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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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.

Xiangming Xu
East Malling Research

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Dr Christopher J Atkinson
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CONTENTS

Grower Summary

Headline

A rose powdery mildew prediction model has been developed and both potassium bicarbonate and Farmfos have provided consistent control.

Background and expected deliverables

Powdery mildews infect a wide range of ornamental plants and result in significant economic losses by disfiguring and blemishing leaves and flowers. The reduction in marketability, often caused by relatively low levels of infection, necessitates stringent control measures, which are currently achieved by intensive spray programmes. Intensive fungicide usage can result in unjustified applications and potential environmental pollution. Such use does not always control the disease satisfactorily due to poor timing or choice of fungicides, and may accelerate the selection of mildew strains that are resistant to fungicides.

On susceptible plants, an appropriate strategy is to intervene with fungicides or even better with alternative control agents, including natural products and biocontrol agents (BCA), but only when treatment is justified. This requires knowledge of when there is a risk of disease. For rose powdery mildew, the key information needed to develop a predictive model can be obtained from published information.

Operating a supervised control strategy requires a better understanding of the physical mode of action of available products: protectant, anti-sporulant or curative, so the appropriate products can be selected at the right time to control mildew. Several alternative products have recently been shown to be effective against powdery mildew on other crop species.

There are two expected deliverables from this project:

1. A model which forecasts mildew development on rose, delivered as prototype computer software that can be used directly by growers.

2. Identification of alternative chemicals that are effective against powdery mildew on rose and their main physical mode of action.

Summary of the project and main conclusions

To date, a prediction model has been developed for rose powdery mildew. It has been implemented as a stand-alone computer programme, which can use weather data (text) files of various formats generated by common data loggers. Epidemic data is now being collected to validate the model.

Trials have been conducted to investigate the protectant, curative and anti-sporulant effects of several alternative products against powdery mildew. Preliminary results suggest that only potassium bicarbonate and Farmfos have shown consistent effects in reducing mildew development. These trials will be repeated in 2009.

Financial benefits

This project has produced a model forecasting mildew development on rose, which, after validation, can be used by growers to time fungicide applications, and to select the fungicide and its dose. The project will identify alternative chemicals that are effective against powdery mildew on rose and their main physical mode of action. This research should lead to less fungicide input while maintaining effective mildew control.

Action points for growers

- No action points can be offered at this stage in the project, as all results are preliminary in nature and need confirmation.

Science Section

Introduction

Powdery mildews infect a wide range of ornamental plants and result in significant economic losses by disfiguring and blemishing leaves and flowers. The reduction in marketability, often caused by relatively low levels of infection, necessitates stringent control measures, which are currently achieved by intensive spray programmes. Because of the explosive nature of mildew epidemics, even a low level of disease can lead to severe outbreaks over a very short period of time and hence may lead to 100% loss of plants. Such intensive fungicide usage can result in unjustified applications and potential environmental pollution, does not always control the disease satisfactorily due to poor timing or choice of fungicides, and may accelerate the selection of mildew strains that are resistant to fungicides.

On susceptible plants, an appropriate strategy is to intervene with fungicides or even better with alternative control agents, including natural products and biocontrol agents (BCA), only when treatment is justified. This requires knowledge of when there is a risk of disease, which is often provided by a disease prediction system implemented as computer software. Recent research at East Malling Research (EMR) has demonstrated a supervised disease control approach to be successful on apple. This strategy reduced fungicide input by up to 45% when compared with a conventional programme whilst maintaining comparable disease control (1).

Much information on rose powdery mildew epidemiology is available from published studies; a considerable amount of qualitative information was published in 1970-80s (2-6). While, more recently, a Defra-funded project at EMR investigated quantitative aspects of rose powdery mildew (7, 8). There is now sufficient published information to develop a simple prediction scheme. Thus, key model parameters can be obtained from these published studies and incorporated into the existing apple mildew model structure/software (9), developed at EMR, for commercial use.

