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| **Project title** | Managing downy mildew epidemics in rose by early detection and treatment of infection sources |
| **Project number:** | HNS 150 |
| **Project leader:** | Dr J E Thomas, NIAB |
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| **Key words:** | Rose, downy mildew, early detection, treatment, cultivar risk |

**AUTHENTICATION**

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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# Grower Summary

## Headline

Downy mildew was found in some rootstock stem material, but not detected in any budwood samples.

The products Signum and Valbon proved most effective at controlling rose downy mildew.

Resistant varieties were identified and a diagnostic procedure detected down mildew before obvious or typical symptoms were observed.

## Background and expected deliverables

Rose downy mildew (*Peronospora sparsa*) is a serious problem in rose production systems, causing reddish angular lesions on leaves, but also a range of other symptoms on leaves, stems, petioles and petals which are less obvious and difficult to diagnose accurately. The disease can cause rapid and sudden defoliation, with consequent loss of plant quality and vigour.

New information on the likely sources of infection within the production environment would help to aid prevention and control measures. New fungicides developed for potato blight, or for control of downy mildews on other plant species, may be effective against rose downy mildew. They would provide a means of developing improved control, contributing to anti-resistance strategies, and integrating with biostimulant plant products evaluated in HNS 135. Investigating whether variation exists in the resistance of a range of current commercial varieties could help to identify high and low risk material.

The overall objectives of this project were therefore to identify potential sources of downy mildew in production environments, to identify and evaluate potential new fungicide products for downy mildew control and investigate the potential of varietal resistance to disease control.

## Summary of the project and main conclusions

<u>Detection and diagnostics</u>

Rose samples were collected from a number of production sites in areas where growers suspected downy mildew to be present, but also from plants which were thought to be healthy. The samples consisted of whole plants (field grown, container plants, and standard roses) which were divided into different tissue types for analysis, or various leaf, stem, and petiole samples taken *in situ*. Budwood tissue from two sources was also collected. The appearance of all tissue types was noted, and a number of digital images taken. A molecular diagnostic technique, based on the methods developed in the US, was applied to the samples. Of the 408 individual samples tested, 63 were positive. None of the budwood material tested was found to contain downy mildew. This finding does not eliminate budwood

as a source of infection, though it does help to reduce grower concerns that budwood could frequently be introducing infection.

Of the positive samples, most were leaves and stems. Several woody stem samples were positive (e.g. Figure 1), including stems of some rootstock plants. The presence of downy mildew here indicates that rootstock material may act as a source of infection for the developing grafted plant, though it does not prove internal systemic infection. Fewer woody stem samples, leaf petioles and buds were positive. Stem samples which were positive did not always show clear or typical symptoms of downy mildew, and sporulation was very infrequent. However, slightly raised, reddish brown patches (e.g. Figure 2, 3 and 4) on buds and petioles were generally positive for downy mildew, and sporulation was occasionally seen on these.

Figure 1 Infected woody stem shavings

Figure 2 Infected bud and bud stalk

Figure 3 Infected petioles

Figure 4 Infected petioles

These results suggest that downy mildew infection may be overlooked because symptoms are not typical or obvious, particularly on stems and petioles, and these infections could contribute to inoculum sources. Samples of stem from overwintered plants which had shown foliar infection in the previous year also tested positive, though no sporulation was seen on these plants. However, the presence of infection still leaves the possibility that active sporulation may subsequently occur, providing a source of inoculum.

Fungicide evaluation

Of the fungicide actives tested, Valbon (benthiavalicarb + mancozeb) and Signum (boscalid + pyraclostrobin) were most effective overall, though in the first year, this was not apparent until the later stages of the experiment (see Figures 5 and 6). These two products were also the most effective in the second year trial (Figure 7) and markedly reduced leaf fall (Figure 8).

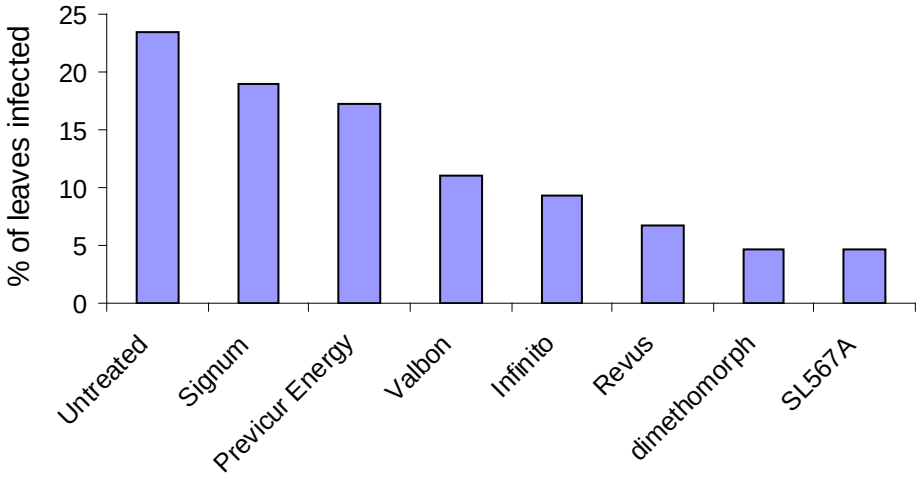


Figure 5 Incidence of downy mildew 4 weeks after first spray (2007)

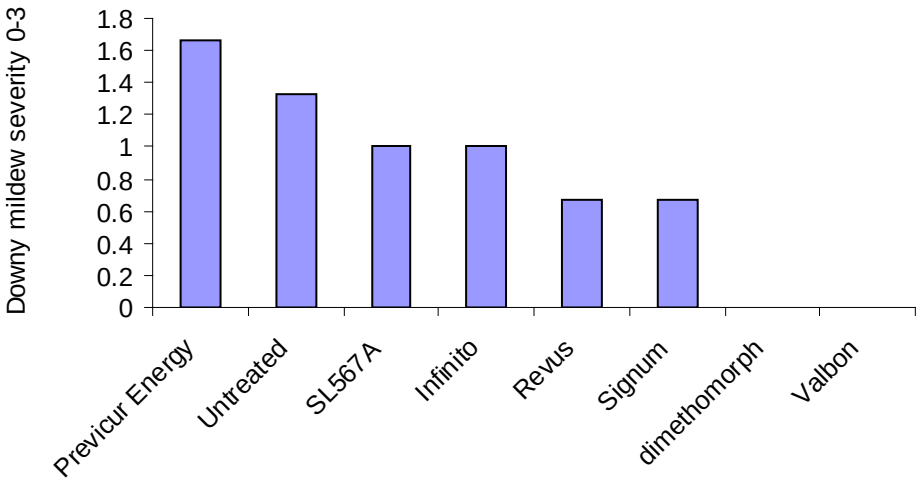


Figure 6 Severity of downy mildew 13 weeks after first spray (2007)

