 58, 621-635.

Magarey RD, Coffey BE and Emmett RW (1993) Anthracnose of grapevines, a review. *Plant Protection Quarterly* 8, 106-110.

Wilcox W (2003) Grapes: Black rot (*Guignardia bidwelli* (Ellis) Viala and Ravaz.). Cornell Cooperative Extension Disease Identification Sheet No. 102GFSG-D4, Cornell University.

3.2. Efficacy of burning grapevine material infected with E. ampelina

Authors

M. Sosnowski (SARDI), R. Emmett (DPI Vic), T. Vu Thanh (Uni of Adelaide), T. Wicks (SARDI) and E. Scott (Uni of Adelaide)

Introduction

Burning infected plant material is widely used in the control and eradication of endemic and exotic pathogens. However, there is little scientific evidence to confirm that pathogens are eliminated during this process (Sosnowski *et al.*, 2009). During the burning of infected plant material in the black spot eradication trial, the opportunity was taken to set up an experiment to assess the efficacy of burning as a means of eradicating *Elsinoe ampelina* from grapevine. Black spot (anthracnose), caused by *E. ampelina*, is an important disease of grapevines worldwide (Magarey *et al.*, 1993). The fungus infects leaves, stems, petioles and berries.

Methods

An experiment was conducted in the Sunraysia district of Victoria. Vines (cvs Red Globe, Christmas Rose, Blush Seedless and Fantasy Seedless) were inoculated in spring 2007 by spraying a suspension of *E. ampelina* conidia on new shoots with 2-4 unfolded leaves. The shoots were covered with polyethylene bags overnight to provide high humidity for spore germination and infection.

In July 2008, vines were drastically pruned in an experiment to eradicate the disease. On treated vines, all plant material above the crown was removed and placed in a pit (5 x 3.5 x 0.5 m; Figure 6A). In August 2008, six steel poles were placed upright at random within the pit. Steel mesh bags, containing infected vine canes (approx 30 g each; Figure 6B) and temperature crayons (Tempilstik) in glass Petri dishes (Figure 6C) were attached to the poles at 20 and 50 cm above the pit floor. Another set of mesh bags was buried 5 cm below the soil surface on the floor of the pit. After the vine material was burnt using accelerants with assistance from the Victorian Department of Sustainability and Environment (Figure 6D,E), the mesh bags (Figure 6F) were collected and the ash was transferred to plastic tubes. Unburnt canes from untreated control material and buried samples were grated using a cheese grater. All samples were stored at 3-4°C until they were used.

A bioassay was conducted in a glasshouse at 22-28°C in December 2008 using potted grapevines (cv. Thompson Seedless). The three youngest expanded leaves on each shoot were sprayed with deionised water and dusted with the ash or grated vine material. Each treatment was applied to 3-4 shoots per vine and the inoculated shoots were covered with polyethylene bags (Figure 2H). After 48 hours, the bags were removed, the leaves were sprayed again with deionized water and the bags were replaced and left overnight. The experiment was arranged as a completely randomized design with two replicate vines per treatment. Twelve days after inoculation, the vines were assessed for symptoms of black spot.

Results

The temperature crayons indicated that the fire reached in excess of 250°C and variable temperatures up to 60°C occurred 5 cm below the soil surface (Figure 7). No leaf lesions developed on plants inoculated with ash whereas significant numbers of lesions developed

Figure 6. A. Pit filled with infected grapevine material and steel poles marking the location of samples, B. Steel mesh bag containing infected cane material, C. Steel mesh bag containing Petri dish with glass tubes containing temperature crayons, D. Victorian Department of Sustainability and Environment officer stoking the fire with accelerant, E. Ashes remaining after the fire and F. Mesh bags containing temperature crayons and ash remains of infected canes.

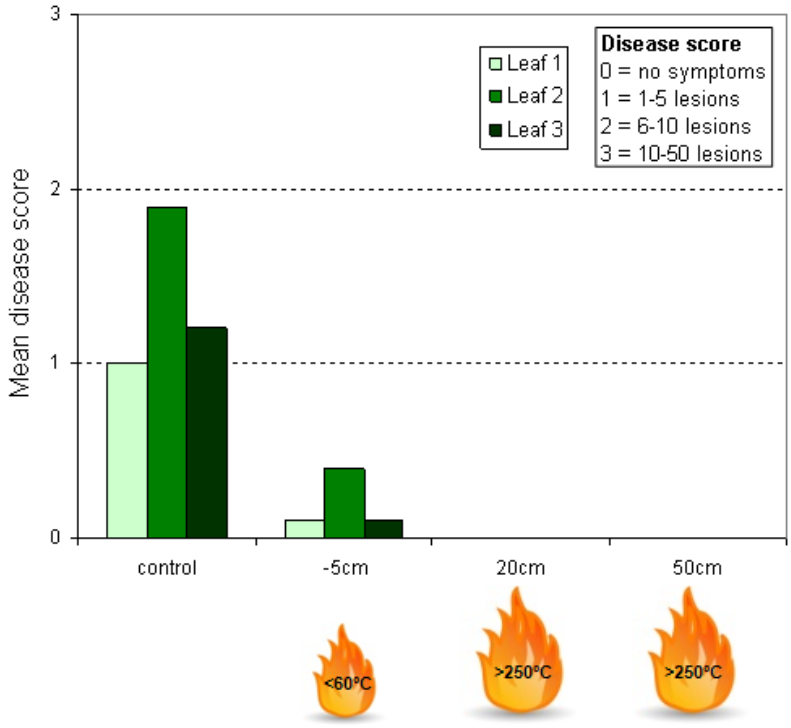

Figure 7. Mean disease score on the three newest leaves on grapevine shoots (cv. Thompson Seedless) inoculated with ash from burnt vine material, infected with *Elsinoe ampelina.* Samples were positioned 20 and 50 cm above the floor of the bonfire, buried 5 cm below the soil surface under the fire or left untreated (control). Data are presented for each leaf individually, with Leaf 1 being the oldest. Temperatures reached at each position are indicated in the flame icons.

on plants inoculated with grated material from the controls. Fewer lesions developed on plants inoculated with cane material that was buried 5 cm below pit floor.

Conclusions

These results confirm the efficacy of burning infected vine material, as temperatures exceeded those that are lethal to most fungi (50-72ºC; Agrios 1988) and the bioassay verified that this was the case. Buried samples only reached a maximum of 60ºC, so any pathogen on debris which penetrates the soil may not be eliminated.

Acknowledgements

We thank Chris Dyson (SARDI) for statistical support and members of the Department of Sustainability and Environment Victoria for assisting with the fire.

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Magarey RD, Coffey BE and Emmett RW (1993) Anthracnose of grapevines, a review. *Plant Protection Quarterly* **8**, 106-110.

3.3. Validation of drastic pruning eradication protocol in the USA

Authors

M. Sosnowski (SARDI), R. Emmett (DPI Vic), W. Wilcox (Cornell University) and T. Wicks (SARDI)

Introduction

In Section 3.1, the endemic disease of grapevine, black spot (*Elsinoe ampelina*), was used as a model to develop and evaluate a drastic pruning eradication protocol, for the exotic disease black rot (*Guignardia bidwellii*). These pathogens have similar biology and epidemiology, infecting fruit, leaves and shoots of grapevines (Magarey *et al.* 1993; Wilcox 2003). A trial was conducted in New York State, in collaboration with Cornell University, to validate the drastic pruning protocol for the eradication of black rot.

Methods

A trial was established on a block of cv. Concord (*Vitis* x *labruscana*) grapevines at the Robbins Research Farm, New York State Agricultural Experimental Station, Cornell University, Geneva, NY, USA. In July 2008, 10 developing clusters on each vine designated for the trial were inoculated by spraying with a conidial suspension of *G. bidwellii* (2×10^5 spores/ml). In September 2008, these vines were assessed for symptoms of black rot to determine whether diseased berries, many of which would fall to the ground to serve as sources of overwintering inoculum, were distributed evenly throughout the trial site.

Each vine was visually assessed for the mean percent disease severity of the inoculated clusters, using on a 0 to 4 scale (0 = no disease; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%). For data analysis, the site was divided into three blocks on either an east/west (E/W) or north/south (N/S) basis, providing an equal number of vines within each block. The mean values were subjected to analysis of variance for a completely randomized block design. There was no significant block effect when considered on either an E/W or N/S basis (P = 0.56 and 0.72, respectively).

In May 2009, most vines were removed from the block by cutting trunks 20 cm above the ground with a chainsaw and treating the stumps with herbicide, to establish discrete two-vine plots. A minimum distance between plots of 5 m reduced the likelihood of disease spread from retained vines assigned to the control treatment (Figure 8). The plots were assigned to one of two treatments (i) controls (pruned to leave approximately 50 buds per vine and two infected bunches for disease carry over, as a positive check on environmental suitability for disease development; Figure 9A) or (ii) the eradication protocol, using a completely randomised design with nine replications. The eradication protocol consisted of the following steps:

- vines were cut at the crown using a chainsaw (Figure 9B)
- all excised vine material above the crown was removed (Figure 9C) and placed in a heap about 1200 m from the trial plots
- the vineyard floor around treated and control vines was raked (Figure 9D) and the debris removed placed in the heap and burnt (Figure 9E)
- lucerne hay was rolled out over the vineyard floor to provide a 10-20 cm thick layer of mulch (Figure 9F&G)
- watershoots emerging below 30 cm from the ground were removed on 7 July
- one vine in each plot was sprayed with a combination of the fungicides Nova 40W at 0.4 g/L (ai. 40% myclobutanil) and Penncozeb 75DF at 4.8 g/L (a.i. 75% mancozeb) six times on a fortnightly basis from 2 June until 13 August, whereas the other remained unsprayed.

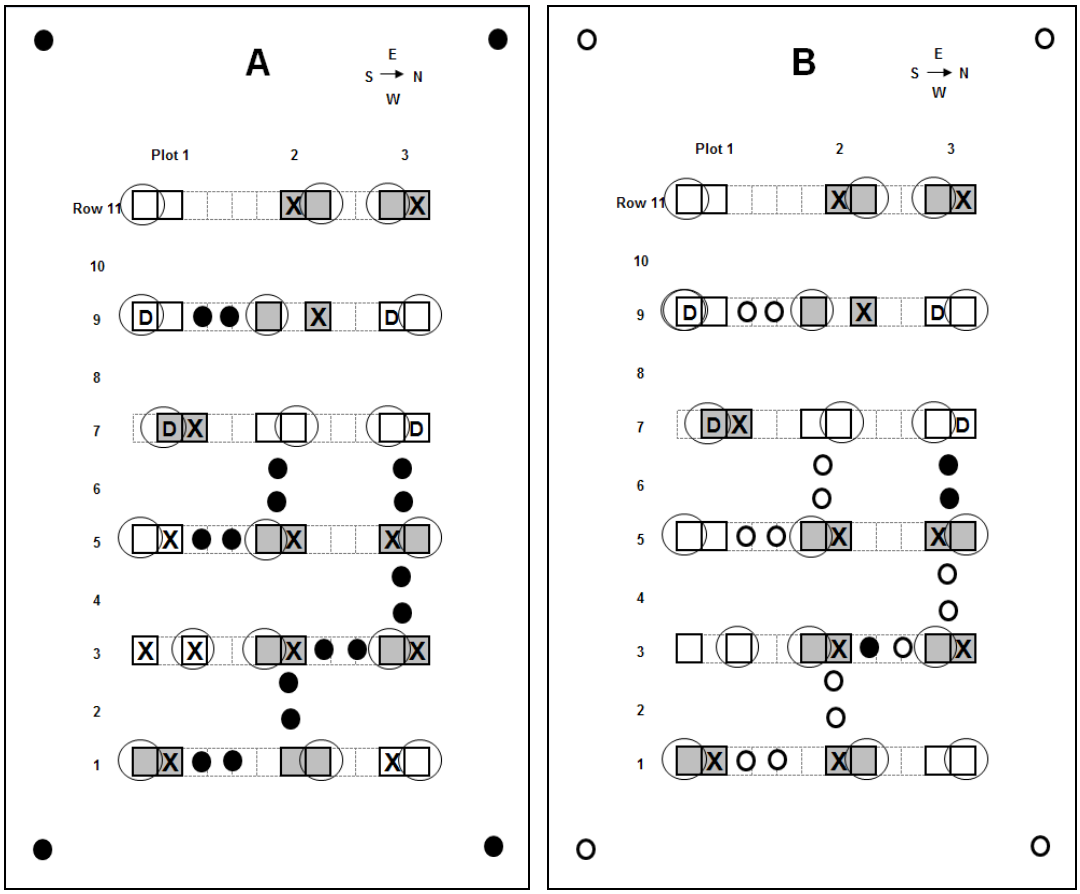

Figure 8. Black rot field trial located in a vineyard block (cv. Concord) at the Robbins Research Farm, NY, USA showing results in A. 2009 and B. 2010. Plots consist of two vines (solid framed boxes) and were randomly assigned as control (grey) or treated (white). Rows 2, 4, 6, 8 and 10 and vines between plots (grey dashed boxes) were cut and stumps killed with herbicide. Vines observed with black rot symptoms are indicated (X). Vines which died during the experiment are indicated (D) and were not included in data analysis. Sentinel vines (○) were strategically positioned to monitor disease spread from control plots and those recorded with symptoms are indicated (●). Fungicide (myclobutanil + mancozeb) was applied to foliage on one vine of each treated plot (circled). Figure not to scale.

Vines were assessed for black rot symptoms in August 2009 using the following scale: 0 = no infection, 1 = slight infection (< 6 leaf lesions), 2 = low infection (6 leaf lesions - 5 infected bunches/leaves), 3 = moderate infection (5-20 infected bunches/leaves) and 4 = severe infection (20+ infected bunches/leaves).

Potted sentinel vines (cv. Chardonnay) were placed strategically around the trial site, radiating at 2 m intervals in different directions (north, south, east and west) from the control plots (Figure 9H), to assess the potential for spore movement from the plots. Between 3 June and 15 July, 20 potted vines were taken from an outdoor nursery (Figure 9I), which was purposely isolated from any vineyards, and placed in the trial site for two weeks, and then retrieved and replaced with a fresh set of vines. Following removal from the trial site, vines were incubated in an isolated location for a further two weeks before black rot lesions on leaves were counted.