Operating a supervised control strategy also requires better understanding of the physical mode of action of available products: protectant, anti-sporulant or curative, so the right products can be selected at the right time to control mildew. Several alternative products, including potassium bicarbonate, have been shown to be effective against powdery mildew in several other crop species. But only one study reported testing of alternative products against rose powdery mildew (10). *Bacillus subtilis* strain QST 713 is a biological bactericide (BCA) sold as Serenade® and has shown activity against many pathogens. The manufacturer of this product (Agraquest) claims that Serenade® has significant control effects against powdery mildew on several crops: grape, cherry, hops, leaf vegetables, cucumber and pepper. This product was registered in the UK in 2008.

This project has two specific objectives: (1) to develop and validate a model (system) that predicts risks of rose powdery mildew in relation to environmental conditions, and (2) to determine the efficacy of several new alternative chemicals for controlling active colonies of rose powdery mildew, focusing on the protective, curative and anti-sporulant activities.

Materials and methods

Development of prediction models

We have developed a predictive model, which simulates epidemics of secondary mildew on vegetative shoots at daily intervals and predicts the percentage of mildewed leaf area. The model simulates the epidemic in daily steps from 09:00 A.M. to 08:59 A.M the following day, rather than from midnight to midnight, to be consistent with the definition of 'daily record' used by the British Meteorological Office. The model consists of a series of sub-models, which estimate percentage of leaf area with infectious disease, the length of latent period and infection rate. It generates daily forecasts of the severity of new infections and of total mildewed leaf area with sporulating lesions. The model is driven by hourly ambient relative humidity and shade temperature (°C). Vapour pressure deficit (mmHg) is calculated from temperature and relative humidity.

The first sub-model deals with infection and it relates the rate of infection of host tissue by powdery mildew to ambient temperature and relative humidity. Depending on model validation results in 2009, we may include rainfall to more accurately estimate the rate of infection as well. The model assumes that after conidia have germinated successfully, they will infect and colonise host tissue; thus the infection rate is directly estimated from the rate of conidial germination. The effects of temperature and relative humidity on conidial germination are described by models developed from published data. Any spores that do not germinate within one day of their release are regarded as non-viable, i.e. only the effects of the current day weather conditions on germination are considered. The model first calculates the percentage of conidia germinated based on temperature only; it then estimates the percentage of conidial mortality from temperature and relative humidity. From these two indices, a new index is calculated to estimate the percentage of conidia germinated with a 24 h period.

Colonies are assumed to sporulate immediately after becoming visible. Thus the incubation period (the time from infection to visible symptoms) is equivalent to the latent period in the model. The hourly rate of colony development during the incubation period is calculated using the model of Xu (7). This model describes the effects of temperature on the rate of fungal development during the median incubation period (the time from infection to when more than 50% colonies become visible). Relative humidity is assumed not to affect the fungal growth rate during the incubation period. Once colonies have started to sporulate, they are assumed to sporulate for 14 days and thereafter cease sporulation. The model tracks incubation development of new infections on each day and estimates the total sporulation area.

<u>Testing alternative products</u>

A number of products were tested in 2008, including: Serenade®, Wetcit, potassium phosphate (Farm fos), potassium bicarbonate, Enzicur, and Chitoclear. In addition, at least Systhane was included as a treated control; this fungicide was used after consultation with advisors. The application rate is the full label-recommended rate. It was not possible to include Milsana because it was out of stock due to the shortage of its ingredients in 2008; but it will be included in 2009 tests. Enzicur was only tested once in 2008 because its use-by-date was late July. In addition, an untreated (but inoculated) control was included. Each product was applied to the plants (shoot/leaves) until run-off using a 500 ml hand-held sprayer. Two plants were used for each treatment; there were 3-5 five shoots assessed for mildew on each plant. Initially two cultivars were used in testing: one Hybrid Tea **(**Prima Ballerina) and one climber (Zephirine Drouhin). However, powdery mildew did not develop quickly on cv. Prima Ballerina. So all subsequent tests were done on cv. Zephirine Drouhin. In all trials, percentage of mildewed leaf area was estimated for both upper and lower leaf surfaces. Disease was only assessed on the top four leaves (the youngest fully unrolled leaves and three rolled leaves at the time of inoculation/treatment).