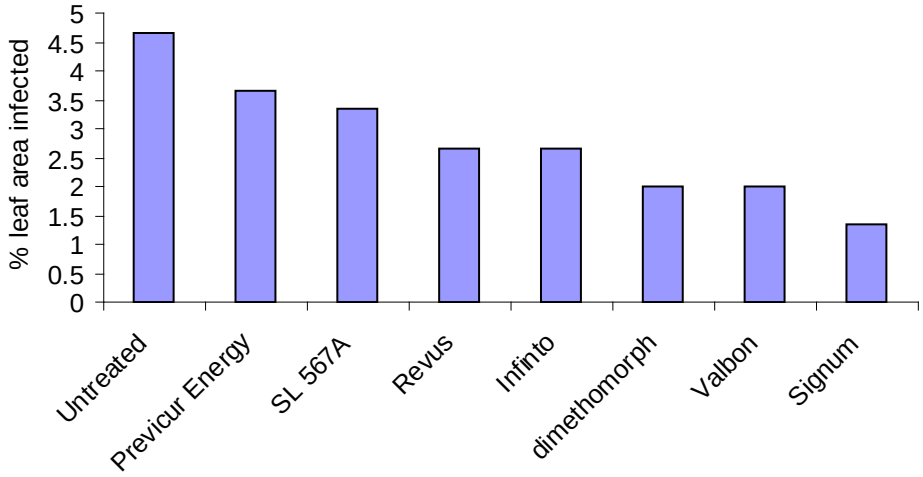


Figure 7 Severity of downy mildew, 6 weeks after first spray (2008)

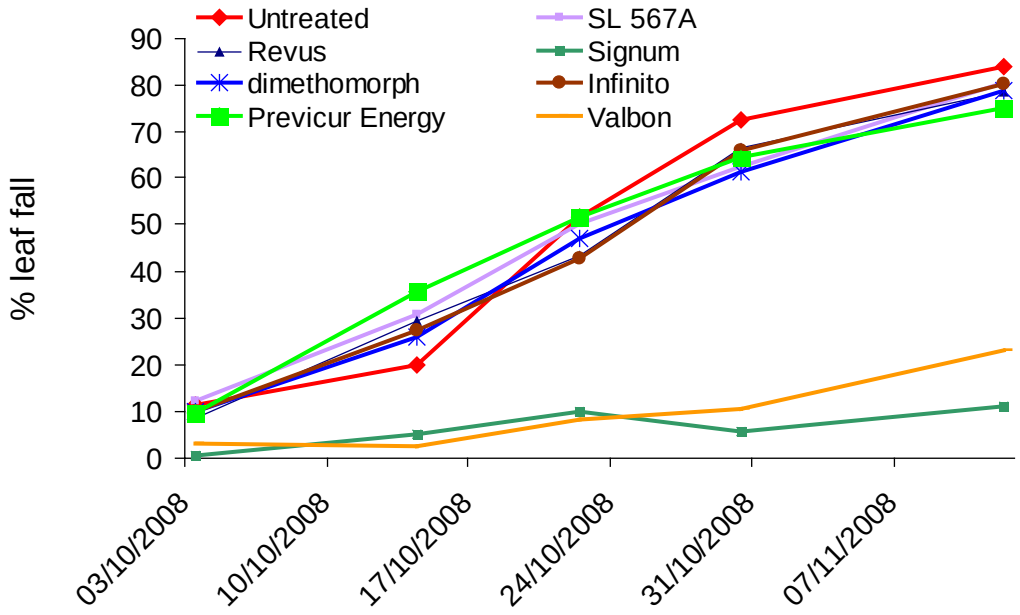


Figure 8 Leaf fall in fungicide trial (2008)

Varietal resistance

There were significant differences between the levels of downy mildew on a range of rose varieties in an inoculated test. Though it was not possible to test a large number of varieties, some relatively high levels of partial resistance were recorded. The most resistant varieties were Hot Chocolate, Glamis Castle. The most susceptible varieties were Rhapsody in Blue, Lili Marlene, Silver Jubilee, Silver Anniversary and Blue Moon. Susceptible varieties may develop downy mildew very rapidly and should be monitored carefully.

## Financial benefits

Rose downy mildew can result in sudden leaf drop which leads to complete loss of saleable produce. Growers and producers have highlighted downy mildew as a major problem. Improved knowledge of infection sources will help to target control measures before extensive disease spread. Efficiency of disease control measures is likely to be improved if they are used before significant establishment of disease in the growing environment, and the number of sprays needed may be reduced. The new off-label product Valbon (benthiavalicarb + mancozeb) which is approved for use against downy mildew on hardy ornamental stock was one of the most effective products tested for rose downy mildew control, and improved leaf retention.

## Action points for growers

- Examine plants regularly for downy mildew. Classical symptoms are angular red leaf lesions, but pale green leaves are often the most actively sporulating tissue.

- Include checks for symptoms which are less obvious, such as raised reddish brown patches on stems and petioles, and red leaf lesions with diffuse margins.

- Check overwintered plants which showed leaf symptoms the preceding season – these may be a source of new infection.

- Check rootstock plants for signs of infection.

- Treat suspect material with a protectant fungicide early in the growing season and maintain control programmes. Alternate available products with different modes of action, and consider integrating fungicides with biostimulant products (see HNS 135).

- Remove fallen leaf material and trimmings from areas where infection has occurred.

- Monitor varieties individually and target control measures carefully on high risk types.

- Read the new factsheet 14/09 which has been produced on rose downy mildew and its control.

# Science Section

## Introduction

Rose downy mildew (*Peronospora sparsa*) has caused extensive losses in rose production systems before plants reach the retail sector. The disease develops rapidly and even intensive spray programmes may be unable to achieve effective control. Similar losses have occurred in the US. Aegerter et al., (2002) developed DNA primers and an extraction system capable of detecting *P. sparsa* from rose tissue, and found that the pathogen was present in the cortex of stem, crown and root tissue of material used to propagate rootstocks. Symptomless tissue was found to contain infection, suggesting that infection sources in the growing environment were not being identified and treated accordingly.

Previous work in the UK (Defra funded project HH1749SHN) found some mycelium and large numbers of resting spores (oospores) in leaf and petiole material by light microscopy, and the report concluded that the major source of overwintering infection was leaf debris However, there was no examination of other plant tissues. Good hygiene was considered essential for reducing epidemic development.

Despite good hygiene practice by growers, rapid and severe disease development is still occurring and, together with the recent results from the US, this suggests that other sources of primary infection in propagating material may be involved. The advent of a high-throughput diagnostic test, compared to the limitations of light microscopy, now presents an improved opportunity for the detection of infection.

This project will use the diagnostic test developed in the US to investigate the incidence of *P. sparsa* within propagating material, and on the basis of the results obtained, investigate the potential of early detection and treatment or removal of infection to improve disease management.

The project will also evaluate potential new conventional products for downy mildew control. This work will complement that of HNS 135 for the retail sector where a number of biostimulants are being investigated for downy mildew control.

The relative susceptibility of selected cultivars to downy mildew will be evaluated in order to determine whether advice on disease management is robust for different cultivar material.

## Materials and Methods

<u>Variety resistance</u>

Inoculated tests were carried out in each year of the project. Varieties for testing were selected on the basis of some limited previous data on susceptibility, grower interest and comment on relative levels of infection seen in commercial production, and some randomly selected material. Some varieties were included in each year to provide data on reproducibility of the test method.

Spores of *P. sparsa* were obtained by shaking infected leaves with sterile distilled water. Infected material was obtained from growers each year. Spores were either used directly from fresh sporulating leaves, or from infected leaves frozen at -20 ° C. Spore concentrations were $10^4$ per $ml^{-1}$ in each case.