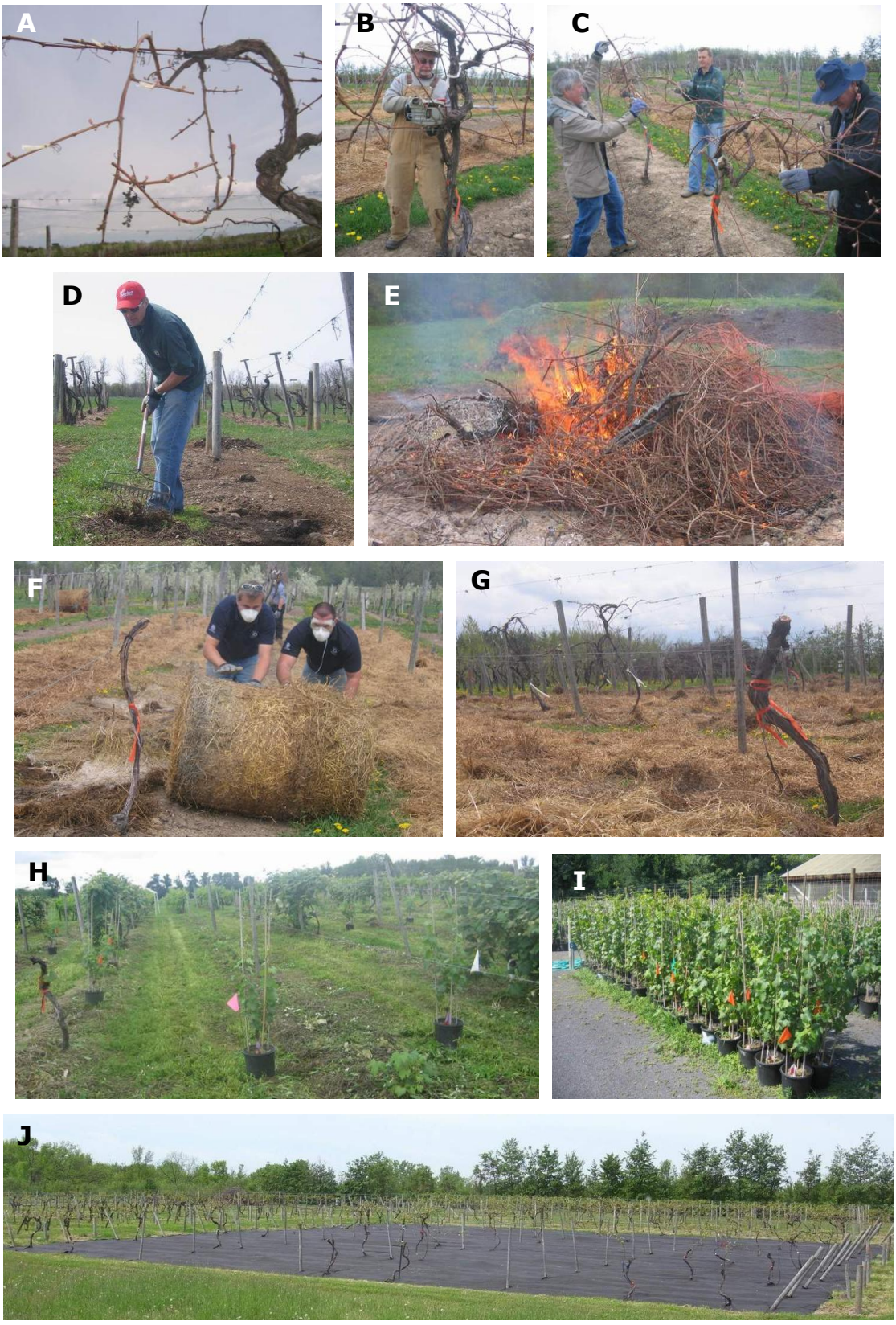

Figure 9. Drastic pruning validation trial at Robbins Research Farm, New York Agricultural Experiment Station, Cornell University, Geneva, NY, USA. A. Control vine with infected bunch, B. Cutting vines at the crown with a chainsaw , C. Removing infected vine material D. Raking the ground beneath vines, E. Burning excised plant material, F. Rolling out the lucerne hay mulch, G. Treated vine (right) and control vines (left), H. Sentinel vines in the trial, I. Sentinel vines in the nursery, and J. Eradication trial site with landscape fabric mulch.

The trial was continued for a second season. In April 2010, vines in all plots were pruned as normal, leaving 50 buds per vine and retaining two infected clusters on each control vine. In 2010, Geotex landscape fabric (non-woven polypropylene) instead of lucerne hay was laid on the vineyard floor (Figure 9J) as a mulch to prevent spore movement from debris on the ground. As in 2009, low watershoots were removed (24 June) and five fungicide sprays were applied at fortnightly intervals. Sentinel vines (cv. Pinot Noir) were also installed in the vineyard as previously described. On 21 July 2010, vines in all plots were assessed for symptoms of black rot, as described above.

Results

In the first season following the eradication treatments in 2009, all sentinel vines were infected showing that spores were readily spread between plots by wind and rain (Figure 8A). In the significantly drier second season, leaf lesions were recorded on only three sentinel plants, revealing minimal spread of spores between plots (Figure 8B).

In August 2009 control plots had a dense canopy (Figure 10A) compared with drastically pruned vines (Figure 10B). Assessment of vine plots revealed that all unsprayed control vines were infected with black rot (Figure 10C&D), with a mean severity rating of 2.9 (Figure 11). One of the eight control vines sprayed with fungicide was also infected, but with a mean rating of 0.2. Three of the 18 drastically pruned vines failed to produce watershoots and subsequently died. Of the surviving treated vines, three of the seven unsprayed vines were infected, with a mean rating of 0.4. None of the treated vines sprayed with fungicide developed black rot.

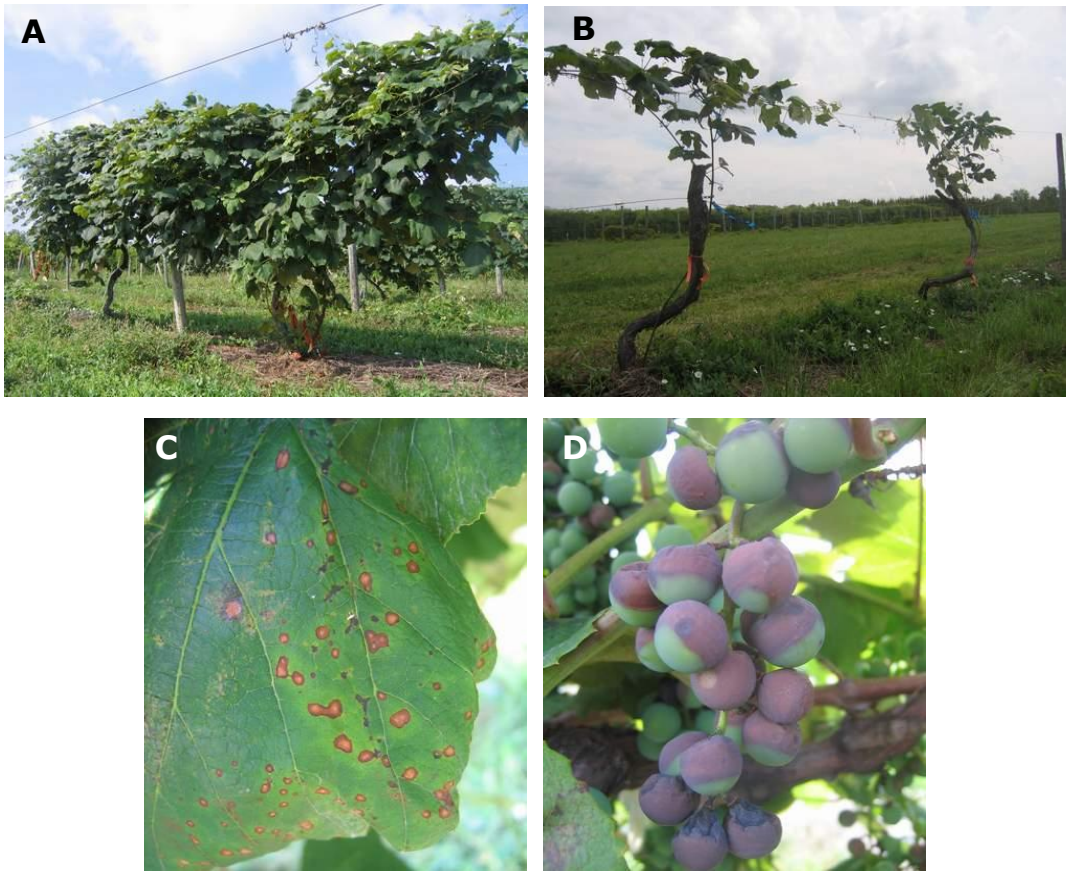

Figure 10. Dense canopy of control vines (A) compared with the sparse canopy of drastically pruned vines (B) in August 2009 and black rot symptoms on leaves (C) and bunches (D).

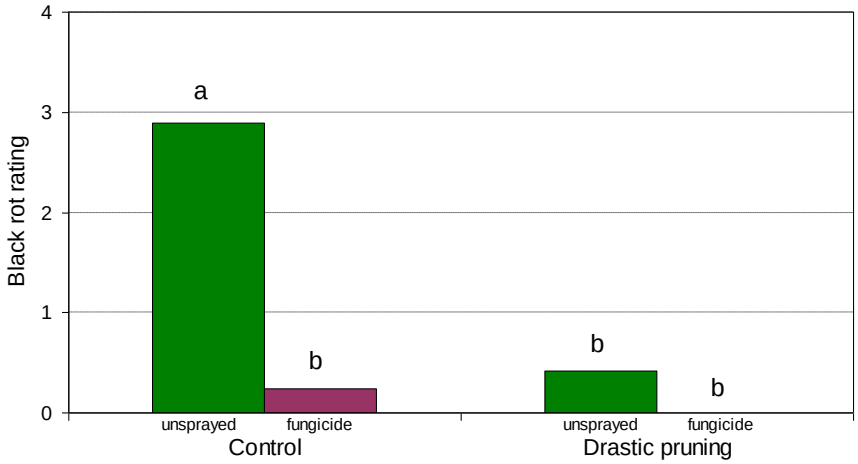

Figure 11. Mean black rot rating of vines in 2009 subjected to drastic pruning compared with controls when either sprayed with fungicide or left unsprayed. 1 = no symptoms, 4 = severe infection. Mean ratings with the same letter (a or b) are not significantly different (LSD; P = 0.05).

The final assessment on 21 July 2010 showed all unsprayed controls were infected, with a mean rating of 3.6 (Figure 12). No black rot developed on the control vines sprayed with fungicide or on drastically pruned vines, with or without fungicide.

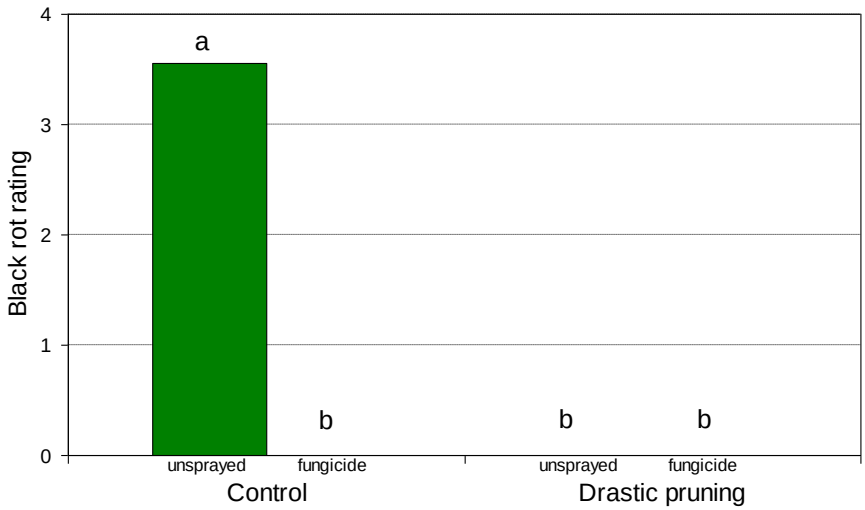

Figure 12. Mean black rot rating of vines in 2010 subjected to drastic pruning compared with controls when either sprayed with fungicide or left unsprayed. 1 = no symptoms, 4 = severe infection. Mean ratings with the same letter (a or b) are not significantly different (LSD; P = 0.05).

Conclusions

The validation trial undertaken in New York demonstrated the efficacy of the eradication protocol for black rot on grapevines. In the first season, when environmental conditions were extremely conducive for black rot development, symptoms were recorded on all vines except those subjected to drastic pruning and a fortnightly fungicide spray program. The significant reduction in disease severity on sprayed control vines highlights the efficacy of the fungicides myclobutanyl and mancozeb for the control of black rot, which are recommended in north-eastern USA (Wilcox, 2010). However, black rot developed on some vines highlighting the necessity of an intensive inoculum removal program in a

successful eradication program. The low level of leaf and fruit infection detected in the unsprayed, drastically pruned vines shows that a fungicide spray program may be necessary to ensure eradication. However, it should be recognized that these infections were possible artefacts of our experimental design, as inoculum was retained in the control vines. The latter would not occur in an eradication program. In the second season, the drastic pruning treatment completely prevented black rot from developing on the treated vines. Similarly, fungicide application eliminated infection even from control vines with retained inoculum.

If an incursion of black rot occurred in Australia, the drastic pruning protocol has potential to save the wine industry many millions of dollars in lost production and vineyard re-establishment costs. The eradication protocol will be included in the Viticulture Industry Biosecurity Plan. With further validation, the protocol also has potential for eradication of other pathogens of grapevines as well as other perennial crops.

Acknowledgements

We thank C. Austin, J. Burr and D. Riegel (Cornell University) for technical assistance and trial maintenance and in-kind contributions of Cornell University with vineyard and laboratory facilities.

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3.4. Decision reference tool

Following validation of the drastic pruning protocol for black rot of grapevine in this project, it was considered that this strategy may be effective for other exotic pathogens of perennial crops. A decision reference tool has been developed to provide a guide for consideration of non-destructive eradication by scientific assessment panels convened as part of consultative committees during incursions. Based on a list of high-priority plant pathogens compiled by the Subcommittee on Plant Health Diagnostic Standards (SPHDS), pathogens are rated on the likelihood of eradication using a drastic pruning protocol based on pathogen epidemiology and past experience in eradications. A preliminary draft of the decision reference tool is included on the following page. It excludes non-perennial hosts, insects, nematodes, viruses, viroids and phytoplasmas, which are not relevant for this type of eradication strategy. Of the listed fungal and bacterial pathogens, each is classified as a surface pathogen (inhabiting fruit, leaves and green shoots) or systemic pathogen (inhabiting all parts of plant including trunks and/or roots). The likelihood of eradication using this strategy is indicated by rating as: yes, possible, maybe or no. This document also identifies the 'possible' plant-pathogen systems as candidates for validation through further research.