Protective activity, i.e. the ability to prevent mildew spores from infecting healthy leaves. Healthy plants of cv. Zephirine Drouhin were maintained in a glasshouse compartment, free of powdery mildew, and randomly allocated to each treatment. They were sprayed with an appropriate product first and moved to a polythene tunnel after spray deposits had dried. For the next four days, these plants were inoculated by shaking leaves with sporulating mildew colonies over the top of the shoots thus dispersing conidia. The plants were then moved back to the glasshouse compartment (i.e. on day 4) for incubation. This experiment was carried out three times: July 2008, September 2008, and February 2009. Mildew was assessed 7-10 days later. In February 2009, three plants were used in each treatment.

Curative activity, i.e. the ability to kill the young developing, but symptomless, colonies thereby preventing them from forming visible lesions. Healthy plants cv. Zephirine Drouhin were first inoculated using the same method described above and left in the polythene tunnel compartment with mildewed plants for two days. They were then moved back to a glasshouse compartment, randomly assigned to a treatment and sprayed with an appropriate product. Mildew was assessed 7-10 days later. This experiment was done twice: July 2008 and February 2009. In February 2009, three plants were used in each treatment.

Antisporulant activity, i.e. the ability to reduce spore production. Plants were first inoculated with mildew conidia and left for mildew to develop. About 14 days later, two plants were randomly allocated to a treatment. Fresh colonies were treated with an appropriate product. Then, 7-10 days later, a cellotape imprint of a lesion was taken and mounted on a slide for assessment of conidia morphology under a microscope (X 100). For each plant, two tapes were taken from two randomly chosen treated lesions. In February 2009, only one plant was available for each treatment.

Repeated applications. An additional experiment was conducted to evaluate the effect of repeated application of each individual product on the development of powdery mildew. Five products were used for this trial: potassium bicarbonate, potassium phosphate, Chitoclear, Systhane and Serenade. In addition, an untreated control was included. Two plants of cv. Zephirine Drouhin were randomly allocated to each treatment; this experiment was conducted in a glasshouse compartment. The first application was made in September 2008 and thereafter every 10-14 days. In total, four applications were made. Powdery mildew on leaves assessed twice: one week after the second spray and two weeks after the last spray.

<u>Data analysis</u>

Total number of leaflets inoculated and infected per shoot as well as average leaflet area infected per shoot were analyzed. Generalized non-linear mixed modeling was used to analyze the data where the product was treated as a fixed factor, and plants and shoots within plants were treated as random factors. For the incidence of leaflet infection, a binomial distribution was assumed for the error distribution. Then Fisher's least significant differences (LSD) was used to compare treatments.

Results

<u>Model development</u>

A prediction model has been developed for rose powdery mildew and implemented as a computer programme (Figure 1).

Figure 1. A screen shot of the main window of the rose mildew prediction model

This model uses ASCII text files as input files (i.e. containing weather data). All weather data loggers should be able to produce ASCII text files. The programme provides a very flexible data format definition facility to define the exact data format for each specific data file (Figure 2). This is essential for the model to read weather data accurately. The data format definition and subsequent data handling by the model have been tested for data files from several different types of weather data loggers used in the UK, e.g. Skye, Davis, Tinytag.