Inoculum was applied either by pipetting five 0.01ml droplets on the abaxial leaf surface (fresh spores) or by spraying a suspension over the whole surface until run-off (frozen spores). For each test, healthy leaves which had just become fully expanded were taken from field grown plants of the test varieties, surface sterilised and the rinsed in several changes of sterile distilled water. Three replicates of between 10-20 leaves were used.

Tests were carried out in shallow (1cm) lidded Perspex trays, lined with damp filter paper, or Petri dishes, sealed with Parafilm and incubated after inoculation at between 15-18 C, 16 h day provided by Warmwhite fluorescent tubes.

Leaves were inspected for the appearance of red or yellowish red angular spots at intervals after inoculation and scored by counting number of spots.

Following information that a severe infection of downy mildew had occurred, a visit was made to Royal National Rose Society Gardens during 2007. However, though significant powdery mildew was seen, no downy mildew was recorded. Samples of leaves from Silver Jubilee, Savoy Hotel, Paul Shirville, Countess of Wessex, Together Forever, National Trust, Hot Chocolate, Desert Island and Freedom, together with the species *rugosa alba, roxbergii*, *forrestiana, suffulata, arvensis,* and *rubiginosa* were brought to Cambridge and firstly incubated in damp chambers to encourage sporulation of any downy mildew that might have been present and not visible, and then inoculated as before.

Fungicide trials

Fungicide evaluation was carried out in 2007 and 2008. Plants of cv Silver Jubilee were planted in field soil covered by Mypex matting (2007) or maintained in containers (2008). Each plot consisted of three plants, and there were three replicate plots for each treatment. Inoculum of downy mildew was introduced into the trials by placing infected plants at intervals in the trial area (one infector plant every three plots) and by foliar applications of spore suspensions from infected material collected during for other parts of the project. Trials were irrigated by sprinklers during dry conditions (0.5 h per day during late afternoon). Plants were treated in each year with a winter wash of sulphur to reduce interference from blackspot. Pests were controlled as necessary through each season using Decis (aphids), Gazelle (aphids) and Majestik (red spider). Test fungicides, active ingredients and rates of application are shown in Table 1.

Infector plants were introduced into the year 1 trial on 14th June, with a spore suspension (< $1 \times 10^2$ spores/ml) also being applied directly to foliage at the same time on the lower third of the plant. Sprays were initiated on 22nd June with subsequent sprays of all products on 5th July, 23rd July, 10th August, 23rd August, 6th September and 29th September. In year 2, infector plants were introduced on 27th August, and spraying initiated on the 28th August, with subsequent sprays of all products on 11th September, 25th September, 9th October, and 24th

 

October. A foliar inoculation (1 x $10^3$ spores/ml) over the whole plants was applied on 15th September

Plots were scored for downy mildew (red or yellow angular leaf spots) either as incidence or severity, and for yellowing and leaf fall in the year 2 trial.

Table 1 Products used in fungicide evaluation 2007 and 2008

| Product name | Active ingredient | Rate of application | Approval Status |
|---|---|---|---|
| SL 567 A | metalaxyl M | 1.3l/ha | - |
| Revus | mandipropamid | 0.6l/ha | - |
| Signum | boscalid and pyraclostrobin | 1.5l/ha | - |
| --------- | dimethomorph | 0.6kg/ha | - |
| Infinito | fluopicolide and propamocarb | 1.6l/ha | - |
| Previcur Energy | propamocarb and fosetyl Al | 2.5l/ha | - |
| Valbon | benthiavalicarb + mancozeb | 1.6kg/ha | SOLA |

The plant hormone, brassinosteroid, which was initially included in the proposed treatment set due to its potential disease control properties and inclusion in some “plant tonic” type products, was omitted in favour of Valbon, which was judged to have more immediate practical relevance to growers.

Detection of downy mildew using molecular diagnostics

Samples of rose plants and tissue were received during summer 2006, summer and autumn 2007, and late winter 2007/8 from a number of growers, breeders and ADAS experimental sites carrying out work on control of rose downy mildew with biostimulant products (HNS 135). A total of seven different growing environments (locations) were sampled. Symptom types on different material were recorded, and a number of digital images were also taken. Tissue was frozen at -20°C if necessary before processing.

PCR was carried out on a range of suspect tissue, as well as apparently healthy tissue. Multiple sections of budwood samples were taken to include any suspect areas as well as normal sections. DNA preparations were performed using the Nucleospin Plant mini-kit. Genomic DNA of *P. sparsa* was prepared from a bulk of conidia washed from leaves with abundant sporulation. All sampled tissue was washed thoroughly in distilled water to remove any surface structures of *P. sparsa* and avoid cross contamination of samples. Tissue was then ground in liquid nitrogen. Tough stem material was shredded first using an automatic pencil sharpener. For further cellular disruption, material was transferred to a 1.5 ml centrifuge tube along with 100 µl of the lysis buffer and a 3 mm tungsten bead and was shaken on a Qiagen Tissuelyser for 1.5 minutes at 30 Hz. Following this step the kit protocol was followed. DNA was eluted from the column in 50 µl $H_2O$ and 2 µl was used directly in a PCR reaction.

Primer sequences from the *P. sparsa* ITS region were as follows: forward primer PS3 ATTTTGTGCTGGCTGGC and reverse primer PS1 TGCCACACGACCGAAGC (Aegerter,

2002). These primers amplified a 660 bp product with the following cycling conditions: 1 x reaction buffer, 1 mM dNTP, 2 mM $MgCl_2$, 0.4 µM each primer in a volume of 20 µl. PCR reactions were performed on a Perkin Elmer 9600 PCR machine. Products were visualised on a 1 % agarose gel. Reactions conditions were 1 Cycle 95 $^{o}$C for 5 minutes; 40 Cycles 95 $^{o}$C 30s, 60 $^{o}$C 30s, 72 $^{o}$C 30s, 1 Cycle 72 $^{o}$C 10 minutes. Sensitivity was determined as 10 pg fungal DNA in 60 ng rose DNA from100 mg of rose tissue.

**Results**

Variety resistance

Red spotting appeared under the points of inoculation in the first year experiment after 6 days and was scored after 10 days. In the second experiment, spotting was scored when it first appeared after 6 days, then again after 20 days. Some sporulation was seen, though not on every lesion. There were significant differences between the varieties tested in the second experiment, though not in the first (Tables 2 and 3)

Table 2 Mean number red spots per leaf on rose varieties, experiment 1

| Variety | Mean number red spots/leaf |
|---|---|
| Blue Moon | 0.92 |
| Silver Jubilee | 0.80 |
| Auskeppy Grace | 0.78 |
| Sexy Rexy | 0.61 |
| Sunset Boulevard | 0.55 |
| Princess Alice | 0.45 |
| Buxom Beauty | 0.31 |
| Glamis Castle | 0.28 |
| Rhapsody in Blue | 0.25 |
| Super Fairy | 0.16 |
| lsd (p=0.05) | NS |

Table 3 Mean number of red spots per leaf on rose varieties, experiment 2

| | 6 days after inoculation | 20 days after inoculation |
|---|---|---|
| Lili Marlene | 3.67 | 2.00 |
| Velvet Fragrance | 2.33 | 2.00 |
| Shocking Blue | 2.00 | 2.00 |
| Sexy Rexy | 1.00 | 1.67 |
| Blue Moon | 1.00 | 1.00 |
| Deep Secret | 0.67 | 2.00 |
| Silver Anniversary | 0.67 | 9.00 |
| Rhapsody in Blue | 0.33 | 1.67 |
| Sunset Boulevard | 0.00 | 7.00 |
| Hot Chocolate | 0.00 | 0.00 |
| Glamis Castle | 0.00 | 0.67 |
| lsd (p=0.05) | 0.720 | NS |

There was no sporulation detected in any of the detached leaves collected from RNRS, and attempts to inoculate the material did not produce any downy mildew symptoms. Powdery mildew increased rapidly, and the leaves senesced.