Decision Reference Tool

for use of drastic pruning for eradication

Scientific Name	Exotic disease	Perennial host	Pathogen type	Pathogen inhabitation	Likelihood of eradication	Comments
Anisogramma anomala	Hazelnut blight (Eastern Filbert Blight)	Hazelnut	Fungus	Systemic	no	
Apiosporina morbosa	Black Knot	Plum, stone fruit	Fungus	Systemic	maybe	Diseased wood evident by knots
Blood Disease Bacterium (*Pseudomonas celebensis*)	Blood Disease	Banana	Bacteria	Systemic	no	
Candidatus Liberibacter africanus	Huanglongbing (african strain)	Citrus	Bacteria	Systemic	no	
Candidatus Liberibacter americanus	Huanglongbing (american strain)	Citrus	Bacteria	Systemic	no	
Candidatus Liberibacter asiaticus	Huanglongbing (asiatic strain)	Citrus	Bacteria	Systemic	no	Experiment by Lopes et al. (2007) failed to eradicate HLB
Ceratocystis ulmi (=Ophiostoma ulmi)	Dutch Elm Disease	Elm	Fungus	Systemic	no	
Ciborinia camelliae	Camellia Petal Blight	Camelia	Fungus	Surface	possible	
Cladosporium caryigenum	Pecan scab	Pecan	Fungus	Surface	possible	
Colletotrichum acutatum SGO strain	Post bloom fruit drop	Citrus	Fungus	Surface	possible	
Cryphonectria parasitica	Chestnut blight	Chestnut	Fungus	Systemic	no	
Elsinoe australis	Sweet orange scab	Citrus	Fungus	Surface	possible	
Elsinoë mangiferae	Mango Scab	Mango	Fungus	Surface	possible	
Endocronartium harknessii	Western gall rust	Pine	Fungus	Systemic	maybe	Diseased wood evident by galls
Erwinia amylovora	Fireblight	Pome fruit	Bacteria	Systemic	no	
Erwinia herbicola	Avocado blast complex	Avocado	Bacteria	Surface	possible	
Erwinia pyrifoliae	Black stem blight	Pear	Bacteria	?	?	
Fusarium circinatum	Pine Pitch Canker	Pine	Fungus	Systemic	maybe	Effects all parts of tree but does not move internally
Fusarium mangiferae	Mango Malformation	Mango	Fungus	Surface	possible	
Fusarium oxysporum f.sp. cubense	Panama disease Tropical race 4	Banana	Fungus	Systemic	no	
Guignardia bidwellii	Black rot	Grapevine	Fungus	Surface	yes	Scientifically validated (Sosnowski et al. 2011)
Guignardia musae	Freckle disease	Banana	Fungus	Surface	possible	
Gymnosporangium juniper-virgininae	Cedar apple rust	Apple, cedar	Fungus	Surface	possible	
Monilinia fructigena	Brown rot	Apple	Fungus	Surface	possible	
Mycosphaerella eumusae	Eumusae leaf spot	Banana	Fungus	Surface	possible	
Mycosphaerella fijiensis	Black Sigatoka	Banana	Fungus	Surface	likely	Successful eradication in Tully (Peterson et al. , 2005)
Neonectria ditissima (Nectria gallegina)	European canker, Nectria canker	Beech, Birch, Maple, Apple	Fungus	Systemic	possible	*N. galligena* was eradicated from apples in Tasmania (Ransom 1997)
Oidium citri	Powdery mildew	Citrus	Fungus	Surface	possible	
Oidium tingitanium	Powdery mildew	Citrus	Fungus	Surface	possible	
Phakopsora euvitis	Grapevine leaf rust	Grapevine	Fungus	Surface	possible	
Phymatotrichum omnivorum	Texas Root Rot	Grapevine, others	Fungus	Systemic	no	
Phytophthora ramorum	Sudden Oak Death	Oak, others	Fungus	Systemic	no	
Pseudocercospora purpurea	Cercospora spot	Avocado	Fungus	Surface	possible	
Pseudomonas syringae pv. *papulans*	Blister spot of Apples	Apple	Bacteria	Surface	possible	
Pseudomonas syringae pv. *syringae*	Avocado Blast Complex	Avocado	Bacteria	Surface	possible	
Pseudopezicula tetraspora	Angular leaf scorch of grape	Grapevine	Fungus	Surface	possible	
Pseudopezicula tracheiphila	Rotbrenner	Grapevine	Fungus	Surface	possible	
Puccinia psidii	Eucalyptus Rust	Eucalypts, others	Fungus	Surface	possible	
Sphaceloma perseae	Avocado Scab	Avocado	Fungus	Surface	possible	
Uredo rangelli	Myrtle rust	Myrtles	Fungus	Surface	possible	
Verticillium dahliae	Verticillium wilt (defoliating strain)	Many	Fungus	Soil-borne	no	
Xanthomonas alfalfa subsp. *citrumelonis*	Bacterial spot	Citrus	Bacteria	Surface?	maybe	
Xanthomonas ampelina / Xylophilus	Bacterial blight	Grapevine	Bacteria	Surface	possible	
Xanthamonas campestris (avocado strain)	Bacterial canker complex	Avocado	Bacteria	Systemic	no	
Xanthomonas campestris (vasicola) pv. musacearum	Banana bacterial wilt (BXW)	Banana	Bacteria	Systemic	no	
Xanthomonas citri subsp. *citri*	Citrus Canker	Citrus	Bacteria	Surface	possible	
Xylella fastidiosa	Pierces disease	Grapevine	Bacteria	Systemic	no	

Surface - tend to only inhabit leaves, fruit and young shoots

Systemic - affects all parts of plant including trunk

Excludes non-perennial hosts, insects, nematodes, viruses, viroids and phytoplasmas

3.5. Postgraduate study – Fusarium wilt of bananas

The project CRC60097 "Epidemiological and biological studies of the exotic plant pathogen Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* 'tropical' race 4 (Foc TR4)" commenced in 2008. PhD candidate: Ms Rachel Meldrum, Supervisors: Dr Elizabeth Aitken (Uni of Qld), Mr Andrew Daly and Ms Lucy Tran-Nguyen(NT DPIFM).

Key findings of the project will be reported separately to CRCNPB.

3.6. Postgraduate study – Efficacy of burning and burial for eradication

The project "Pathogen eradication using pistachio dieback as a model" was funded by an Endeavour Postgraduate Award and PhD student Tu Anh Vu Thanh is in the final stages of the project. Supervisors are Prof Eileen Scott (University of Adelaide), Drs Daniele Giblot Ducray and Mark Sosnowski (SARDI).

Pistachio dieback is caused by the bacterium *Xanthomonas translucens* pv. *pistaciae* (Xtp), that invade the vascular system of pistachio plants. Burial and burning are two accepted means of disposal of diseased plant material. However, there is little or no information on the survival of bacterial pathogens following burial or burning of infected wood. This project study aimed to evaluate the efficacy of burial and burning as means of safe disposal of diseased wood using pistachio dieback as a model.

Infected pistachio wood was buried in orchard soil in pots or placed on the soil surface in August 2008 and retrieved monthly to assess survival of Xtp. Viable Xtp was detected in some wood samples buried for up to 28 months, but not in wood placed on the soil surface at any time.

A field experiment in 2009 to study survival of the pathogen following burning showed that Xtp survived in some unburned wood. To help explain this result, controlled environment experiments were conducted in which infected wood was subjected to 40-60ºC to study survival of Xtp. The results showed that Xtp survived exposure to 40-55ºC for at least 60 minutes, but was killed after exposure to 50ºC for 180 minutes, 55ºC for 120 minutes and 60ºC for 15 minutes or more.

Experiments were also conducted to identify the lethal temperature for Xtp using two methods: in terms of thermal death time and thermal death point. The time of exposure (thermal death time) to 50ºC required to kill Xtp cells varied from 30 to more than 60 minutes and thermal death point for Xtp lies between 60 and 65ºC.

Tu Anh is currently writing her thesis and two manuscripts are being prepared for publication in scientific journals.

4. Implications for stakeholders

Include industry, community, policy makers and others where relevant

An eradication protocol has been validated for black rot, an exotic fungal disease of grapevines. It has the potential to preserve mature premium vines in the event of an incursion of this disease into Australia, thereby enabling the industry to continue producing superior premium wine and giving Australia a competitive advantage in the international market. A cost-benefit analysis[1] identified potential savings resulting from this protocol, versus the standard technique of complete vineyard removal, in the order of $3.6 million for the infected vineyards if there were an incursion of the disease. This represents a substantial reduction of the economic impact on both the affected producers and the local community.

The validation of the protocol and subsequent publication of the results in an international scientific journal and in the Viticulture Industry Biosecurity Plan documents the validity of the technique and provides confidence in its utility for policy and decision makers in the event of an incursion of black rot into Australia.

The protocol may also be effective against other pathogens of grapevines and pathogens of other perennial crops, but validation will be required for each system in order to provide scientific evidence for policy makers to adopt the strategy for future eradications. A decision reference tool for consideration of non-destructive eradication has been developed from the current list of high priority exotic pathogens compiled by the Subcommittee on Plant Health Diagnostic Standards (SPHDS). This preliminary working document is based on knowledge of the epidemiology of each pathogen as well as from past eradication experiences and will require updating as more research is conducted.

Burning and burial is the best method available for disposal of infected plant material. To ensure the pathogen is eliminated it is important that all material is completely burnt; otherwise, any unburnt, buried material must remain undisturbed for more than 3 years, based on results using pistachio.

[1]McLeod R. (2008) Economic assessment of selected investments of the Cooperative Research Centre for National Plant Biosecurity. Final Report to CRCNPB, 2 July 2008. Agtrans Research and eSYS Development.

5. Recommendations

Include recommendations based on the findings from the research

- The review conducted in Phase 1 of the project outlined the information available on the effectiveness of techniques for treatment, removal and destruction of host material to eradicate exotic pathogens. It recommended areas for further research to provide sound scientific evidence for the use of eradication techniques in the future. Phase 2 of this project has addressed some of these gaps in the literature, and will lead to publication of the scientific evidence generated.
- In the event of an incursion of black rot disease (*Guignardia bidwellii*) of grapevines in Australia, the eradication protocol based on drastic pruning should be considered as part of the overall eradication strategy.
- The decision reference tool should be referred to by any scientific assessment panel convened as part of consultative committee during an exotic incursion by a fungal or bacterial pathogen of a perennial crop. It identifies host/pathogen systems on which the protocol is likely to be effective and will require validation in the future.

- In the event of eradication, it is important that all wood material is completely burnt and buried material remains undisturbed for at least three years or more, to prevent any likelihood of pathogen escape following eradication. Factors such as moisture content and wood density may affect the efficiency of burning and further research is required to establish methods to optimise the burn of different host materials.

6. Abbreviations/glossary

ABBREVIATION	FULL TITLE
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
EPP	Emergency plant pest
DPI Vic	Department of Primary Industries, Victoria
PCN	Potato cyst nematode
RKN	Root knot nematode
SARDI	South Australian Research and Development Institute

7. Plain English website summary

CRC project no:	CRC40139
Project title:	Optimising eradication strategies for exotic plant pathogen incursions on perennial crops (Phase 2)
Project leader:	Dr Mark Sosnowski (SARDI)
Project team:	Dr Robert (Bob) Emmett (DPI Vic), Prof Wayne Wilcox (Cornell University), Dr Trevor Wicks (SARDI),
Project collaborators:	Ms Tu Anh Vu Than (Uni of Adelaide), Prof Eileen Scott (Uni of Adelaide), Dr Daniele Giblot Ducray (SARDI), Dr John Fletcher (Plant & Food Research NZ), Ms Rachel Meldrum (NT DPIFM) Mr Andrew Daly (NT DPIFM) Ms Lucy Tran-Nguyen (NT DPIFM) Dr Elizabeth Aitken (Uni of Qld)
Research outcomes:	• An eradication protocol based on drastic pruning has been developed and validated for use on grapevines in the event of an incursion of the fungal disease, black rot (*Guignardia bidwellii*). • A decision reference tool for consideration of non-destructive eradication to guide scientific assessment panels convened as part of consultative committees during incursions of perennial plant pathogens. • Burning infected grapevine and pistachio material will eliminate *Elsinoe ampelina* and *Xanthomonas translucens* pv. *pistaciae*, respectively, if all material is completely burnt. *X. translucens* may survive on unburnt and buried wood for more than two years.
Research implications:	• The eradication protocol based on drastic pruning may be effective for other exotic pathogens of grapevines and other perennial crops but each will require validation before inclusion in Industry Biosecurity Plans. • Completely burning infected grapevine and pistachio plant material will eliminate fungal and bacterial pathogens; further research is required to develop protocols for effective burns on different host plant material. Pathogens may survive in unburnt and buried material for more than two years so soil must remain undisturbed for three or more years.
Research publications:	Sosnowski MR, Emmett RW, Wilcox WF, Austin CN and Wicks TJ (2011) Eradication of black rot (*Guignardia bidwellii*) from grapevines by drastic pruning. *Plant Pathology* (in preparation) Vu Than TA, Sosnowski MR, Giblot-Ducray D, Taylor C and Scott ES (2011) Effect of burning and temperature on survival of *Xanthomonas translucens* pv. *pistaciae*, a wood-inhabiting bacterial pathogen. *Plant Pathology* (in preparation) Sosnowski MR, Emmett RW, Wilcox WF and Wicks TJ (2011) Eradication of black rot from vineyards using a drastic pruning protocol. *CRCNPB Science Exchange*, Barossa Valley, South Australia 9-11 February 2011 p 75. Sosnowski MR, Emmett RW, Wilcox WF and Wicks TJ (2010) Reducing the impact of eradication for exotic grapevine

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REVIEW

Techniques for the treatment, removal and disposal of host material during programmes for plant pathogen eradication

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Eradication of plant pathogen incursions is very important for the protection of plant industries, managed gardens and natural environments worldwide. The consequence of a pathogen becoming endemic can be serious, in some cases having an impact on the national economy. The current strategy for eradication of a pathogen relies on techniques for the treatment, removal and disposal of affected host plants. There are many examples where these techniques have been successful but many where they have not. Success relies on a sound understanding of the biology and epidemiology of the pathogen and its interaction with the host. Removal and disposal of infected plant material for eradication and containment of plant and soil inhabiting fungal, bacterial and viral pathogens are reviewed by considering black Sigatoka of banana, apple scab, maize smut, fireblight, citrus canker and sharka disease of stone-fruit crops. In examining examples of dealing with plant pathogens and diseased host material around the world, particularly Australasia, various techniques including burning, burying, pruning, composting, soil- and biofumigation, solarization, steam sterilization and biological vector control are discussed. Gaps in the literature are identified and emphasize the insufficient detail of information available from past eradications. More effort is required to produce and publish scientific evidence to support the success or otherwise of techniques and suggestions for future research are proposed.