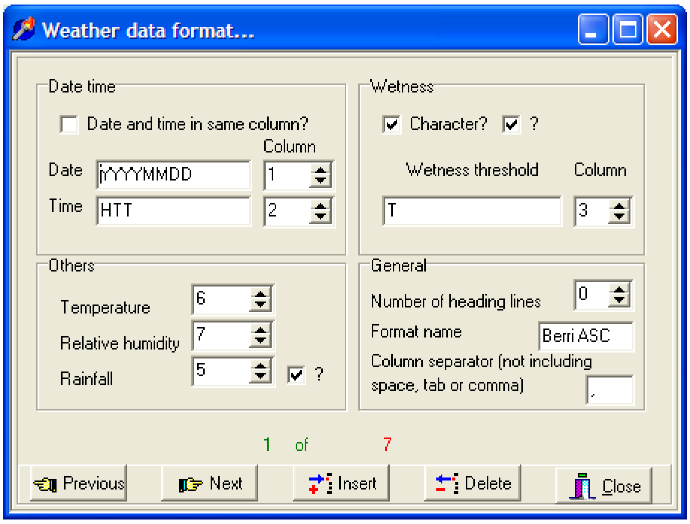

Figure 2. A screen shot of the window used for defining weather data format

Users can run the model for any specified period of time using a particular set of weather data (Figure 3). A rose downy mildew prediction model will also be incorporated into this programme. Thus, in the future, users can run models for rose downy and powdery mildews either separately or simultaneously.

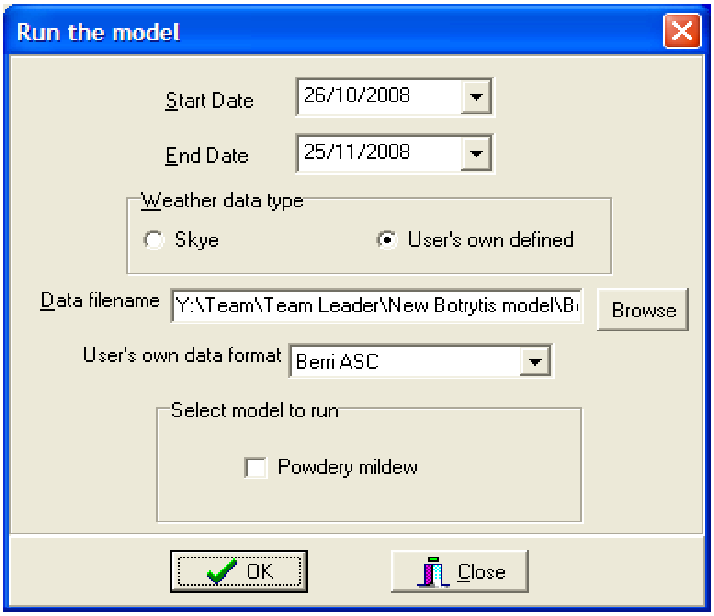

Figure 3. A screen shot of the window used for initiating the predictive model

The model will display predicted mildew development (Figure 4); Figure 4 is for illustrative purpose only. In 2009, we shall collect mildew epidemic data to validate the model.