Fungicide product evaluation

There were large differences between treatments in the % of leaves infected with downy mildew by July in the first experiment, though the differences were not statistically significant. Overall severity of disease measured on 0-5 or a 0-3 scale also differed between treatments, but again not significantly. A score of 5 was equivalent to between 50 and 100 spots in the top 25% of the leaves per plot, and 3 equivalent to 10-25 spots. Valbon and dimethomorph were the most effective treatments by the end of the experiment (Table 4).

Table 4 Incidence and overall severity of downy mildew in the fungicide trial 2007

| Product | % of leaves infected | Severity 0-5 | Severity 0-3 |
|---|---|---|---|
| | 27/07/07 | 20/08/07 | 27/09/07 |
| Untreated | 23.4 | 2.0 | 1.33 |
| SL 567 A | 4.6 | 0.67 | 1.00 |
| Revus | 6.7 | 1.33 | 0.67 |
| Signum | 19 | 1.0 | 0.33 |
| dimethomorph | 4.7 | 2.0 | 0.00 |
| Infinito | 9.3 | 1.67 | 1.00 |
| Previcur Energy | 17.3 | 2.3 | 1.67 |
| Valbon | 11 | 1.7 | 0.00 |
| lsd (p=0.05) | NS | NS | NS |

Leaf drop was significantly affected by fungicide in the second experiment (Table 5). Valbon and Signum showed the lowest levels of leaf drop. All products reduced the level of downy mildew compared to untreated plots (Table 6), with Valbon and Signum being most effective over all. These two products showed the greatest degree of leaf area yellowing towards the end of the experiment (Table 7).

Table 5 Mean % leaf drop in 2008 fungicide evaluation

| | 03/10/08 | 14/10/08 | 22/10/08 | 30/10/08 |
|---|---|---|---|---|
| Untreated | 11.33 | 20.00 | 51.67 | 72.67 |
| SL 567 A | 12.33 | 30.67 | 50.00 | 62.33 |
| Revus | 8.67 | 29.33 | 43.33 | 66.67 |
| Signum | 0.67 | 5.00 | 10.00 | 5.67 |
| dimethomorph | 10.00 | 26.00 | 47.00 | 61.33 |
| Infinito | 10.00 | 27.33 | 42.67 | 66.00 |
| Previcur Energy | 9.67 | 35.67 | 51.67 | 64.33 |
| Valbon | 3.00 | 2.67 | 8.33 | 10.67 |
| lsd (p=0.05) | NS | NS | 20.90 | 17.43 |

Table 6 Severity of downy mildew (% leaf area infected) in year 2 experiment

| | 03/10/08 | 14/10/08 | 22/10/08 | 30/10/08 |
|---|---|---|---|---|
| Untreated | 4.67 | 4.67 | 3.00 | 3.00 |
| SL 567 A | 3.33 | 2.33 | 2.33 | 1.33 |
| Revus | 2.67 | 2.67 | 2.67 | 1.00 |
| Signum | 1.33 | 1.33 | 2.00 | 0.67 |
| Dimethomorph | 2.00 | 2.67 | 2.33 | 1.67 |
| Infinito | 2.67 | 4.00 | 2.00 | 1.33 |
| Previcur Energy | 3.67 | 2.67 | 1.33 | 2.00 |
| Valbon | 2.00 | 1.00 | 1.67 | 1.33 |
| lsd (P=0.05) | NS | 1.73 | NS | NS |

Table 7 Leaf area % yellowing in the year 2 experiment

| | 03/10/08 | 14/10/08 | 22/10/08 | 30/10/08 |
|---|---|---|---|---|
| Untreated | 8.33 | 19.33 | 3.33 | 4.67 |
| SL 567 A | 4.00 | 12.67 | 10.00 | 10.00 |
| Revus | 5.67 | 15.67 | 6.00 | 12.67 |
| Signum | 4.33 | 13.00 | 16.33 | 21.00 |
| dimethomorph | 3.33 | 12.67 | 6.67 | 5.67 |
| Infinito | 10.00 | 16.00 | 7.33 | 8.33 |
| Previcur Energy | 5.67 | 18.00 | 6.67 | 6.00 |
| Valbon | 10.67 | 20.33 | 23.67 | 16.00 |
| lsd (P=0.05) | 4.334 | NS | 8.251 | NS |

Detection of downy mildew using molecular diagnostics

A total of 408 samples were analysed using the molecular diagnostic. Specific samples and symptom description from the ADAS trial sites together with PCR results are shown in detail in Table 8 to illustrate the range of symptoms and their association with diagnostic outcome. Source and tissue or plant type from all producer sites are summarised in Table 9. A small number of samples from NIAB fungicide trials were also analysed. The majority of samples tested were negative for the presence of downy mildew (Table 10). Positive samples did not always correspond to suspect symptoms, but all samples where there was clear sporulation before processing were positive. In some cases, however, positive results were found for tissue which was apparently healthy.

Table 8 Symptom type and PCR outcome for downy mildew presence, ADAS trial sites

| Tissue type | Symptom description | PCR outcome |
|---|---|---|
| Leaf | Patchy yellow/brown ,1 sporangium seen | + |
| Leaf | Mottled green/light green | + (weak) |
| Leaf | Pink patches and pink underside of leaf | - |
| Leaf | Green | + |
| Leaf | Yellow leaf with pink spots | - |
| Leaf | Concave yellow leaf | + (weak) |
| Leaf | Green | + |
| Leaf | Yellow/brown leaves | + |
| Leaf | Green | - |
| Leaf | Green | + |
| Leaf | Yellow / brown | - |
| Leaf | Green with yellow/pink central vein | - |
| Leaf | Brown | - |
| Leaf | Green, mottled yellow | - |
| Leaf | Green, mottled yellow with pink patches ~2mm | - |
| Leaf | Brown | + |
| Leaf | Green | - |
| Leaf | Green | - |
| Leaf | Green / yellow with pink spots | - |
| Leaf | Green | - |
| Leaf | Green with brown patches | + |
| Leaf | Green | - |
| Leaf | Green, mottled yellow / pink | - |
| Leaf | Green | - |
| Leaf | Green with large brown patches | + |
| Leaf | Green | - |
| Leaf | Green with yellow central vein | + |
| Leaf | Green, mottled yellow | - |
| Leaf | Green | + |
| Leaf | Green | - |
| Leaf | Green, mottled yellow / pink | - |
| Leaf | Green | + |
| Leaf | Green with brown patches | + |
| Leaf | Green, mottled yellow / pink | + |
| Stem | Yellow area | - |
| Stem | Green area | - |
| Stem | Base of stem – brown and very woody | + (weak). |