Keywords: Australasia, biosecurity, containment, disease detection, phytosanitation, quarantine

Introduction

An incursion is defined as an isolated population of a pest recently detected in an area, not known to be established, but expected to survive for the immediate future (Anon, 2007) and can threaten the economic viability of plant industries worldwide. The isolation of island nations such as Australia and New Zealand has assisted in the exclusion of many plant pathogens endemic in other parts of the world. In Australia, the farm-gate value of plant industries is over A$15 billion and contributes over A$10 billion to export income (The Australian Bureau of Agricultural and Resource Economics, ABARE). In New Zealand farm gate value of plant industries is approximately NZ$11 billion with export incomes of around NZ$7 billion (Statistics New Zealand). Eradication is defined as the elimination of a pest from an area using phytosanitary measures (Anon, 2007). Eradication programmes can be expensive, requiring a great deal of labour and resources, but often provide great economic benefit. The recent eradication of citrus canker from Queensland cost approximately A$18.5 million. However, this cost was regarded as affordable in order to give long-term benefit and preserve the A$105 million citrus industry in Australia (ABARE; Telford, 2007). In Florida, USA, the cost of citrus canker eradication between 1995 and 2005 was estimated at nearly US$1 billion (Gottwald & Irey, 2007). The eradication of fire blight from Melbourne Royal Botanic Gardens in 1997 cost approximately A$20 million, but this too may have prevented Australian pome fruit industry losses of up to A$870 million had the disease become endemic (Rodoni *et al.*, 2006). Murray & Brennan (1998) estimated potential annual losses of A$491 million should Karnal bunt of

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wheat become endemic in Australia, based on losses in yield, quality and export markets as well as costs of control seed production and research. Predictive costs of an outbreak of Karnal bunt in the European Union have recently been presented (Sansford *et al.*, 2008). The eradication of black Sigatoka from the Tully Valley in Queensland in 2001–3 cost A$17 million (A. Daly, unpublished data). However, the increased cost to the industry in Tully of not eradicating the pathogen, borne from increased de-leafing and chemical application, would have been nearly A$90 million over a 5-year period. In 2006, Cyclone Larry destroyed much of the banana crop in Innisfail, the largest single banana production area of northern Queensland. Queensland production accounts for 95% of bananas entering Australia's central markets. The massive crop loss caused the consumer price index to jump, the price of bananas rising ten-fold. A similar crop loss and resulting impact on the industry and the Australian economy could be experienced should a wide-scale outbreak of an exotic pathogen occur in Australia's relatively small banana production area.

An exotic plant pathogen can be detected in imported material (interception) or as a replicating population following a field outbreak. Ebbels (2003) suggested that the action taken in response to an interception of imported material is often short-term as it is isolated, whereas a field incursion or outbreak detected as a replicating population requires a longer-term approach. The management of an incursion has three alternative responses: eradication, containment or no action. Containment is defined as the application of phytosanitary measures in and around an infested area to prevent spread of a pest (Anon, 2007). The response taken will often depend on the net economic benefit (Fraser *et al.*, 2006). Eradication is likely to be attempted where export markets will be affected and success of the eradication is probable. Sometimes the only option may be to contain or limit spread or, in some cases, do nothing at all despite the potential loss of export markets. Many pathogens are subject to phytosanitary requirements on interceptions and outbreaks or movement of plant material in existing legislation and through the International Plant Protection Convention. According to Simberloff (2003), the success of eradication depends on adequate resources, a commitment to see the programme through to completion, establishment of clear lines of authority, the target species to be detectable at low densities and subsequent intensive management of the system.

The current strategy for eradication of an exotic plant pathogen outbreak involves the following general steps: (i) surveillance to locate source and extent of the infection, (ii) removal of infected and potentially infected plant material, (iii) disposal of plant material (e.g. burn and/or bury), (iv) quarantine and movement controls to prevent the spread of the pathogen, and (v) surveillance to confirm freedom from pathogen. Due to the broad scope of eradications, this review focuses mainly on steps (ii) and (iii), areas requiring more research. Several case studies of eradication or containment of plant and soil inhabiting fungal, bacterial and viral pathogens of both perennial and annual crops are presented. Examples are predominantly taken from Australasia but also draw from cases further abroad. An integrated approach is often employed to contain or eradicate a plant pathogen. Methods for removal and disposal are then evaluated, drawing on information from these and other eradication and control programmes reported worldwide. The objective is to provide information which may lead to the optimization of existing methods or the development of novel eradication methods.

Eradication case studies

Black Sigatoka of banana

Black Sigatoka (black leaf streak) is a disease of banana (*Musa* spp.) caused by the ascomycete fungus *Mycosphaerella fijiensis* and is endemic in many parts of the world (Mulder & Holliday, 1974). The pathogen produces conidia and ascospores on leaf lesions which are spread by rain and wind to infect new banana leaves. Australia remains free of black Sigatoka despite several outbreaks which have occurred in commercial production areas. Jones (1984) reported the first attempt to eradicate black Sigatoka from the Australian mainland and three neighbouring islands following its detection in the northern Cape York Peninsula and the Torres Straight region in 1981. The campaign involved injection of banana plants with herbicide (glyphosate) to kill all bananas in the infested areas. Most leaf and pseudostem residues were destroyed later by burning. Re-planting bananas in the eradication areas was not permitted for 6 months during the wet summer when break-down of remaining plant residues was accelerated. The decision for this host free period was based on data from previous research on the longevity of *M. musicola* (Stover, 1980), the cause of yellow Sigatoka of banana. Conidia, ascospores and perithecia of *M. musicola* did not remain viable on detached banana tissue for longer than 3 to 4 months. Despite these attempts, black Sigatoka reappeared in the northern Cape York Peninsula and on Thursday Island in 1984. This may have been due to survival of the pathogen on banana residue for longer than expected, the failure to locate all stands of bananas near infested areas, or re-introduction of the pathogen via airborne spores or with planting material or leaves from other nearby infested areas north of the mainland (Jones, 1984).

Peterson *et al.* (2005) reported that between 1981 and 2000, black Sigatoka was detected in a further six locations in Cape York Peninsula, most likely resulting from introductions of infected planting material from the Torres Strait region. The outbreaks were eradicated on each occasion using different methods to destroy the infected plants (Peterson, 2002). These included injection with herbicide, removal of plants followed by burial and burning or disc ploughing of felled plants. Resistant cultivars were supplied for re-planting in most cases.

The disease was detected again in 2001 in the Tully area, the major banana-growing region of Australia. A programme of intensive de-leafing was employed to remove the majority of inoculum from plants (Peterson

et al., 2005). Leaves removed from plants were placed on top of one another on the ground to reduce ascospore release, a strategy shown to be effective for *M. musicola* (Peterson *et al.*, 2000). Two months later, leaf material was inspected microscopically and *M. fijiensis* was not detected. To prevent establishment of new infections, mancozeb, propiconazole, difenoconazole, tebuconazole and trifloxystrobin fungicides mixed with mineral oil were applied to plants weekly in rotation for 6 months. Organic growers applied copper-based fungicides plus vegetable oil in rotation with mineral oil alone. The presence of remaining inoculum was monitored using transects of sentinel plants not subjected to fungicides. No further inoculum was detected. Temperature and rainfall data were also analysed to indicate the number of disease cycles that would have occurred during the 6-month period. A statistical model was developed to simulate the multiplication and spread of the pathogen and provided a very high level of confidence that the pathogen had been eradicated (Peterson *et al.*, 2005). The success of the Tully eradication programme was attributed in part to early disease detection, the approaching dry season (suppression of ascospore production) and the biology of the fungus (no alternative hosts and long-term survival structures).

Apple scab

Apple scab, caused by the ascomycete fungus *Venturia inaequalis*, overwinters as pseudothecia in infected leaves on the ground, and ascospores, the primary source of inoculum, are spread by rain and wind to infect new leaves and fruit (MacHardy, 1996). Conidia are then formed and provide secondary infection of more leaves and fruit throughout the season. Apple scab was the subject of several eradication programmes in Western Australia between 1930 and 1996, and since then this Australian state is currently the only apple-producing region in the world free of the disease (MacHardy, 1996; McKirdy *et al.,* 2001). In 1930 apple scab was confirmed in several apple orchards, leading to an eradication programme which involved boiling all apples removed from trees and the orchard floor, application of Bordeaux fungicide and cultivation of the orchard floor (Pittman, 1936). In 1936, introduction of *V. inaequalis* on nursery stock from Victoria prompted eradication measures similar to that in 1930 (Pittman, 1936; Cass Smith, 1940). In addition, all imported nursery stock was dipped in Bordeaux fungicide and the entry of all pome fruit into Western Australia was prohibited. Further outbreaks in 1940 were again subjected to intensive eradication efforts and reported to be successful when the disease was not detected during inspections over the following 5 years (Cass Smith, 1940; Powell & Cass Smith, 1944). In 1947–48, apple scab was detected in orchards and nurseries and subsequently traced back to nursery stock imported from Victoria and Tasmania (Cass Smith *et al.*, 1948). Eradication measures included the complete destruction of all trees with apple scab and the cutting back to basal dormant buds of any adjacent symptomless plants, although details of the methods of destruction were not available. The disease was not detected in Western Australia for a further 40 years.

Between 1989 and 1996, several new outbreaks of apple scab occurred and intensive eradication programmes followed: strategies were developed to interrupt the lifecycle of the fungus (Cripps & Doepel, 1993). Initially, compulsory spraying with the fungicide Baycor 300 (bitertanol) was conducted after harvest and before leaf fall. At 20% leaf fall, 5% urea solution was applied to reduce pseudothecium formation. After leaf fall the whole orchard floor was sprayed with 5% urea to assist microbial breakdown of leaf litter. Leaves were manually raked into rows and mulched using a flail mower. Neglected orchards were removed, all leaf litter treated as above and trees burnt.

Apple scab has not been detected in Western Australia since 1996 and the success of the eradication measures has been attributed to containment of new outbreaks, preventing spread within and between districts, disrupting the lifecycle of the fungus during winter, and preventing infection and disease by applying flusilazole fungicide in the spring-early summer period (Doepel, 1997; McKirdy *et al.*, 2001).

Common smut of maize

Common smut (*Ustilago maydis*) is a basidiomycete fungal disease of maize which survives for up to several years as teliospores in soil or on crop debris. In the presence of moisture in spring or summer, teliospores germinate to form basidia (promycelia) which produce basidiospores. Basidiospores are spread by rain and wind to infect developing plant tissue, eventually forming galls in which new teliospores develop. These either reinfect young plants or overwinter in soil or debris. *Ustilago maydis* was first detected on North Island, New Zealand in a crop at Gisborne in 2006. A survey of the area revealed that smut-affected plants were concentrated in five rows of one crop (Froud *et al.*, 2006). Plants with immature galls were immediately removed, placed into double-layered plastic bags and then incinerated. Four to five medium-sized galls had erupted to various degrees before they could be removed. Soil beneath the infected plants was collected into double bags and incinerated. Plants remaining in the affected crop were mechanically cut into 2–3 pieces using a special harvester causing minimal disturbance. Plants from the affected area of the field were placed at the bottom of a compost heap on black polythene while those from the remainder of the crop around the affected plants (buffer zone) were placed in the centre of the heap. The remainder of the crop with no galls was placed on top and then the entire heap was covered with plastic sheeting and car tyres. Solarization treatment of soil beneath the affected crop was undertaken by securing plastic sheeting over the affected site through spring–summer 2006–7 (Gill, 2008; G. Gill, personal communication). A survey confirmed no further disease was present in the region (Froud *et al.*, 2006) although the presence of *U. maydis* was not assessed

following incineration, ensilage or solarization processes. Thus far, the eradication programme was not complete, but all indications were that the fungus had been eradicated. While no soil testing is planned, a restriction on growing maize at the site exists and will be reviewed in 2009 (G. Gill; M. Bullians, personal communication).

Fire blight of apple and pear

Fire blight, caused by the bacterium *Erwinia amylovora*, is a disease of pear and apple and several ornamental plants in Rosaceae (Hayward & Waterson, 1965). The pathogen overwinters as wood cankers and is spread primarily by bees, flies, ants and rain splash to infect blossoms followed by subsequent infections in shoots, leaves and fruit by rain splash and insects. In parts of northern Europe such as UK and Denmark, the pathogen overwinters in *Crataegus* spp. (Billing *et al.*, 1974). It occurs in 43 countries of North America, Europe, Asia and Africa including New Zealand, although Australia remains free of the disease. *Erwinia amylovora* was found on two trees (*Cotoneaster* and *Sorbus*) in the Melbourne Royal Botanic Gardens (RBG) in 1997 (Jock *et al.*, 2000). This led to the initiation of an eradication programme (Rodoni *et al.*, 1999) which involved the removal of 685 individual host plants (viz. *Pyrus, Malus, Cotoneaster, Crataegus, Pyracantha* and *Sorbus*) within 250 m of the RBG boundary and the removal of 34 feral bee colonies from within 1000 m. Strict quarantine protocols were used for the removal of the plants, and involved the use of disposable clothing and digging the entire plants out of the ground then wrapping them in plastic to avoid plant to plant contamination. Foliage and wood was placed in sealed bags and buried 2 m deep at a site located 30 km from the RBG (Rodoni *et al.*, 1999). The remaining stumps were ground out and the areas from where the plants were removed were steam fumigated. National surveys in the three years following the incursion of *E. amylovora* in the RBG did not detect the disease or the fire blight pathogen (Rodoni *et al.*, 2002).

An incursion of fire blight in Norway occurred in the city of Stavanger, on the south west coast, in 1986 (Sletten, 1990). Diseased plants were found in private gardens, around public buildings, in recreation gardens and along roadsides on species of *Cotoneaster*, *Sorbus* and *Pyracantha*. The nearest commercial apple and pear fruit district was located about 40 km north of the incursion site. The aim of the subsequent eradication programme was to protect the nurseries within the city and prevent the spread of the disease into the commercial production area. A quarantine area of around 700 km^2 was established around the focus of the infection and diseased plants and susceptible hosts were removed, although the number of trees removed was not indicated (Sletten, 1993). Within the quarantine area, the production and sale of all common fire blight hosts was prohibited. More than 60 000 private gardens were checked, in addition to public gardens and roadsides. A mobile wood chipper was used to mulch plant material into fine chips, which were then decomposed in compost heaps for at least one year (Sletten & Melboe, 2004), but no further detail was provided. Bee hives were moved to areas that were free from hosts of *E. amylovora*. Fire blight was detected at around 2000 locations in the quarantine area during the years 1986–1992 although from 1990 there was a significant decline in new outbreaks (Sletten, 1993). The systematic surveillance of the quarantine zone continued every year until 1998 and was expanded to include other areas in Norway. The removal of the main hosts of fire blight greatly reduced the build up of inoculum and also simplified annual surveillance of the disease. It was assumed that the lack of optimal climatic conditions for the pathogen, particularly during spring, may have helped restrict the spread of the disease. Fire blight was not detected in Norway between 1993 and 2000 (Sletten & Melboe, 2004). It is not clear if the recent detection of fire blight within the restriction zone in 2000 is due to the re-emergence of the original inoculum or a more recent introduction. Although no evidence was provided, it was believed that the limited spread from this incursion was due to the illegal movement of contaminated *Cotoneaster* plants and beehives between private gardens. The strict eradication campaign has now been re-established in Norway to prevent the spread of fire blight into the important fruit growing areas and nurseries (Sletten & Melboe, 2004).