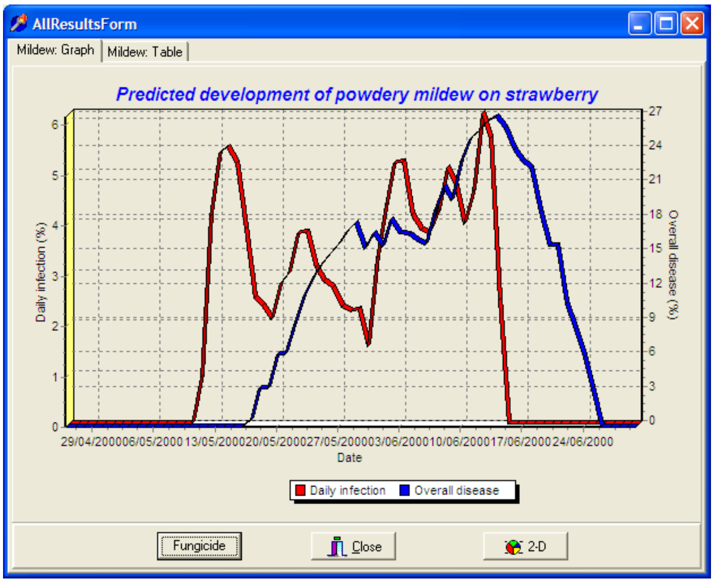

Figure 4. A screen shot of model predictions for illustration purpose only

Testing alternative products

Curative. In the first experiment, there were no significant differences in the incidence of infected leaflets (Figure 5) and the percentage of mildewed leaf area among the treatment. The incidence of leaflets infected ranged from 3% (Systhane and Chito clear) to 14% (Serenade), with 10% for the untreated control.

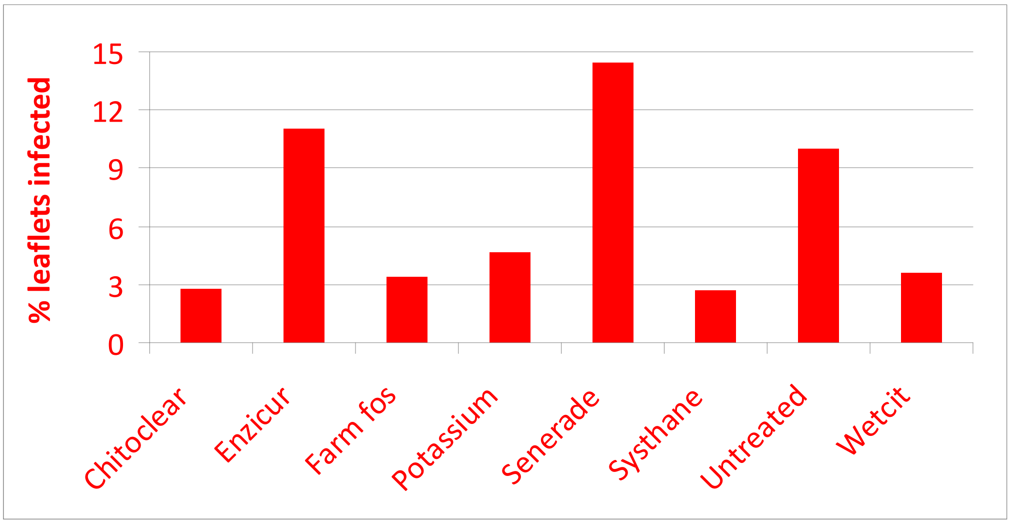

Figure 5. Incidence of rose leaflets infected with powdery mildew in the first curative study

In the second experiment, again overall treatment there were no significant differences in the incidence of infected leaflets (Figure 6) and the percentage of mildewed leaf area among the treatments. However, when comparing individual treatments, Systhane (1%) and Farm fos (3%) had significantly ($P<0.05$) lower incidences than the control (29%).