Table 9 Summary of samples collected from grower/producer sites

| Producer/site | Tissue types collected | Total number of samples tested |
|---|---|---|
| 1 | Whole plants | 16 |
| 2 | Leaf/stem/flower bud/rootstocks/budwood | 45 |
| 3 | Whole plants, containerised plants, standards | 145 |
| 4 | Leaf/stem/flower bud/rootstock/budwood | 143 |
| 5 | Whole plants | 10 |

Table 10 Summary of PCR outcomes, all samples 2006, 2007 and 2008

| Tissue type | PCR positive | PCR negative |
|---|---|---|
| Leaf | 30 | 164 |
| Petiole | 5 | 5 |
| Stem | 18 | 69 |
| Budwood | 0 | 98 |
| Rootstock stem | 6 | 8 |
| Other | 4 (buds and root) | 1(soil) |
| Total | 63 | 345 |

Typical positive symptom types are summarised in Table 11. A relatively high proportion of stem tissue samples were positive. In general, these were not from the same plants that generated positive leaf samples. Many of the positive stem sections were not exhibiting any visible sign of infection, though some had raised brown lesions which are associated with downy mildew infection. Though relatively few petiole samples were tested, those which were positive had symptoms (brownish areas, but no sporulation visible), and negative samples had no symptoms. Several rootstock (*Rosa laxa*) plants from one site tested positive from stem extracts, even though there were no visible symptoms. None of the budwood samples tested (two sites with a total of five separate sources) were positive.

Table 11 Summary of symptom types positive for downy mildew with molecular test

| Tissue | Always positive | Sometimes positive |
|---|---|---|
| Leaf | Reddish angular lesions, green leaf areas with sporulation | Green leaf areas with no sporulation, non angular reddish lesions, brown and yellow leaves |
| Stem/Petiole | Reddish brown solid patches | Raised, woody areas, or green non-woody tissue |

Examples of material which gave positive PCR results are shown in Figures 1 to 6

Figure 1 Mature lesions with pale centers, rose plant site 4

Figure 2 Young, isolated red lesions, becoming angular, site 3

Figure 3 Diffuse red leaf lesion, with red-brown streaks on petiole, site 3

Figure 4 Dark reddish brown patches on green stem, site 3

Figure 5 Woody stem shavings

Figure 6 Bud stem and sepals

*P. sparsa* DNA was detected in stem tissue of two out of eight samples taken from unsprayed overwintered plants which had showed sporulation the preceding year. There was no *P. sparsa* DNA detected in a total of 6 samples of material taken from the most effective fungicide treatment plots in 2007, or from a further 6 taken from the Valbon treated plots in 2008.

**Discussion**

The detached leaf method proved an efficient method of assessing the degree of susceptibility of rose varieties. Symptoms were clearest at 6 to 10 days after inoculation. In the second test, a later assessment showed a decrease in number of lesions for some varieties, as secondary organisms obscured the downy mildew symptoms, but symptoms developed further in other varieties. The differences between varieties in the first test were not significant, though the range of infection levels was relatively high. Differences observed in the first test were not always reproduced in the second test, though the varieties Glamis Castle and Rhapsody in Blue were consistently more resistant. Hot Chocolate was included in the second test and did not develop any downy mildew symptoms. Breeder comment has indicated that this variety has better resistance than the majority, though under high disease pressure some disease will develop. However, even the existence of high levels of partial resistance in available varieties offers scope for further breeding. For growers, identifying the more susceptible types should help to target material where downy mildew control will be critical.

The products accessed from agrochemical companies for use in the fungicide trial were either established or future blight products, or products with activity against downy mildews of other plants. The experiments carried out here were designed to compare the effectiveness of products versus a standard (SL 567A selected in preference to Aliette WG). Each product was applied repeatedly through the season. While this provides a good comparison of efficacy, it is not a commercial practice. In year 1, disease incidence (% of

leaves with downy mildew lesions) was relatively high, but severity (% leaf area infected) was quite low. All products reduced downy mildew compared to untreated plots at the first score in the first experiment, but relative ranking had changed by the end of the experiment, with Valbon and Signum being most effective, and both better than SL 567A. Dimethomorph was highly effective, though this is not available as a straight product. First applications were not made until 8 days after infection was introduced, longer than intended due to weather, and it is likely that effectiveness of protectant products was reduced at the first score, but that with subsequent cycles of infection and spraying, protectant action would have operated. In year 2, overall severity was higher. Valbon and Signum were the most effective products. Severity on untreated plots declined towards the end of the experiment, presumably due to loss of infected leaves.

The PCR diagnostic proved to be an effective method of detecting the presence of *P. sparsa*, and could be developed as a relatively inexpensive test for use commercially, though it requires laboratory facilities. False positive results with the specific primers are highly unlikely to have occurred, since sufficient leaves which appeared healthy tested negative and leaves with classical symptoms tested positive. False negative results may occasionally have occurred, especially with woodier tissue, where some positives showed relatively weak bands. Lateral flow devices, if they were to become available and effective for all tissue types, would be suitable for in field use.

It was clear that many samples which were suspected of having downy mildew were disease free, and also that many samples which did not show typical symptoms were infected. Red streaking on the stems of some standard roses, though it appeared to suggest the presence of downy mildew, was consistently negative. On the other hand, woody stem samples were sometimes positive, as were stems and petioles with raised, reddish brown patches. While sporulation was observed only rarely on or near these tissue types, they may nevertheless act as sources of infection, and plants which show the symptoms should be treated early in the season, or removed if possible. One root sample out of three was positive, and two samples from woody tissue at the crown of two plants were also positive. Rootstock plant material (stems) was found to be infected, and again it is possible that this could sporulate and cause infection on grafted material. The stem material sampled during the project was from the outer layers of tissue, and represented mostly cortex cells rather than vascular strands. True systemic colonization of rose, i.e. via the vascular system, was therefore not proved, but rather the existence of internal, intercellular growth. However, none of the budwood samples tested proved positive, and while infected budwood cannot be ruled out as a source of infection, the material examined here was healthy. Evidence from the US indicated that dipping infected rootstock material in fungicide reduced subsequent epidemic development, even though the exact pathways of infection were not elucidated. In the UK, early treatment of propagating material with systemic foliar fungicide such be considered as a means of reducing epidemic development.

Profuse sporulation of *P. sparsa* was most commonly seen on pale green tissue, with angular lesions just becoming visible. However, spores were still occasionally visible on darker red lesions, and brown necrotic leaves which had fallen from plants, and this type of debris will act as an infection source in at least the short term.

 

## Conclusions

Downy mildew infection persists in various rose tissues which do not show obvious or typical symptoms, and these may act as sources of infection. A new fungicide active for downy mildew control on ornamentals was very effective against rose downy mildew, and should be used as part of protectant spray programmes. Targeted application on suspected infected plants early in the season should be considered, including material intended for propagation. The molecular diagnostic offers growers an opportunity to identify infection in the absence of typical symptoms. A lateral flow device would provide an alternative method which could be used in-field. Though it was not possible to carry out comprehensive testing of rose varieties within the scope of the project, testing of selected varieties revealed a considerable range of partial resistance. The degree of variation observed suggests that it would be worthwhile targeting more susceptible types for careful monitoring and more intensive control regimes.