Fire blight was first reported in Israel in 1985 and within 10 years posed a serious threat to the national pear industry due to the emergence of streptomycin-resistant strains of *E. amylovora* through its routine use for management of fire blight during this period. Although fire blight has not been eradicated, control has been achieved with a management programme called Fire-Man (Shtienberg *et al.*, 2002). Fire-Man aims to reduce the amount of inoculum in the orchards before bloom, protect blossoms from infection and sanitation of infected plant tissues after bloom. Infected tissue was removed by pruning out blighted branches and limbs 30 cm below the margin of the canker (Shtienberg *et al.*, 2002). An alternative method of removing inoculum involved *in situ* burning of blighted tissues by applying a propane torch directly to the pear trees (Reuvini *et al.*, 1999). However, Shtienberg *et al.* (2002) reported that it had no effect on internal populations of *E. amylovora* and the heat created wounds on surviving limbs, increasing chances of reinfection. The Fire Blight Control Advisory system (FBCA) (Shtienberg *et al.*, 1999) uses thresholds of temperature and leaf wetness based on local experience and this proved to be the best fire blight prediction model in Israel compared to 'Maryblyt (Shtienberg *et al.*, 2002). The initial strategies in Israel were aimed at containment and eradication of *E. amylovora*, but since the mid nineties there has been a shift towards intensive management practices to protect the national pear industry from severe losses caused by fire blight. In the USA, fire blight prediction models such as 'Maryblyt' (Steiner, 1990) or 'Cougarblight 98C' (Smith, 1999) have been developed based on temperature and leaf wetness, and are used for the timely application of chemicals to control the disease.

Fire blight became established in the Emilia-Romagna region of Italy in 1997 following failure of measures which included destruction of 500 000 pear trees (Calzolari *et al.*, 1999). Short distance spread of *E. amylovora* from undetected infection foci within the region and the long distance spread of greater than 14 km from neighbouring districts by birds were cited as the source of inoculum (Battilani *et al.*, 1999). These examples demonstrate the difficulty of eradicating fire blight and in some cases containment or control may be the only option. In the European Union, 'protected zones' have been established for *E. amylovora*, preventing further spread by movement of host plant material and bees into countries and areas free of the pathogen (Anon, 2003).

Citrus canker

Citrus canker, caused by the bacterium *Xanthomonas smithii* subsp. *citri*, formerly *X. campestris* pv. *citri* or *X. axonopodis* pv. *citri* (Schaad *et al.*, 2005) has been found in most continents of the world except Europe. The pathogen has been eradicated in South Africa, Australia, Fiji, Mozambique and New Zealand (Schubert *et al.*, 2001) and eradication programmes are continuing in Argentina, Uruguay and Brazil but have been started and stopped in Florida, USA. *Xanthomonas smithii* forms canker lesions on fruit, leaves and twigs of citrus plants and upon wetting, bacteria multiply and ooze to the surface (Gottwald *et al.*, 2001). Wind driven rain can spread the bacteria up to 15 km from the source to infect citrus trees via stomata or wounds.

Citrus canker was detected on lemon (*Citrus limon*), orange (*C. sinensis*) and other *Citrus* spp. in Kerikeri, North Island, New Zealand in 1937 and subsequently on most of the North Island (Reid, 1938). Removal of lateral branches and all green tissue such as leaves, petioles and thorns were removed from affected lemon, orange, grapefruit and citronella trees then sprayed with polysulphide chemicals (0.2% lime sulphur) and wounds treated with bitumen paint or petrolatum. Excised plant material was burned and leaf material remaining on the orchard floor was ploughed in. All citrus trees in affected orchards were treated and, citrus trees with old, infected wood in private gardens were removed and burned. The eradication was largely successful, but the disease appeared in Auckland in 1960 (Dye, 1960) and again in Taranaki (350 km south of Auckland) in 1972 (Pennycook *et al.*, 1989). Citrus canker was again eradicated using methods similar to those described above. Surveys continued until 1993, when New Zealand was declared to be free of citrus canker (Taylor *et al.*, 2002).

In Australia, citrus canker was detected on Thursday Island in Queensland, and an eradication campaign was initiated in 1984 (Jones *et al.*, 1984). Ten citrus trees with symptoms, including lime (*C. aurantifolia*) and orange, were destroyed by lopping and incineration, along with any citrus within 15 m of infected trees. Vegetation under the canopies was burnt to ground level using a flame gun and herbicide applied to citrus stumps to prevent regrowth. Replanting and movement of citrus material was restricted. Surveys led to several detections near the original outbreak up until 1986, however continued monitoring for the following 2 years failed to detect citrus canker, and Thursday Island was declared to be free from the disease in 1988 (Jones, 1991).

In 2004 an outbreak of citrus canker in Emerald, Queensland prompted an eradication programme which involved removal and disposal of infected and suspect plant material, strict quarantine controls and regular surveillance (Telford, 2007). Destruction of 495 000 commercial citrus trees and 4300 residential or ornamental citrus trees was carried out by bulldozing the trees into heaps and burning. An 18-month 'host-free' period (concluded in June 2007) in the quarantine area was undertaken. *Xanthomonas smithii* subsp. *citri* was not detected in a study of potential reservoirs on residual plant material in the soil (Gambley *et al.*, 2007). Continued surveillance every 3 months until December 2008 will determine the success of the eradication.

In the Northern Territory, Australia, citrus canker has been detected and successfully eradicated on two occasions. In 1912, severe disease was observed in limes and lemons at Stapleton (110 km south of Darwin) and on most citrus trees within the vicinity of Darwin. All citrus trees and stock at the Botanic Gardens and the affected trees at Stapleton were destroyed by fire. Movement of citrus trees and fruits out of the Northern Territory was prohibited (Hill, 1918). Further detections of citrus canker in 1918 and then in 1922 led to the decision to destroy all citrus north of latitude 19°S. A successful eradication was achieved by removing and burning trees with quarantine restrictions lifted in 1924 (Mertin, 1952). In 1991 citrus canker was again detected in the Northern Territory on pummelo (*C. maxima*) trees in an orchard in Humpty Doo, 50 km south-east of Darwin. The property and surrounding area were placed under quarantine and trees on the affected property were burned *in situ* (Broadbent *et al.*, 1995). A further outbreak was detected in 1993 on another property 500 m from the first and was concluded to have originated from the previous outbreak (Broadbent *et al.*, 1995). All citrus on this and adjoining properties was destroyed, creating a 400 m buffer zone around the two infection sites. Eradication was declared successful in 1995 (Pitkethley & Ulyatt, 1995).

Citrus canker was first found widespread in Florida, USA in 1912 and eradicated by 1933 through regular nursery and orchard inspection, on-site destruction of infected trees and nursery plants by burning, good sanitary practices by citrus workers and enforcement of strict quarantines (Schoulties *et al.*, 1987). In 1984 an outbreak arose in a nursery in Polk County and following extensive surveys, 20 million young citrus trees were removed and burned (Schoulties *et al.*, 1987). In 1986, citrus canker was found in residential and commercial citrus trees in the Tampa Bay area (Schubert *et al.*, 2001). Eradication was declared in 1994 and was achieved by removing and burning all citrus trees within 38 m of infected trees. In 1995, citrus canker was detected in residential trees in

Miami and then further detections were made in commercial orchards in Manatee County (Graham *et al.*, 2004). The eradication campaign involved removal of all citrus within 579 m of infected trees by chainsaw followed by wood chipping and transport in a covered trailer to a landfill. It was found that some debris infected with *X. smithii* escaped during transport and dumping and have potential to cause re-infection (Graham *et al.*, 2004). Following Hurricane Wilma in 2005, citrus canker spread widely and was declared endemic in Florida and eradication was discontinued (Gottwald & Irey, 2007). Efforts are now concentrated on best management practices for citrus canker and minimizing production losses.

Belasque *et al.* (2005) describe two experiments for the eradication of citrus canker in Brazil. In the first, all diseased plants in five orchards were removed and, when two or more diseased plants were adjacent, the plants within a 30 m radius of the diseased plants were drastically pruned. In the second experiment, diseased plants in 12 orchards were similarly pruned including plants within a 30 m radius of the diseased plants. The drastic pruning protocol consisted of removing all branches, leaves and fruit, including any plant material on the ground, followed by burning and burial on site. The remaining trunks were painted with lime and copper oxychloride. All citrus trees in a 2 km radius of experimental orchards were inspected every 3 months and any plants showing symptoms eliminated. Citrus canker affected plants were found 8–11 months after pruning in six orchards, one in the first experiment and five in the second. In the other 11 orchards, no plants expressing symptoms were found in 2 years of monitoring. It was concluded that drastic pruning could be applied as an effective method of control of citrus canker, but not for complete eradication.

Sharka disease of stone fruit

Sharka disease, caused by *Plum pox virus* (PPV), is one of the most serious viral diseases of stone-fruit crops (*Prunus* spp.) and occurs throughout Europe, the Middle East, Africa and South America (Brunt *et al.*, 1996). The virus is spread by vegetative propagation, aphid vectors and seed.

PPV was first detected in North America in 1999 in Pennsylvania in peaches (*P. persica*) (Levy *et al.*, 2000). The PPV incursion was confined to several counties in Pennsylvania due to a national surveillance programme and aggressive eradication measures (Hughes *et al.*, 2002). The latter involved removing all trees within a production block where a PPV-infected tree had been detected plus all trees within a 500 m radius of the infected tree, resulting in the removal of 650 hectares of commercial orchards. Targeted surveys conducted in Pennsylvania from 2003–2005 on over one million commercial nursery and residential trees showed that the incidence of PPV detection in these areas had declined significantly (Levy, 2006).

Monitoring and eradication of PPV involved the removal of infected trees from plum (*P. domestica*) and apricot (*P. armeniaca*) orchards over a 15-year period in the Puglia region of Italy, where it was found in the province of Lecce (Myrta *et al.*, 2006). The programme supported by decree of mandatory control involved annual monitoring of orchards and nurseries by visual observation, leaf sampling and testing. Orchards with more than 30% of trees infected were completely removed, including roots. No orchards exhibited 10–30% infection, and when infection did not exceed 10%, removal was limited to infected and adjacent trees. All trees removed were burned (A. Myrta, personal communication). Continued surveillance since 1994 indicates that the Puglia region is now free of PPV and the use of certified virus-tested plants is advised by the regional phytosanitary service together with the collaboration from stone fruit nurserymen.

PPV was discovered in stone fruit orchards in Ontario and Nova Scotia, Canada in 2000 (Thompson, 2006). Individual trees were tested for PPV and only infected trees were removed, unless the incidence of PPV in a block was greater than 10%, in which case the entire block was removed. However, Thompson (2006) did not report the means of disposal of plant material. Over time the threshold value has been lowered to 1.5% and a certification scheme has been designed to provide clean replacement trees, in the hope that PPV will be eliminated from Canada by 2010.

In 1996, PPV was detected in plum trees in the Netherlands (Verhoeven *et al.*, 1998). All infected trees (cv. Jubileum) had been imported from elsewhere in the European Union since 1994. All orchards planted with material of this origin in the Netherlands were surveyed using visual diagnosis and enzyme-linked immunosorbent assay (ELISA). Fourteen percent of all trees were infected in 29 of 43 orchards. Inspection of the 12 500 plum trees remaining in these orchards revealed 13 infected trees of five other cultivars. All infected trees were removed and burned during the autumn and winter of 1996–97 (Verhoeven *et al.*, 1998; J. Verhoeven, personal communication). The planting of certified virus-free propagation material, followed by inspections in nurseries and orchards, and large scale ELISA testing for PPV also contribute to the limited occurrence of up to just 50 plants per year with PPV in the Netherlands at present (Verhoeven *et al.*, 2008).

Although discovered in 1961 an eradication programme for PPV in Poland was begun in 1996. This programme included elimination of PPV from nursery material and commercial orchards as well as from other potential hosts, especially in the areas surrounding nurseries (Zandarski & Zych, 2005). Infected trees were removed and propagation from affected orchards was prohibited until declared free from the virus. Aphid control was achieved using insecticide sprays. Although eradication was not completely successful, Zandarski & Zych (2005) reported that PPV was almost eradicated from nurseries and orchards and the movement of the infected plant material has virtually ceased.

Techniques for eradication and containment of plant pathogens

The case studies described above are generally well documented and demonstrate the integration of numerous techniques for eradication and containment of plant pathogens. There are many other examples of eradication, containment and control of plant pathogens of which specific techniques are discussed below.

Burning

Burning is often the preferred method of disposal as it eliminates the affected material and immediately kills any pathogens it may contain, according to Ebbels (2003). However, there are numerous examples where burning has reduced incidence or contained pathogens, but not eradicated them. Burning has been used in both successful and unsuccessful eradication programmes already described but testing for viable pathogens in ash and debris remaining after burning has not been reported. Similarly, the temperature and duration of burning are rarely documented. Temperature may be important in pathogen mortality, as was shown in a study in which rice stubble was burnt in an attempt to eradicate sheath diseases of rice caused by *Rhizoctonia oryzae* and *R. oryzae-sativae* in New South Wales, Australia (Lanoiselet *et al*., 2005). Whilst the amount of inoculum was reduced, the variable temperatures and duration of heat exposure within the rice straw may not have killed all of the sclerotia of *R. oryzae*. A subsequent experiment revealed that a temperature above 107°C for at least 90 s was required for 100% mortality of sclerotia (Lanoiselet *et al*., 2005).

Burning of crop residue has been an effective means of controlling fungal pathogens, most often in combination with other methods such as chemical tillage or crop rotation, and has been used to control fusarium disease in wheat and sorghum (Burgess *et al*., 1996), eyespot (Boer *et al*., 1993) and Karnal bunt in wheat (Singh *et al*., 1993) and ascochyta blight in chickpea (Gan *et al*., 2006). In the case of Karnal bunt, unpublished data has also been reported on the collection of *T. indica* teliospores in aerial samples above burning wheat stubble as well as insufficient temperatures to kill spores at the soil surface (Sansford *et al*., 2004). In a review by Hardison (1976), control of plant disease by 'thermosanitation' through application of fire and/or flame indicated a number of cases where burning had reduced the incidence of plant pathogens. Examples included brown spot needle blight (*Scirrha acicola*) and fusiform rust (*Cronartium fusiforme*) in pine trees; apple scab (*V. inaequalis*); dieback (*Diaporthe vaccinii*) and canker (*Godronia cassandrae*) of blueberries; flag smut (*Urocystis agropyri*), stem eyespot or foot rot (*Pseudocercosporella*), root rot or take-all (*Gaeumannomyces*) of wheat; scald (*Rhynchosporium secalis*) and net blotch (*Pyrenophora teres*) of barley; *Septoria avenae* on oats and brown spot (*Pleiochaeta setosa*) of lupin. In the USA, ergot (*Claviceps purpurea*) and blind seed disease (*Gloeotinia temulenta*) were effectively controlled in grass seed crops by burning stubble (Hardison, 1980). In the case of ergot, fire destroyed most of the sclerotia in crop residues and on the soil surface. In an attempt to eradicate *Moniliophthora* (*Crinipellis*) *perniciosa* on cocoa in Brazil, removal and burning of trees resulted in containment of witches' broom disease to the state of Bahia, Brazil (Pereira *et al*., 1996). Powdery mildew (*Erysiphe flexuosa*) of horse chestnut was controlled in Poland by burning fallen leaves and fruit to limit the spread of spores (Adamska *et al*., 2002). Control of fusarium wilt of bananas (*Fusarium oxysporum* f.sp. *cubense*) was achieved in China by removing and burning infected plants, and spraying the soil with the fungicide triadimefon three times at 25-day intervals (Lin, 2004). No information on the temperature or duration of burns in any of these examples was provided. Walduck & Daly (2007) conducted field experiments in Australia to monitor the effect of burning on heat penetration through the soil profile and the ability to eliminate inoculum of the 'Tropical' race 4 strain of *F. oxysporum* f.sp. *cubense*, the cause of banana fusarium wilt within the soil and buried pieces of infected banana tissue. Separate laboratory experiments had shown that temperatures of 65°C and 90°C were necessary to eliminate microconidia and chlamydospores, respectively. Temperature recordings showed that heat did not reliably penetrate the soil below 200 mm at the temperature required to kill the pathogen in banana residue. This was confirmed by isolations from the buried tissue. Temperatures at just 10 cm below the surface were not high enough to eliminate the chlamydospores. Lateral transfer of heat within the profile was also minimal.