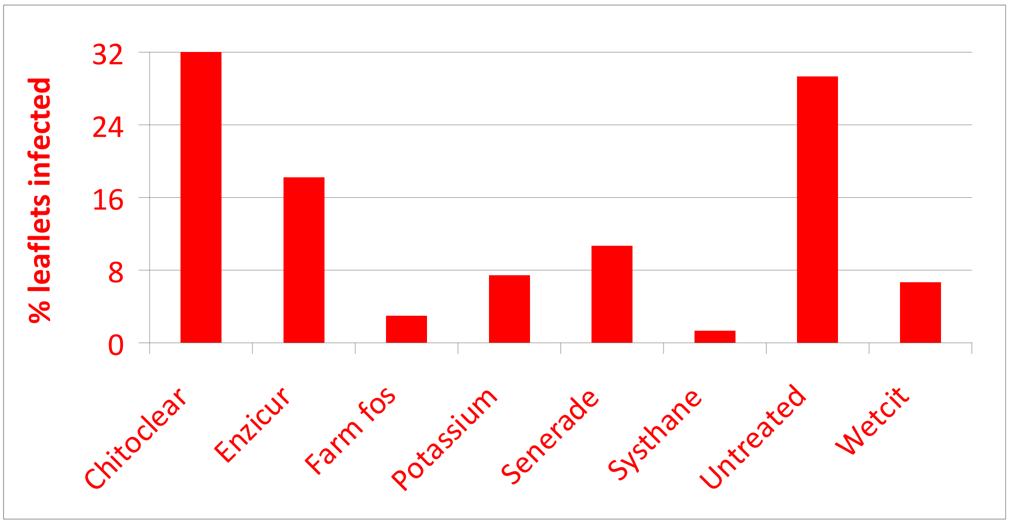

Figure 6. Incidence of rose leaflets infected with powdery mildew in the second curative study

The third curative experiment has just completed and data are still to be analyzed.

Protectant. In the first experiment, there were no significant differences in the incidence of infected leaflets (Figure 7) and the percentage of mildewed leaf area among the treatments. The incidence of leaflets infected ranged from 3% (Serenade and Enzicur) to 13% (Chitoclear), with only 4% for the untreated control.

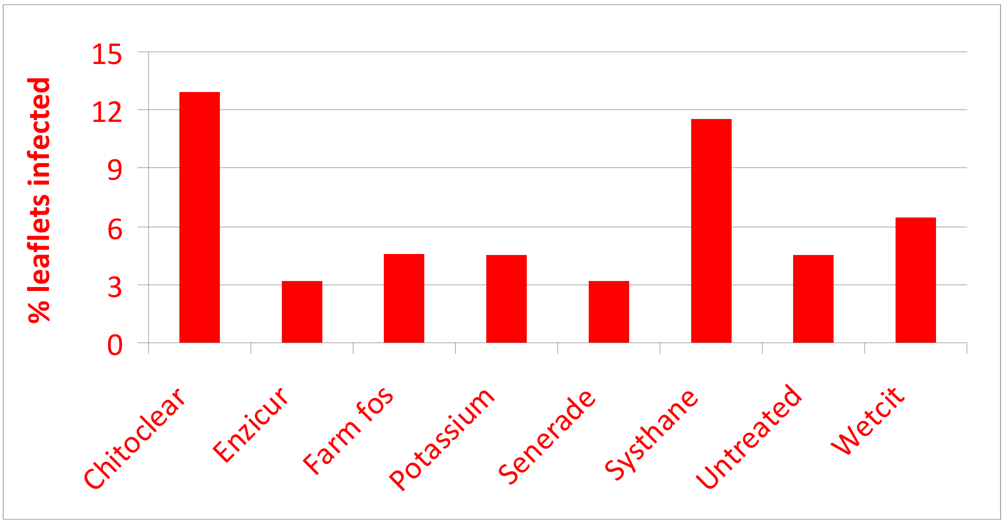

Figure 7. Incidence of rose leaflets infected with powdery mildew in the first protectant study

The second protectant experiment has just completed and data are still to be analyzed.

Antisporulant. We have conducted two anti-sporulant trials: September 2008 and February 2009. Slides are still being microscopically assessed.

Repeated applications. We have conducted one repeated application trial: September-November 2008. The second has just started from February 2009.

For the first assessment, after two applications, there were significant ($P < 0.01$) differences in the incidence of leaflets infected and in the percentage of leaf area infected. Of all the treatments, only potassium bicarbonate significantly reduced the incidence of leaflet infection (Figure 8); about 66% of leaflets treated with potassium bicarbonate were infected, compared to nearly 97% for the untreated control. All treatments, except the Chitoclear, all significantly reduced the percentage of leaf area infected when compared with the control; among them potassium bicarbonate and Farm fos had significantly less disease than Systhane (Figure 9).