## Technology Transfer

1. Presentation of findings to HDC/HTA Roses R&D Forum, November 2007
2. Updated presentation to HDC/HTA Roses R&D Forum, December 2008
3. Fact Sheet " Control of rose downy mildew" in preparation 2009 (HNS 135 and HNS 150 outputs)

## References

Aegerter, B. J., Nunez, J.J. and Davis, R.M. (2002). Detection and management of downy mildew in rose rootstock. Plant Disease **86**, 1363-1368

Biology and epidemiology of rose downy mildew (*Peronospora sparsa*). Defra Final project report HH1749SHN, 2002

## Acknowledgments

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## APPENDIX

1. Replicate data, variety test 1, mean spots/leaf (out of 5 inoculation points)

| | | | | Mean |
|---|---|---|---|---|
| Blue Moon | 0.63 | 0.88 | 1.25 | 0.92 |
| Silver Jubilee | 0.80 | * | * | 0.80 |
| Auskeppy | 0.33 | 1.75 | 0.25 | 0.78 |
| Sexy Rexy | 0.33 | 1.50 | 0.00 | 0.61 |
| Sunset Boulevard | 0.25 | 1.00 | 0.40 | 0.55 |
| Princess Alice | 0.43 | 0.57 | 0.33 | 0.44 |
| Buxom Beauty | 0.33 | 0.33 | 0.25 | 0.31 |
| Glamis Castle | 0.33 | 0.50 | 0.00 | 0.28 |
| Rhapsody in Blue | 0.33 | 0.33 | 0.00 | 0.22 |
| Super Fairy | 0.17 | 0.17 | 0.14 | 0.16 |

2. Replicate data, variety test 2, mean total spots/leaf, 6 days after inoculation

| | | | | Mean |
|---|---|---|---|---|
| Lili Marlene | 4 | 5 | 2 | 3.67 |
| Velvet Fragrance | 1 | 3 | 3 | 2.33 |
| Shocking Blue | 3 | 2 | 1 | 2.00 |
| Sexy Rexy | 1 | 1 | 1 | 1.00 |
| Blue Moon | 1 | 2 | 0 | 1.00 |
| Deep Secret | 2 | 0 | 0 | 0.67 |
| Silver Anniversary | 1 | 1 | 0 | 0.67 |
| Rhapsody in Blue | 1 | 0 | 0 | 0.33 |
| Sunset Boulevard | 0 | 0 | 0 | 0.00 |
| Hot Chocolate | 0 | 0 | 0 | 0.00 |
| Glamis Castle | 0 | 0 | 0 | 0.00 |

3. Replicate data, variety test 2, mean total spots/leaf, 20 days after inoculation

| | | | | Mean |
|---|---|---|---|---|
| Lili Marlene | 1 | 1 | 4 | 2.00 |
| Velvet Fragrance | 1 | 3 | * | 2.00 |
| Shocking Blue | 1 | 3 | * | 2.00 |
| Sexy Rexy | 1 | 1 | 3 | 1.67 |
| Blue Moon | 1 | 1 | * | 1.00 |
| Deep Secret | 2 | 2 | * | 2.00 |
| Silver Anniversary | 3 | 15 | * | 9.00 |
| Rhapsody in Blue | 1 | 2 | 2 | 1.67 |
| Sunset Boulevard | 12 | 2 | * | 7.00 |
| Glamis Castle | 1 | 1 | 0 | 0.67 |
| Hot Chocolate | 0 | 0 | 0 | 0.00 |

4. Replicate data, fungicide trial year 1

Incidence of downy mildew (% of leaves infected) 27/07/07

| | | | | Mean |
|---|---|---|---|---|
| Untreated | 26 | 10 | 34 | 23.33 |
| SL 567 A | 7 | 5 | 2 | 4.67 |
| Revus | 9 | 9 | 2 | 6.67 |
| Signum | 40 | 7 | 10 | 19.00 |
| dimethomorph | 5 | 4 | 5 | 4.67 |
| Infinito | 12 | 7 | 9 | 9.33 |
| Previcur Energy | 23 | 17 | 14 | 18.00 |
| Valbon | 24 | 5 | 4 | 11.00 |

Severity of downy mildew 0-5 20/08/07

| | | | | Mean |
|---|---|---|---|---|
| Untreated | 2 | 3 | 1 | 2.00 |
| SL 567 A | 0 | 1 | 1 | 0.67 |
| Revus | 1 | 2 | 1 | 1.33 |
| Signum | 2 | 0 | 1 | 1.00 |
| dimethomorph | 2 | 2 | 2 | 2.00 |
| Infinito | 2 | 2 | 0 | 1.33 |
| Previcur Energy | 2 | 3 | 2 | 2.33 |
| Valbon | 1 | 3 | 1 | 1.67 |

Severity of downy mildew, 0-3 27/09/07

| | | | | Mean |
|---|---|---|---|---|
| Untreated | 2 | 1 | 1 | 1.3 |
| SL 567 A | 2 | 1 | 0 | 1.0 |
| Revus | 0 | 2 | 0 | 0.7 |
| Signum | 0 | 0 | 1 | 0.3 |
| dimethomorph | 0 | 0 | 0 | 0.0 |
| Infinito | 0 | 1 | 2 | 1.0 |
| Previcur Energy | 2 | 2 | 1 | 1.7 |
| Valbon | 0 | 0 | 0 | 0.0 |

5. Replicate data, fungicide trial year 2 (% leaf drop, % leaf yellowing, % leaf area infected with downy mildew)

| | | Drop | Yellowing | Downy mildew | Drop | Yellowing | Downy mildew | Drop | Yellowing |
|---|---|---|---|---|---|---|---|---|---|
| | | 03/10/2008 | 03/10/2008 | 03/10/2008 | 14/10/2008 | 14/10/2008 | 14/10/2008 | 22/10/2008 | 22/10/2008 |
| Untreated | 1 | 12 | 5 | 5 | 12 | 18 | 4 | 45 | 2 |
| | 2 | 5 | 10 | 5 | 16 | 20 | 5 | 60 | 4 |
| | 3 | 17 | 10 | 4 | 32 | 20 | 5 | 50 | 4 |
| SL 567A | 1 | 18 | 2 | 4 | 50 | 5 | 1 | 57 | 15 |
| | 2 | 12 | 3 | 4 | 26 | 15 | 4 | 50 | 10 |
| | 3 | 7 | 7 | 2 | 16 | 18 | 2 | 43 | 5 |
| Revus | 1 | 4 | 4 | 4 | 5 | 22 | 4 | 15 | 8 |
| | 2 | 12 | 8 | 2 | 23 | 15 | 2 | 55 | 4 |
| | 3 | 10 | 5 | 2 | 60 | 10 | 2 | 60 | 6 |
| Signum | 1 | 0 | 1 | 0 | 0 | 15 | 0 | 2 | 23 |
| | 2 | 1 | 5 | 2 | 10 | 10 | 2 | 15 | 8 |
| | 3 | 1 | 7 | 2 | 5 | 14 | 2 | 13 | 18 |
| dimethomorph | 1 | 10 | 2 | 2 | 28 | 15 | 2 | 50 | 2 |
| | 2 | 5 | 3 | 2 | 20 | 10 | 3 | 35 | 8 |
| | 3 | 15 | 5 | 2 | 30 | 13 | 3 | 56 | 10 |
| Infinto | 1 | 15 | 10 | 2 | 35 | 18 | 4 | 48 | 5 |
| | 2 | 10 | 5 | 5 | 37 | 7 | 4 | 50 | 5 |
| | 3 | 5 | 15 | 1 | 10 | 23 | 4 | 30 | 12 |
| Previcur Energy | 1 | 7 | 2 | 2 | 30 | 12 | 3 | 57 | 2 |
| | 2 | 7 | 3 | 5 | 25 | 20 | 2 | 55 | 6 |
| | 3 | 15 | 12 | 4 | 52 | 22 | 3 | 43 | 12 |
| Valbon | 1 | 5 | 7 | 2 | 5 | 25 | 2 | 5 | 18 |
| | 2 | 2 | 10 | 2 | 1 | 10 | 0 | 8 | 23 |
| | 3 | 2 | 15 | 2 | 2 | 26 | 1 | 12 | 30 |