Control of bacterial diseases has also been achieved using management programmes which involve burning of infected plant material. Examples include bacterial leaf spot of strawberry (*Xanthomonas fragariae*) in Belgium (Lieten, 1998) and a number of diseases of cucumber, melon and squash caused by species of *Pseudomonas, Xanthomonas* and *Agrobacterium* in Brazil (Oliveira & Moura, 1994). Again, information on temperature and duration of burning was not provided.

Burying

Burying infected plant material is appropriate in cases where burning is not practical, such as large volumes of potatoes or root vegetables (Ebbels, 2003). Burial of both burned and unburned plant material has been used extensively in disposal of infected material as part of the eradication of plant pathogens, as described earlier. This method was employed as part of the successful eradication of fire blight from Australia. However, in the unsuccessful eradication of citrus canker in Florida, escape of debris during transport to the burial site may have compromised the programme. Ebbels (2003) highlights the importance of preventing the escape of pathogens using sealable containers or impermeable covers on high-sided trucks in transit. An incursion of grapevine leaf rust (*Phakopsora euvitis*) in the urban area of Darwin in the Northern Territory of Australia in 2001 (Weinert *et al*., 2003) led to

an eradication programme which involved cutting diseased vines at ground level, applying herbicide to the stump to prevent regrowth, and disposal of excised material by deep burial (West, 2005). Material was transported in a covered truck to a burial site remote from Darwin (A. Daly, personal communication). Continued surveillance led to two separate detections (and removal) in 2006. There were no further detections during the following 12 months, so Australia was declared free of the disease (Carroll, 2007).

The endemic diseases net blotch (*Pyrenophora teres*) and leaf scald (*Rhynchosporium secalis*) of winter barley were initially observed to be less severe where carry-over stubble of a previous crop was burnt or incorporated by cultivation than where it was only partially buried in experiments at Rothamsted in the UK (Jenkyn *et al.*, 1995). However, by summer in the same crops both diseases were usually more severe where straw had been burnt than where it had been incorporated into soil.

Pruning and selective removal

For eradication of pathogens from perennial plants, an alternative to complete crop removal is pruning and selective removal of infected plants. This will reduce production loss and has potential to eradicate the pathogen in some cases. The successful eradication of canker, caused by the fungus *Nectria galligena*, from apple trees on the island of Tasmania in Australia, began in 1954 and spanned 20 years (Ransom, 1997). The programme combined complete removal of severely infected trees and pruning of infected wood from slightly infected trees. Bordeaux mixture fungicide was used to reduce risk of further infection and excised plant material was burned on site. Three surveys over the following 17 years failed to detect the disease. Twig canker, caused by *Phomopsis arnoldiae*, was eradicated from Bohemian olive in Arezzo, Italy by removal and elimination of infected plant parts and application of copper treatment after leaf fall (Marino, 2005). Anthracnose (*Colletotrichum* sp.) on tamarillo (*Solanum betaceum*) was controlled in Colombia by pruning of infected branches, removal of infected fruit and the use of fertilizers and protectant fungicides (Gomez Hurtado, 1993). However, the means by which infected material was destroyed was not reported.

Huanglongbing (HLB) disease of citrus trees, caused by the phytoplasma '*Candidatus* Liberibacter asiaticus' and '*Ca*. Liberibacter americanus', was first reported in Brazil in March 2004. Lopes *et al.* (2007) conducted experiments to determine if pruning of branches with symptoms or the entire canopy would eliminate the disease. Orange trees with different degrees of symptom severity as well as symptomless controls were pruned, although no detail on the method of disposal of infected plant material was provided. Caging and treatment with insecticides was used to control the psyllid vector, *Diaphorina citri*. Symptoms reappeared following pruning, with greater severity on trees with a greater degree of symptom severity before pruning. The failure of pruning to eradicate HLB may be due to the systemic nature of the pathogen, as only plant material showing symptoms was removed and the phytoplasma may have remained in symptomless tissue.

In a review of eradication measures in Ghana for over 40 years to control or contain the spread of *Cocoa swollen shoot virus* (CSSV), Thresh & Owusu (1986) concluded that removing only trees expressing symptoms was effective only in small outbreaks. It was more effective to selectively remove trees with symptoms as well as adjacent symptomless trees, although detail on the disposal of plant material was not offered.

Composting

Composting is the decomposition of biodegradable organic matter and generally consists of an initial mixing period with mesophilic (15–40°C) growth, a high temperature thermophilic (> 45°C) phase where 'sanitization' occurs, and another longer and lower temperature mesophilic phase for maturation or stabilization (Day & Shaw, 2001).

Composting has been the subject of research to eradicate fungal pathogens from contaminated plant debris. *Fusarium oxysporum* f.sp. *melonis*, the cause of vascular wilt of cucurbits, was eradicated after infected tissue was composted for at least 4 days at above 55°C (Suarez-Estrella *et al.*, 2003). Club root (*Plasmodiophora brassicae*) was eradicated from infected brassica wastes after 7 days of composting at 54–73°C, assessed using a Chinese cabbage bioassay (Fayolle *et al.*, 2006). The oomycete *Phytophthora ramorum*, causal agent of sudden oak death, could not be detected by PCR assay on inoculated leaves of California bay laurel following composting at 55°C (Swain *et al.*, 2006). However, the fungus that causes dry root rot of beans and other crops in warm climates, *Macrophomina phaseolina*, survived a peak compost temperature of 60°C for 21 days (Lodha *et al.*, 2002). A review by Noble & Roberts (2004) revealed that 33 of 38 fungal pathogens examined were reduced to levels below detection limits when exposed to peak composting temperatures of 64–70°C for 21 days.

Green waste such as tree, shrub, turfgrass and other landscape plant trimmings and weeds from home gardens and commercial landscapes is ground and used as mulch for ornamental landscapes and in avocado orchards in the USA. Downer *et al.* (2008) assessed survival of introduced plant pathogens in unturned piles of fresh and aged green waste which reached temperatures of 70°C and 45°C, respectively. Sclerotia of *Sclerotinia sclerotiorum* survived for 8 weeks in both fresh and aged green waste. In fresh green waste, *Armillaria mellea* and the nematode *Tylenchulus semipenetrans* did not survive more than 2 days and *Phytophthora cinnamomi* persisted for over 21 days. Composted aged green waste was less effective at reducing pathogen viability, most likely due to the cooler temperatures of 45°C rather than 70°C. It was concluded that sclerotium-forming pathogens are the most difficult to eradicate from undisturbed piles of green waste, and that intermittent turning of piles would increase the

likelihood of eradication through increased temperature, microbial attack and chemical degradation.

Noble & Roberts (2004) listed four bacterial plant pathogens reported from the literature to be eradicated during composting. Composting conditions required for eradication were: 7 days at 40°C for *E. amylovora* on cotoneaster shoots; 77 days at 60°C for *Erwinia chrysanthemi* on chrysanthemum bark; 4 days at 35°C for *Pseudomonas savastanoi* on bean leaves; and 16 h at 59°C for *Ralstonia solanacearum* on potato. Noble & Roberts (2004) also reported the potential of composting for eradication of plant viruses quoting various examples. *Tobacco mosaic virus* (TMV) was eradicated from infected plant material after peak compost temperatures in excess of 68ºC and composting for longer than 28 days, although it is important to note that the conditions required for eradication vary in the literature. Other examples included the viral/fungal complexes *Lettuce big vein virus* (LBVV)/*Olpidium* sp. and *Tobacco necrosis virus* (TNV)/*Olpidium* sp. eliminated by composting at 50°C for 7 and 50 days, respectively, and *Melon necrotic spot virus*, TNV and *Tomato spotted wilt virus* eradicated by a peak composting temperature of 65°C and a composting duration of up to 28 days.

There is evidence that certain plant pathogens, with resting spores that may be heat tolerant, can survive composting, sometimes through inadequate methods or failures in the treatment process (Anon, 2008b). Examples reported from the literature include some *formae speciales* of *Fusarium oxysporum*, *Olpidium brassicae*, *Plasmodiophora brassicae*, *Streptomyces scabies*, TMV, *Tobacco rattle virus* (TRV) and *Xanthomonas malvacearum* (Noble & Roberts, 2004). In the European and Mediterranean Plant Protection Organization (EPPO) region, it has been recommended that plant material of origin known or suspected to contain any quarantine pathogens should receive additional heat treatment of 74°C for 4 h, 80°C for 2 h or 90°C for 1 h before or after composting (Anon, 2008b).

Soil fumigation

Methyl bromide has been used as a soil fumigant for almost 50 years and has a wide spectrum of activity against plant pathogens and pests, including fungi and bacteria. Its volatility allows good penetration of the soil through vapour diffusion and it has been used extensively to prepare soil for planting strawberry, tomato, pepper, tobacco, melons, grapes, ornamentals and turf grass for the successful control of pathogens such as *Verticillium*, *Phytophthora*, *Pythium*, *Cylindrocarpon* and *Rhizoctonia* spp. (Wilhelm & Paulus, 1980; Ristaino & Thomas, 1997; Porter *et al*., 1999; Duniway, 2002). Due to the environmental impact of methyl bromide on depletion of ozone, the use of this fumigant has been phased out as part of the Montreal Protocol, with an exception for use in the event of eradication (Duniway, 2002).

Alternatives to methyl bromide have been the subject of much research since the Montreal Protocol and several reviews (Ristaino & Thomas, 1997; Porter *et al*., 1999; Duniway, 2002; Ajwa *et al*., 2003; Ruzo, 2006) identify alternative chemical fumigants. Strawberry diseases caused by *Rhizoctonia*, *Phytophthora* and *Verticillium* spp. can be controlled using chloropicrin, 1,3-dichloropropene (Telone), or a mixture of both. Methyl iodide and sodium azide have efficacy against fungi similar to methyl bromide, but require application of greater rates. Dazomet (Basamid) also gave similar efficacy against soil fungi to methyl bromide. Methyl isothiocyanate, the primary active agent of metam sodium (Vapam), has activity against plant pathogenic fungi. However, distribution in soil is limited and if soil temperature and moisture are not optimal, methyl isothiocyanate may be unreliable. Application of chemical fumigant requires covering of the soil with plastic to contain the gas (Matthiessen & Kirkegaard, 2006).

Biofumigation

Biofumigation refers to the suppression of selective soil-borne organisms by volatile isothiocyanates released by hydrolysis of glucosinolates from the decomposing tissues of *Brassica* spp. incorporated into the soil as a green manure (Matthiessen & Kirkegaard, 2006). An Australian study demonstrated that Indian mustard (*B. juncea*) green manure reduced the severity of bacterial wilt (*R. solanacearum*) in the following tobacco crop (Akiew & Trevorrow, 1999). Following greenhouse trials in Israel, Tsror *et al*. (2007) concluded that biofumigation can reduce the population of soil-borne pathogens such as *F. oxysporum*, *R. solani*, *V. dahliae* and *Pythium* spp. but not necessarily eradicate them. *In vitro* experiments by Fan *et al*. (2008) in China showed that powdered tissue of *B. oleracea* var. *caulorapa* on agar suppressed the growth of a wide range of soil-borne fungal species. Biofumigation reduced the number of viable microsclerotia of *Verticillium* spp. in field soil by 19–47% in Switzerland (Michel *et al*., 2007).

Limitations of biofumigation include containment of isothiocyanates within the soil, timing of incorporation so that soil moisture and temperature are optimal, and the increased occurrence of pathogens common to *Brassica* spp. (T. Wicks, personal communication). Biofumigation does not provide 100% mortality and therefore may not be suitable for eradication, but could be used as part of an integrated programme to assist in containing soil-borne disease without adverse impact on the environment.

Soil solarization

Solarization, also known as polyethylene or plastic mulching, is the process in which clear polyethylene is placed over soil and utilises solar energy to raise the temperature for the control of soil-borne pests and diseases (Katan, 1981). Katan *et al*. (1976) suggested that biological as well as thermal activity may be involved in the suppression of soil-borne pathogens. Solarization is more economical and less hazardous than chemical fumigation.

There are numerous examples of the use of solarization in both the eradication and control of plant pathogens. *Phytophthora nicotianae* and *R. solani* were eradicated from tomato seed beds using double-layer solarization (Rodriguez Perez *et al*., 2005). Maximum temperatures at 5 cm depth in double layer-solarized seed beds were 70 and 73°C in 2002 and 2003, respectively, over 20°C higher than the uncovered control. In both years, temperatures higher than 60°C over nine consecutive hours were achieved. Sclerotia of *Corticium rolfsii* were eradicated when solarization treatment was applied for 1 day at 60–80°C (Martins *et al*., 2003) and, in another study, were eliminated in potted soil at depths to 10 cm by solarization with polyethylene sheets for up to 21 days (Rao & Maity, 2003). Amendment of field soils with cabbage leaf residues in combination with soil solarization resulted in complete elimination of *F. oxysporum* f.sp. *gladioli* (gladiolus wilt) at 5 cm soil depth (Harender & Sachin, 2005). *Verticillium dahliae* was eliminated from soil to a depth of 25 cm after 2 weeks under polyethylene sheets in Israel (Katan *et al*., 1976). *Fusarium oxysporum* f.sp. *lycopersici* populations were reduced by 54–100% at varying depths to 25 cm and maximum soil temperatures in both cases were 49 and 42°C at 5 and 15 cm, respectively. Wilt of strawberry caused by *F. oxysporum* f.sp. *fragariae* was controlled under glasshouse conditions by soil solarization combined with solar-heated irrigation when heated at 40°C for 7 days or at 45°C for 2 days (Sugimura *et al*., 2001).