## 6 Summary of PCR outcome with short description of tissue

| Comment/variety | Tissue type | pcr |
|---|---|---|
| ADAS | Stem | neg |
| ADAS | Dead leaf (2) | Positive |
| ADAS | Green leaf (2) | neg |
| ADAS | Yellow Section of leaf | Positive |
| ADAS | 4mm dia red lesion | Positive |
| | Mixture | neg |
| ADAS 2 | Stem | neg |
| ADAS 2 | Green leaf (2) | Positive |
| ADAS 2 | Brown leaf | neg |
| ADAS 2 | Yellow leaf | Positive |
| ADAS 2 | Red lesions | Positive |
| ADAS 2 | Green leaf | neg |
| ADAS 2 | Green leaf/Red spots | Positive |
| | lesion | Positive |
| | Green leaf | neg |
| | Purple lesion | neg |
| | Mixture stem leaf | neg |
| | Mixture stem leaf | neg |
| | Mixture stem leaf | neg |
| | Mixture stem leaf | neg |
| | Mixture stem leaf | Positive |
| | Mixture stem leaf | Positive |
| | Mixture stem leaf | neg |
| | Mixture stem leaf | neg |
| | Mixture stem leaf | neg |
| | Mixture stem leaf | neg |
| | Mixture stem leaf | Positive |
| | Mixture stem leaf | neg |
| | Mixture stem leaf | neg |
| Angelina | Plant1 Leaf;small black lesions | neg |
| Angelina | Plant1 Woody stem 7mm dia | neg |
| Angelina | Plant1 Leaf; large black blotches | neg |
| Angelina | Plant1 stem with brown lesions | neg |
| Angelina | Plant1 non-woody stem | neg |
| Angelina | Plant 2 Woody stem | Positive |
| Angelina | Plant 2 non-woody stem | neg |
| Angelina | Plant 2 Leaf;small black lesions | neg |
| Ghislane de Feligonde | Green leaf | neg |
| Ghislane de Feligonde | Woody stem | neg |
| Ghislane de Feligonde | Leaf; large black blotches | neg |
| Ghislane de Feligonde | Flower buds | neg |
| Chevy Chase | Flower buds | neg |
| Chevy Chase | Green leaf | neg |
| Chevy Chase | Red lesions | neg |
| Chevy Chase | Woody stem | neg |
| | Green leaf/Red spots | neg |
| | red spots on leaf | neg |
| | red spots on leaf | neg |
| | red stem | Positive |
| | red leaf spots | neg |
| | red leaf spots | neg |
| | red leaf spots | neg |
| | red leaf spots | neg |
| | red leaf spots | neg |
| | red leaf spots | neg |

| | | |
|---|---|---|
| | red leaf spots | neg |
| | red leaf spots | neg |
| | red leaf spots | neg |
| | red leaf spots | neg |
| | red leaf spots | neg |
| | brown stem section | Positive |
| | small flecks downy mildew | Positive |
| | suspect red symptoms on green leaf | weak |
| | leaf spots | neg |
| | leaf spots | neg |
| | Filtree leaf | neg |
| | std stem | neg |
| | bush stem scrapings | neg |
| | red lesions | neg |
| | brown leaf spots | neg |
| | red leaf spots | neg |
| | dead leaf from suspect plant | neg |
| Stem 1, variety 1 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 1 variety 2 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 4 | budwood | neg |

| | | |
|---|---|---|
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 1 variety 3 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 1 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 2 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 3 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 4 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| Stem 5 | budwood | neg |
| | Bark | neg |
| | Leaf | neg |
| | Bark | neg |
| | Leaf | neg |
| | Bark | neg |
| | Leaf | neg |
| | Dead stem | neg |
| | Dead leaf | neg |
| | Dead Flower | neg |
| | Green Stem | neg |
| | Red leaf sections | neg |
| | Green Leaf | neg |
| | Stem | neg |
| | Root | neg |
| | Stem base | neg |
| | Angular lesion on leaf | neg |
| | Green stems (pool) | neg |
| | Green leaves | neg |
| | Green stem section | neg |
| | Green stem section | neg |
| | Green stem section | neg |
| | Green stem section | neg |
| | Green stem section | neg |
| | Brown lesions on stem | neg |

| | | |
|---|---|---|
| | Brown lesions on stem | neg |
| | Brown lesions on stem | neg |
| | Brown lesions on stem | neg |
| | Stem 1 | neg |
| | Stem 2 | neg |
| | Stem 2 red lesion | neg |
| | Green leaf | neg |
| | Leaves with Blackspot | neg |
| | Flower | neg |
| laxa rootstock plants | Asymetric lesion on leaf | positive |
| laxa rootstock plants | Red leaf lesion | neg |
| laxa rootstock plants | Green leaf | neg |
| laxa rootstock plants | Stem sections | positive |
| laxa rootstock plants | Stem sections | positive |
| laxa rootstock plants | Stem sections | positive |
| laxa rootstock plants | Stem sections | positive |
| laxa rootstock plants | Stem section inc. lesion | neg |
| | Asymetric lesion on leaf | neg |
| | Red leaf lesion | neg |
| | Green leaf | neg |
| | Stem sections | neg |
| | Stem sections | neg |
| | Stem sections | positive |
| | Stem sections | positive |
| | Stem section inc. lesion | neg |
| | Asymetric lesion on leaf | neg |
| | Red leaf lesion | positive |
| | Green leaf | neg |
| | Stem sections | neg |
| | Stem sections | neg |
| | Stem sections | neg |
| | Stem sections | neg |
| | Stem section inc. lesion | neg |
| | Green/new leaves | negative |
| | Red lesions | Positive |
| | Stem base | Positive - weaker than lesions |
| Rosa Multiflora (2) | Stem/brown lesion | neg |
| Rosa Multiflora (2) | Stem/green | positive |
| Rosa Multiflora (2) | Green leaf | neg |
| Rosa Multiflora (2) | Brown/Yellow leaves | neg |
| | Stem section inc. lesion | neg |
| | Stem section green | neg |
| laxa rootstock plants | root section1 | neg |
| laxa rootstock plants | root section2 | neg |
| laxa rootstock plants | Stem at base | neg |
| laxa rootstock plants | Upper stem | neg |
| laxa rootstock plants | Green leaves | neg |
| laxa rootstock plants | Stem at base | neg |
| laxa rootstock plants | Green leaf | neg |
| laxa rootstock plants | Red lesions on leaves | neg |
| | yellow leaves | neg |
| | Red leaves | positive - weak band |
| | Stem section -green | neg |
| | Stem section -lesion | neg |
| | Flower | neg |
| | lesions on stem | neg |
| | lesions on leaf | neg |

| | |
|---|---|
| Red spots on stem | neg |
| Red spots on leaf | neg |
| Blackened thorns | neg |
| buds | neg |
| stem section 1 | neg |
| stem section 2 | neg |
| stem section 3 | neg |
| stem section 4 | neg |
| flower and bud1 | neg |
| green leaf and lesions1 | neg |
| green leaf | neg |
| green leaf | neg |
| 2 leaves + lesions | Positive |
| stem | Positive |
| green stem | neg |
| pot soil samples | neg |
| unopened bud | neg |
| green leaf +blackspots | Positive |
| green leaf+lesions | Positive |
| green leaf + lesions | positive - weak band |
| green leaves + stem | positive - weak band |
| green leaves + stem | neg |
| green stem | neg |
| green leaf + lesions | neg |
| main green branch | neg |
| brown stem | Positive |
| green stem with lesion | Positive |
| green stem with lesion | positive - weak band |
| small dead stem off main bud stem | neg |
| stem peel | neg |
| dead leaves | neg |
| stem main | neg |
| root 1 | positive - weak band |
| root 2 | neg |
| fibrous root | neg |
| Brown stem peel | neg |
| Green stem peel | neg |
| Green stem above graft | neg |
| Green side shoot above graft | neg |
| Root just below graft | neg |
| Root bottom below graft | positive - weak band |
| Stem scrape 1 | neg |
| Stem scrape 2 | neg |
| Stem scrape 3 | neg |
| Stem scrape 4 | neg |
| Stem scrape 5 | neg |
| Stem scrape 6 | neg |
| Green leaves | neg |
| Green leaves | neg |
| Green stem peel 1 | neg |
| Green stem peel 2 | neg |
| Brown Stem peel 1 | neg |
| Brown Stem peel 2 | neg |
| yellow leaf 1 | neg |
| yellow leaf 2 | neg |
| pooled leaves stem 1 | neg |
| pooled leaves stem 2 | neg |
| 4 outer petals showing lesions | neg |

| | |
|---|---|
| 4 outer petals showing lesions | neg |
| red stem peel 1 | neg |
| red stem peel 2 | neg |
| green leaves | neg |
| Green stem | neg |
| red leaf lesion - 1a | neg |
| red leaf lesion - 2a | positive - weak band |
| red leaf lesion - 3a | neg |
| red leaf lesion - 4a | neg |
| red leaf lesion - 5a | neg |
| Stem from leaf 1a | positive - weak band |
| red lesion -1b | positive - weak band |
| green leaf -1b | neg |
| red lesion -2b | neg |
| green leaf -2b | positive - weak band |
| red lesion -3b | neg |
| green leaf -3b | positive - weak band |
| leaf+brown spot lesion 5b | positive - weak band |
| stem from leaf 5a+b | neg |
| red lesion -4b | positive - weak band |
| green leaf -4b | positive - weak band |
| Red leaf lesion 1c | neg |
| Green leaf section 1c | neg |
| Red leaf lesion 2c | neg |
| Green leaf section 2c | neg |
| Red leaf lesion 3c | neg |
| Green leaf section 3c | neg |
| Red leaf lesion 4c | neg |
| Green leaf section 4c | neg |
| Red leaf lesion 5c | neg |
| Green leaf section 5c | neg |
| dotted lesions leaf 1d | neg |
| dotted lesions leaf 2d | neg |
| dotted lesions leaf 3d | neg |
| dotted lesions leaf 4d | neg |
| dotted lesions leaf 5d | neg |
| Stem 1d | neg |
| Stem 2d | neg |
| Stem 3d | neg |
| Stem 4d | neg |
| stem from d leaves | neg |
| Shoots from stem -1 | neg |
| Leaves from stem -1 | neg |
| Stem -1 | neg |
| Shoots from stem -2 | neg |
| Leaves from stem -2 | neg |
| Stem -2 | neg |
| Shoots from stem -3 | neg |
| Leaves from stem -3 | neg |
| Stem -3 | neg |
| Shoots from stem -4 | neg |
| Leaves from stem -4 | neg |
| Stem -4 | neg |
| Shoots from stem -5 | neg |
| Leaves from stem -5 | neg |
| Stem -5 | neg |
| Thin stem from leaf -5 | neg |
| 1.1 Leaf | Positive |

| | | |
|---|---|---|
| | 1.2 Stem near leaf attachment | neg |
| | 2.1 Stem from base | Positive |
| | 2.2 Bud - sepals with sporangia | Positive |
| | 2.3 Bud - petals | neg |
| | 3.1 Small lesion on stem | neg |
| | 3.2 Large lesion on stem | Positive |
| | 4.1 Green Stem - a section from middle | neg |
| | 5.1 Dead stem | neg |
| | 6.1 Red stem | Positive |
| | 6.2 Green stem just below bud | neg |
| | 7.1 root + stem base -pool from 5 fragments | neg |
| | 8.1 green petiole 1 | Positive |
| | 8.2 green petiole 2 | Positive |
| | 8.3 green petiole 3 | neg |
| | 8.4 green petiole 4 | neg |
| | 8.5 green petiole 5 | neg |
| | 8.6 stem section 1 | neg |
| | 8.7 stem section 2 | neg |
| | 8.8 stem section 3 | neg |
| | 8.9 stem section 4 | neg |
| | 8.10 stem section 5 | neg |
| | 9.1petiole section 1 top | neg |
| | 9.2 petiole section 2 bottom | neg |
| | 10.1 bud-end of bud stem | Positive |
| | 10.2 other end of bud stem | Positive |
| | 10.3 middle of bud stem -sporangiophores | Positive |
| | 10.4 outer petals of bud | Positive |
| | 10.5 inner petals of bud | Positive |
| | 11.1 stem scraping of raised brown area on stem | neg |
| | 11.2 stem scraping of raised brown area on stem | neg |
| | 11.3 stem scraping of raised brown area on stem | neg |
| from fungicide trial | bud | neg |
| from fungicide trial | leaf | neg |
| from fungicide trial | stem 1 | neg |
| from fungicide trial | stem 2 | neg |
| from fungicide trial | stem 3 | neg |
| from fungicide trial | stem 4 | neg |
| from fungicide trial | lesion on bud stem | neg |
| from fungicide trial | bud from stem | neg |
| from fungicide trial | bud 2 | neg |
| from fungicide trial | leaf 1 | neg |
| from fungicide trial | leaf 2 | neg |
| from fungicide trial | leaf 3 | neg |
| sprouts from budwood | leaf V shaped lesion | neg |
| sprouts from budwood | leaf 2 red lesion | neg |
| sprouts from budwood | leaf 3 | neg |
| sprouts from budwood | Stem 1 | neg |
| sprouts from budwood | Stem 2 | neg |
| sprouts from budwood | Stem 3 | neg |
| sprouts from budwood | Stem 4 | neg |
| sprouts from budwood | Stem 5 | neg |
| sprouts from budwood | Stem 6 | neg |
| sprouts from budwood | Stem 7 | neg |
| old budwood | dry stem 1 | neg |
| old budwood | dry stem 2 | neg |
| old budwood | dry stem 3 | neg |

| | | |
|---|---|---|
| old budwood | dry stem 4 | neg |
| old budwood | dry stem 5 | neg |
| old budwood | dry stem 6 | neg |
| old budwood | dry stem 7 | neg |
| old budwood | dry stem 8 | neg |
| old budwood | dry stem 9 | neg |
| old budwood | dry stem 10 | neg |