Effective solarization depends on soil type, moisture and pH and also requires warm, sunny days, limiting the timing and location of its use (T. Wicks, personal communication). A further limitation of soil solarization is the depth of penetration of temperatures necessary to kill the pathogen and it may therefore be more effective in combination with fumigation. In Spain, *R. solanacearum* and *Tomato mosaic virus* in tomato crops were successfully controlled by biosolarization (biofumigation with solarization), although temperature and time of exposure were not reported (Zanon *et al*., 2006).

Steam sterilization

Steam sterilization of soil surrounding infected trees (*Cotoneaster* and *Sorbus*) was used in the successful eradication of fire blight in Melbourne described previously. In Italy *Sclerotium rolfsii*, *Fusarium oxysporum* and *Rhizoctonia solani* were eradicated from field soil by steam sterilization during 1999–2006 using an Ecostar 600 self-propelled steriliser and a system of spray booms for steam (Perruzi, 2007). Whilst little information can be found on use of this method, it offers potential for use in eradicating soil-borne pathogens in the future.

Vector control

Vectors such as insects may spread some plant pathogens, therefore controlling the vector is a viable method of containing a pathogen and could be used in an eradication programme in combination with cultural management techniques and destroying infected plant material. Although vectors occasionally spread bacterial and fungal pathogens, viral pathogens are commonly spread this way and there are many examples of vector control. The use of an insecticide for controlling mealybug vectors improved the likelihood of eradication of CSSV along with the adoption of virus-resistant cocoa cultivars (Thresh & Owusu, 1986). The use of aphid vector monitoring systems to assist the control of the spread of *Potato virus Y*, *Barley yellow dwarf virus* and *Beet yellows virus* is now well established as a tool to manage virus disease outbreaks (Sigvald, 1998; Anon, 2008a). Control is achieved through focused use of insecticides, crop-sowing timing, and the planting of resistant cultivars. A combination of resistant cultivars and cultural management of beet crops to provide early plant emergence and development, and a highly coordinated beet leafhopper vector scouting and spray programme have achieved adequate control of *Beet curly top virus* in California, USA (Wisler & Duffus, 2000). Similarly, soil-borne viruses such LBVV, TNV and TRV can be managed using host resistance combined with chemical treatment of the soil-borne vector (Walsh, 1998).

Biological control

Biological control is the use of beneficial organisms and their products, such as metabolites, which reduce the negative effects of plant pathogens and promote positive responses by the host plant (Vinale *et al*., 2008). *Trichoderma* spp. and *Bacillus* spp. are among the most commonly isolated soil microorganisms which produce biologically active compounds, such as cell wall degrading enzymes and secondary metabolites. Their ability to protect plants and contain pathogen populations has led to these fungi being widely studied (Cannon, 1996; Vinale *et al*., 2008). Biological control of Pierce's disease (*Xylella fastidiosa*) through inoculation with benign strains of the bacterium was reported to have potential for commercial vineyards according to Hopkins (2005).

Bacteriophages are viruses that multiply inside bacterial cells and may offer a method of biological control of bacterial pathogens. In the field, reduced bacterial populations of *X. campestris* pv. *juglandis* were associated with substantial increases in phage numbers, suggesting involvement of the latter in controlling walnut blight (McNeil *et al*., 2001). Citrus canker and bacterial spot caused by *X. axonopodis* pvs. *citri* and *citrumelo* were controlled in the greenhouse by bacteriophage treatment (Balogh *et al*., 2008). However, the efficacy of phages, as is true of many biological control agents, depends greatly on prevailing environmental factors as well as on susceptibility of the target organism (Jones *et al*., 2007).

Biological control is not normally appropriate for eradication of a pathogen. However, if used for this purpose it would require inundative application of the biocontrol agent in numbers sufficient to overwhelm the pathogen population and possibly combined with other treatments in an integrated programme (Ebbels, 2003).

Discussion

Eradication or containment of exotic plant pathogens requires great effort from all those involved. Along with many successful eradication schemes there have been failures. Usually due to the integrated approach required in undertaking eradication it is often difficult to identify specific reasons for success or failure. In this review, examples from the literature have been described, methodologies summarized and available evidence examined for some of the successes.

Burning infected plant material has been widely used in the eradication and control of exotic and endemic pathogens. However, there appears to be little or no scientific evidence available to confirm that pathogens are eliminated during this process. Murray (1998) suggested that destroying wheat crops with Karnal bunt by burning could be counterproductive, as spores of the pathogen may rise in the heat currents and be dispersed, effectively spreading disease which, along with survival of spores in soil, was reported from unpublished data (Sansford *et al.*, 2004). Several studies have shown temperature is a crucial factor in pathogen mortality, so burn-temperatures need to be above a certain threshold over a period of time. In his review, Hardison (1976) concluded that the effectiveness of burning for disease control needs to be determined for individual diseases, and that a limitation of this strategy is the incomplete burning of residues. Further research is necessary to increase confidence in burning for elimination of pathogens.

Burial of infected plant material, with or without burning, is also commonly used in eradication programmes. The likelihood of pathogen dispersal and survival or decline on either buried or exposed material needs to be considered. There is a great deal of published information on epidemiological factors affecting survival of specific plant pathogens. The most important decision when burying is where to bury, the depth at which material should be placed, and should include consideration of drainage or seepage (T. Wicks, personal communication). The buried material should be covered with a depth of soil which prevents disturbance by birds, animals or the elements; Ebbels (2003) suggests at least 2 m. Burial at 2 m was used in the fire blight eradication in Melbourne (Rodoni *et al.*, 1999), but burial depth was not specified in literature concerning any other examples. Further research on pathogen survival on different plant material, soil types and under different environmental conditions could provide evidence for the optimal burial conditions for a successful eradication. Burial is an effective method of disposal but, where possible, should be done on-site. Processing of the material, such as chipping, may increase the decomposition of wood, but also increases chances of pathogen dispersal. On-site disposal of plant material is sometimes not practical in urban situations. For example, during the eradication of grapevine leaf rust in Darwin, plant material was removed, isolated and transported carefully to a remote site to avoid dispersal of infected material (A. Daly, personal communication).

Complete removal of perennial crops for eradication of pathogens can lead to substantial economic loss to industries. The use of severe pruning to remove infected parts of the plant and reworking from the trunk minimises the time taken to restore it to commercial productivity. This method was used as part of the successful eradication of black Sigatoka on bananas in Tully and nectria canker from apples in Tasmania, and was reported to contribute to the control of citrus canker and huanglongbing in Brazil, as well as anthracnose of tamarillos in Columbia. Eradication of non-systemic exotic pathogens such as these in other hosts requires research to develop specific protocols.

Biological control has been commonly employed to control endemic plant pathogens, but due to its variable nature has not been considered for eradication of exotic pathogens. However, there may be potential for its use as part of an integrated approach, especially for containing an outbreak. Bacteriophage may provide a promising strategy in the control of fire blight. Bacteriophages isolated from *E. amylovora* have shown a high degree of lytic activity against *E. amylovora* in the laboratory, but there have been no studies to determine the effectiveness of the phage as a control agent in the orchard environment. To date, *E. amylovora* lytic phage has been investigated only in areas where fire blight is endemic in the USA (Schnabel *et al.*, 1999) and Canada (Svircev *et al.*, 2002). Australian orchards have remained free of fire blight and this may be due in part to the presence of some natural defence mechanisms in those orchards in the form of a natural antagonist of *E. amylovora* such as an aggressive bacteriophage. There is evidence for a unique microflora consisting of closely related saprophytic *Erwinia* species in Australian orchards, which requires further investigation (Rodoni *et al.*, 2003).

Plant viruses and virus diseases have been studied for more than 100 years and much attention has been given to their control (Thresh, 2003). Chemotherapy, thermotherapy and meristem-tip culture can be successful, but they cannot be routinely used on a large scale. Consequently, the main approach has been to prevent or delay a virus infection or to ameliorate its effects. Various means have been used to achieve these objectives, including phytosanitation (involving quarantine measures, crop hygiene, use of virus-tested planting material and eradication), timing of crop planting, use of pesticides to control vectors, mild strain protection and the deployment of resistant or tolerant cultivars. These measures can be used singly or in combination to exploit synergistic interactions. The strategy of removing infected plants for eradication of virus diseases has often been attempted with perennial crops such as fruit trees or grapes. According to Thresh (1988), the success of this strategy for eradication or containment depends on the rapidity of implementation following an incursion and the extent of knowledge on the epidemiology of the disease and the availability of detection methods.

Composting plant material may also provide an alternative to burning and/or burying during the eradication

of exotic pathogens, however, additional heat treatment is recommended in the EPPO-region to ensure sterilization of plant material containing quarantine pathogens (Anon, 2008b). The success of composting is largely dependent on the exposure of pathogens to certain temperatures and time periods sufficient to kill the pathogen. If successful this strategy has the potential to be integrated into a containment and/or eradication programme.

Soil-borne pathogens are particularly difficult to eradicate. The use of one, or a combination of, solarization, chemical fumigation or biofumigation may provide support in controlling a pathogen incursion but efficacy and consistency remain uncertain.

In conclusion, there are many examples to draw upon in considering methods of eradication and a sound understanding of the biology and epidemiology of a pathogen is paramount. The information available from past eradications is not always sufficiently detailed and so more effort is required to produce and publish scientific evidence to support the success or otherwise of attempts of containment and eradication of incursive plant pathogens and diseased plant material.

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Controlling plant pathogens – practical experiments in eradication

Introduction

- Simple practical tools are needed to eradicate a plant pathogen incursion successfully.
- Our reviews (Fletcher et al. 2008; Sosnowski et al. 2009) suggest that a range of eradication methods could be useful, depending on the type of incursion.

Two scenarios were selected for trialling eradication technologies:

- a nematode pathogen that may have entered New Zealand on contaminated footwear or on nursery stock – potato cyst nematode (PCN) *Globerodera rostochiensis*
- seed-borne pathogens arriving by mail – *Septoria apiicoli* and *Erwinia carotovora* in celery seed.

Potato cyst nematode, *Globerodera rostochiensis*.

Method

Nematode eradication

- Heat treatments and nematicide chemicals were applied to control PCN in a shade house experiment:
- In a replicated trial 'Ilam Hardy' potato tubers were sprouted and placed in each pot with a sachet of standardised inoculum of PCN cysts.

Seed-borne disease eradication

- Celery plants ('Safir') were grown from two celery seed lines: one with 30% seed-borne incidence of *Erwinia carotovora*, the other with 11% *Septoria apiicoli*.
- Replicated trials (five plants/plot (one infected) in four reps) using plastic bag solarisation and chemical treatments were established (Figure 1).
- Streptomycin was sprayed weekly to control *Erwinia carotovora* and carbendazim and chlorothalonil to control *Septoria apiicola*.
- The *Erwinia* experiment ran through summer (December through January) while the *Septoria* experiment ran from autumn into winter (April to June).
- Temperature and humidity within treatments were monitored using Hobo® data loggers.

Figure 1. Celery spray and solarisation treatments.

Results

Table 1. Estimated means for 10 eradication treatments to control potato cyst nematode in potato ('Ilam Hardy') for three measurements from a shade house trial.

Treatment		Stem height (cm)	Tuber weight (g)	Mean number of cysts
T50	50°C 24 h	71.3	96.5	2461.5
T75	75°C 24 h	69.8	120.5	814.0
T100a	100°C 24 h	62.5	163.0	1.0
T100b	100°C 1 h	63.2	186.5	4.3
T100c	100°C 30 min	63.5	156.8	3.0
T100d	100°C 10 min	64.8	94.0	2397.3
No treatment		64.3	104.0	3573.0
Fenamiphos	recommended rate	62.2	114.8	436.5
Below rec.		73.8	103.0	26.5
Above rec.		68.3	76.5	4.0
Lsd 5%		13.8	71.4	

Erwinia carotovora

Table 2. Percentage infected or dead plants of celery plants ('Safir') infected with *Erwinia carotovora* using plastic solarisation and agromycin treatments.

	Pre treatment	Post treatment
Control	35	85 infected
Plastic	45	100 dead
Sprayed	25	75 infected
Spray + Plastic	24	100 dead

- Daily temperatures ranged from 10 to 30°C, under plastic from 17 to 100°C.
- Most plants under plastic died (Figure 2).
- Sprayed plants were not significantly less infected than control plants ($P > 0.5$).
- Some plants remained un-infected (25% of sprayed plants and 15% of controls) in plots without plastic.
- A *Stemphyllium botryosum* infection developed in control and sprayed plots; this too appeared to be eradicated by solarisation (Figure 3).

Figure 2. Collapsed celery plants following spray chemical and plastic solarisation treatments.

Figure 3. Leaf symptoms of *Erwinia carotovora* and *Stemphyllium botryosum* on celery plants.

Septoria apiicoli

Table 3. Percentage infected or dead plants of *Septoria apiicoli* infected celery plants ('Safir') using plastic solarisation and carbendazim/chlorothalonil treatments.

	Pre treatment	Post treatment	Mean infection score
Control	40	100 infected	3.2
Plastic	45	85 infected	1.0
Sprayed	35	65 infected	0.0
Spray + Plastic	30	40 infected	0.4

- Over the period, daily temperature measurements ranged from -1 to 25°C and under plastic from 6 to 27°C.
- Few plants died under plastic.
- Sprayed plots had lower infection levels than unsprayed.

Conclusions

Potato cyst nematode, *Globerodera rostochiensis*

- Chemicals are not very effective against PCN.
- Heat treatments gave good results but did not entirely eradicate PCN.

Erwinia carotovora

- Solarisation (temps up to 100°C) will kill infected plants, with good potential for eradication.
- Chemicals had almost no effect.

Septoria apiicoli

- Sprayed plants had lower infection rates than unsprayed, but chemical treatment did not eradicate the fungus.
- Solarisation temperatures from April to June were too low to eradicate the fungus.

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SEAWEED EXTRACTS SLIGHTLY REDUCE EFFECTS OF ROOT KNOT NEMATODES ON TOMATO PLANTS

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INTRODUCTION:

- Root knot nematodes (RKNs), from the genus *Meloidogyne*, are economically important pests worldwide, attacking nearly several species of crop and fibre plants. Typical symptoms of RKN infection are the formation of galls on the roots (Fig 1 and 2), causing substantial damage to the root systems that resulting in poor yield and plant development.
- Control of RKNs has typically been by using chemical pesticides, but restrictions on their use have driven a need to find alternative controls. These restrictions are due to high human toxicity and deleterious environmental effects (see the Montreal Protocol; United Nations Environment Programme 1992).
- Plant-based products have been investigated for nematode control, as they are often non-toxic and non-persistent in the environment. Several of these, including neem extract, rapeseed, Sudan grass, marigold, castor oil cakes, sugarcane bi-product (furfural) and extracts from several species of seaweed have been shown to be effective in different crops infested with RKNs.
- In the present study, glasshouse pot experiments were conducted to monitor the effects of seaweed concentrates on RKNs and plant growth, and to compare activity with that of the traditional nematicide fenamiphos (Nemacur™).

MATERIALS AND METHODS:

Seedlings of tomato (*Solanum lycopersicon*; cv. Moneymaker) were raised in a glasshouse. Seedlings (height approx. 10 cm) were transplanted into 5 L capacity plastic pots filled with potting mix heavily infested with *Meloidogyne fallax*. Single applications both of seaweed extract and fenamiphos were applied at the time of seedling transplant. Individual pots were treated with either 1 ml of seaweed extract or fenamiphos (in 500 ml of water). Control pots received 500 ml water. There were six replicates of each treatment. The trial was laid out in two adjacent 3 × 3 Latin Squares on a single glasshouse bench. The pots were watered regularly as required. Plants were harvested 16 weeks after planting. Data of plant height, fresh and dry weights of shoots and roots, and galling severity index were recorded at plant maturity. The galling index of Orion et al. (2001) was used, where 0 = no galls through to 5 = 76−100% galled roots.

Statistical analyses

Data from the first experiment were subjected to analysis of variance, taking into account the design of the experiment (two adjacent Latin squares).

RESULTS

Fresh and dry weights of shoots were not significantly affected (P=0.55 and 0.73 respectively) by seaweed extract or fenamiphos. However, these parameters tended to be greater with these treatments (Tables 1). Root galling index for tomato plants was not significantly affected (P=0.3) by the treatments. Root systems of seaweed extract- and fenamiphos-treated plants were less affected by RKN than those in untreated controls (Tables 1). Low nematode root galling indices in plants treated with the seaweed extracts could be due to the presence of kinetin

TABLE 1: Mean plant parameters and galling indices for tomato plants grown in pots to which different treatments were applied.

Treatment	Plant height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Galling index
Untreated	53.2	90.63	14.95	9.15	1.43	3.25
Seaweed extract	64.5	120.03	17.65	12.17	1.92	2.50
Fenamiphos	79.0	112.55	17.63	9.95	1.54	2.00
LSD 5% (d.f.=6)	20.2	64.54	9.39	4.40	0.93	1.82
F Prob. for treatments (d.f.=2; 6)	0.055	0.546	0.732	0.294	0.456	0.309

CONCLUSIONS

- Seaweed extracts suppressed nematode activity to a level similar to that from fenamiphos, resulting in fewer galls on plant roots and slightly improved growth.
- The efficacy of seaweed extracts should be further explored using different combinations of treatments, before they can be incorporated into integrated nematode management.
- Further experimental work is continuing to explore the efficacy of other naturally derived chemicals, and to compare them with standard synthetic nematicides.

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A

B

Fig 1. Tomato root infected by root knot nematode (*Meloidogyne fallax*), (A) early in the season (B) late at harvest.

Seed-borne celery disease eradication trial -data analysis - DRAFT

John Fletcher Virginia Marroni Ruth Butler, April 2011

1. Objective

Following on from a small scale experiment in 2008-9 (Fletcher et al 2009) a small field trial was established to determine if seed-borne fungal and bacterial infections in celery seed might be controlled and/or eradicated using agrichemicals or solarisation.

2. Experiment and Data

Celery cv Safir (line 410483) plants grown from seed infected with *Septoria apiicola* (11%) and *Erwinia carotovora* (30%) initially grown in the glasshouse and then transferred to a randomised field plot. Controls were non infected Safir line 410488.

There were five treatments: Solarization (clear polythene), Fungicide (carbendazim and chlorothalonil) and Antibiotic (agromycin), Fungicide +Antibotic and an untreated Control. Five replicates of each treatment were used and plots consistent of 20 plants. The plots were laid out using a Latin square design.

Plants were scored both before and after treatment. They were scored for whether they were symptom less (n), dead (d), had bacteria (*Erwinia carotovora*) (b) and/ or *Septoria* (s) present, and whether they had margin necrosis (mn) (margin necrosis either did not appear with bacteria or *Septoria*, or else if one occurred the other was not scored).

3. Methods of Analysis

For each assessment, the number of plants per plot with each of n, d, b, s or mn was calculated. These 10 summaries were analysed separately with a binomial generalized linear model with a logit link (McCullagh & Nelder 1989). The analysis of deviance done as part of these analyses included contrasts to assess the effects of the various treatments, including assessing any interaction between antibiotic and fungicide. In the results, the estimated percentages for each treatment are presented, along with 95% confidence limits. These were obtained in the transformed (logit) scale, and back-transformed to percentages.

The analyses were carried out with GenStat (GenStat Committee 2010).

4. Results

Pre-treatment, 171 plants (34%) showed no symptoms, no plants had margin necrosis, 44 plants (9%) were dead, 208 (42%) had bacterial symptoms, and 117, *Septoria* (23%). The distribution of none of these varied significantly with the treatments ($0.74<p<0.99$ for the overall treatment effect for all except marginal necrosis, which was not tested because none was present).

Table 1: Pre-treatment, percentage of plants with each criteria for each treatment (95% confidence limits)

Treatment	none	dead	bacteria	septoria
Solarisation	38.0 (21.5,57.8)	7.0 (2.3,19.5)	44.0 (23.8,66.4)	21.0 (13.1,32.0)
Fungicide	36.0 (20.0,55.9)	10.0 (4.0,23.1)	37.0 (18.5,60.3)	25.0 (16.3,36.3)
Antibiotic	30.0 (15.5,50.1)	10.0 (4.0,23.1)	42.0 (22.2,64.7)	27.0 (18.0,38.4)
Fung+Antibotic	31.0 (16.2,51.1)	10.0 (4.0,23.1)	41.0 (21.5,63.8)	19.0 (11.5,29.8)
Control	36.0 (20.0,55.9)	7.0 (2.3,19.5)	44.0 (23.8,66.4)	25.0 (16.3,36.3)

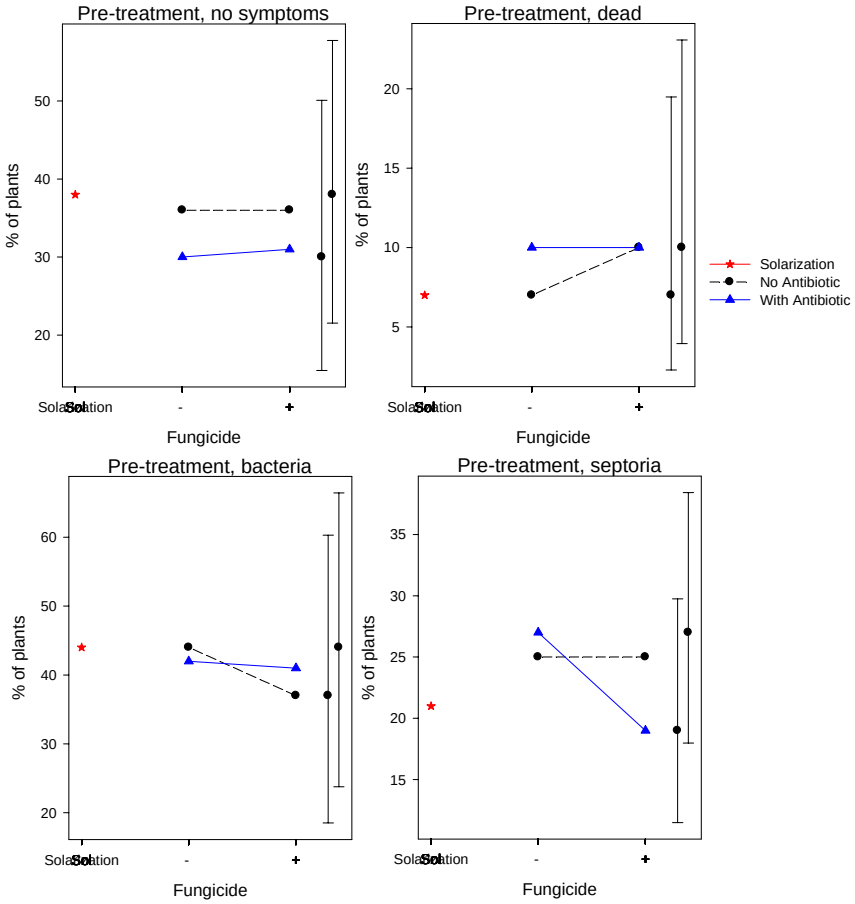

Figure 1: Pre-treatment percentage of plants with no symptoms, dead, or had bacteria or *Septoria*. Error bars in each plot are 95% confidence limits for the smallest and largest percentage in the plot.

Post-treatment, there were some significant differences between treatments in the percentage of plants for each of no symptoms, dead, bacteria, *Septoria* and margin necrosis ($p<0.001$ for the overall effect of treatments for no symptoms, dead and bacteria; $p=0.015$, 0.013 for the overall treatment effect for *Septoria* and margin necrosis respectively). The pattern of differences varied between the five types. No plants had no symptoms with the solarization treatment, a significantly lower percentage than for the other treatments ($p<0.001$), which all had at least 15% of plants with no symptoms. Both fungicide and antibiotic had significant effects on the percentage of plants with no symptoms ($p= 0.014$, 0.003 respectively for the two main effects), and the effects were relatively independent of each other ($p=0.476$ for the fungicide by antibiotic interaction). Both Fungicide and Antibiotic increased the percentage of plants with no symptoms, by around 15-18%.

Table 2: Post-treatment, percentage of plants with each criteria for each treatment (95% confidence limits)

Trt	none	dead	bacteria	septoria	margin necrosis
S	0.0 (0.0,4.4)	100.0 (94.6,100.0)	0.0 (0.0,4.2)	0.0 (0.0,4.7)	0.0 (0.0,5.9)
F	32.0 (21.3,44.9)	40.0 (26.0,55.8)	11.0 (5.4,21.1)	1.0 (0.1,13.4)	16.0 (6.9,32.8)
A	36.0 (24.8,49.0)	34.0 (21.0,49.9)	0.0 (0.0,4.2)	6.0 (2.0,16.7)	24.0 (12.3,41.5)
F+A	50.0 (37.4,62.6)	29.0 (17.0,44.9)	0.0 (0.0,4.2)	2.0 (0.3,12.4)	19.0 (8.9,36.1)
C	15.0 (7.9,26.6)	38.0 (24.3,53.9)	14.0 (7.5,24.6)	13.0 (6.3,25.1)	26.0 (13.8,43.6)

Numbers dead were significantly higher with the solarization treatment than for all other treatments ($p<0.001$), with all plants dead with this treatment. However, neither fungicide nor antibiotic significantly affected the numbers dead ($0.3<p<0.84$ for the main effects and interaction), with between 29 and 40% of plants dead.

No plants showed bacteria when antibiotic was applied, and, since no plants were survived with the solarization treatment, there were no plants with bacteria for this treatment either.

The other two treatments had 11-14% of plants with bacteria (significantly greater than 0, p<0.001). The effect of fungicide was negligible (p=0.597).

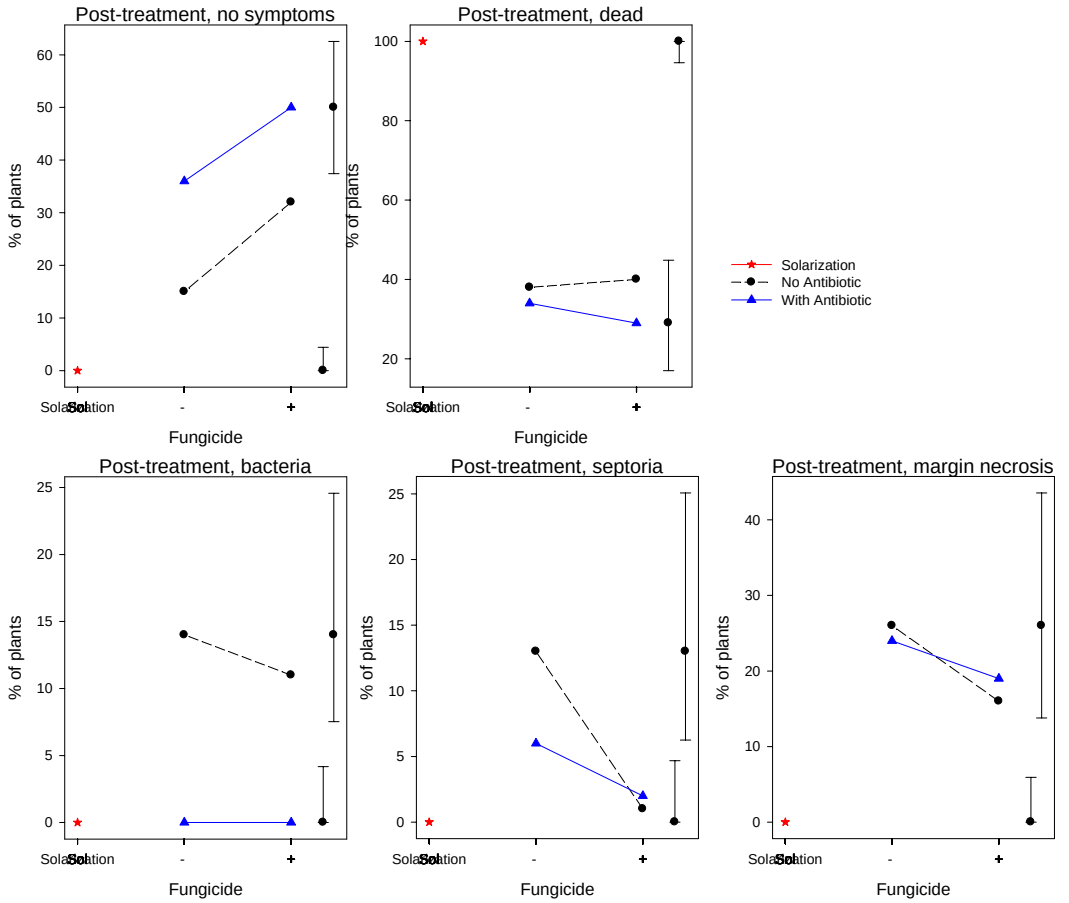

Figure 2: Post-treatment percentage of plants with either no symptom, dead, or had bacteria or *Septoria*. Error bars in each plot are 95% confidence limits for the smallest and largest percentage in the plot.

The percentage of plants with *Septoria* was lower when fungicide was applied than when it was not applied (p=0.010), at around 1-2%, compared with 6 or more %. Antibiotic had a negligible effect (p>0.3 for the antibiotic effect and the interaction with fungicide).

Margin necrosis was lower when fungicide was applied than when it was not applied, but the effect was not significant (p=0.280), Antibiotic had a negligible effect on margin necrosis (p>0.7 for the main effect and the interaction).

5. Conclusions

Solarization using polythene shows promise as a method of containing and eradication a seed-borne bacterial or fungal infection

6. References

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