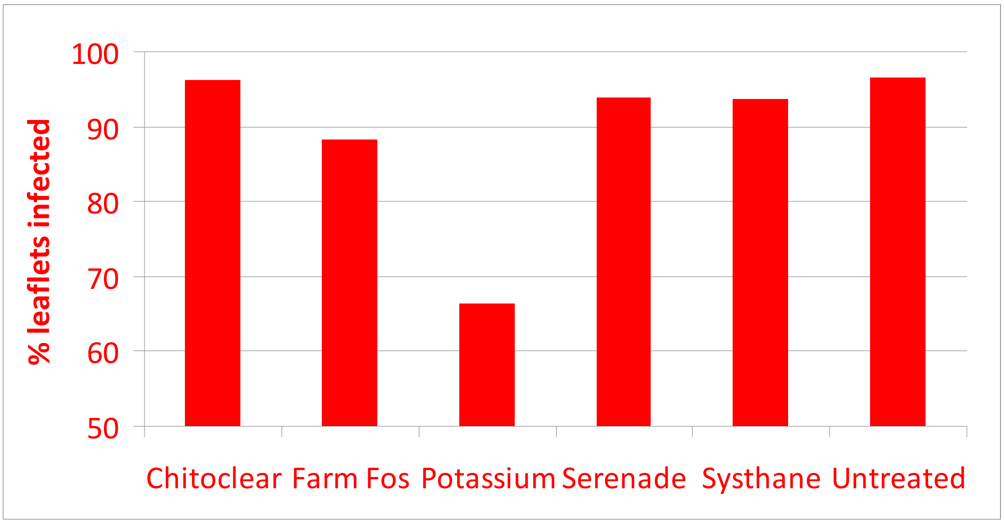

Figure 8. Incidence of rose leaflets infected with powdery mildew of the first assessment after two applications of each product in the first repeated-application study

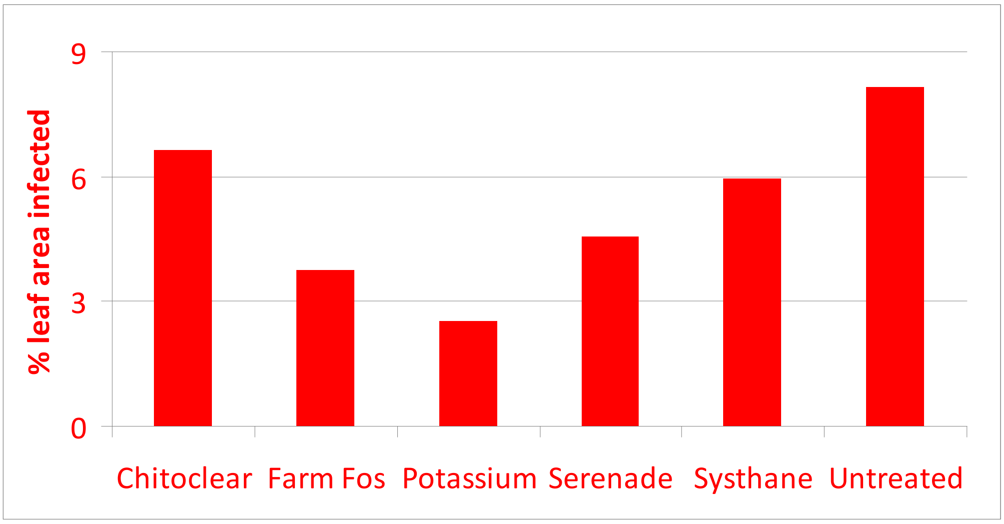

Figure 9. Percentage of leaf area infected with mildew of the first assessment after two applications of each product in the first repeated-application study

In the second assessment (two weeks after the last applications). There were significant ($P<0.01$) differences in the incidence of leaflets infected and in the percentage of leaf area infected among the treatments. Only plants treated with potassium bicarbonate and Farm fos had less ($P < 0.01$) disease than the untreated control (Figure 10)

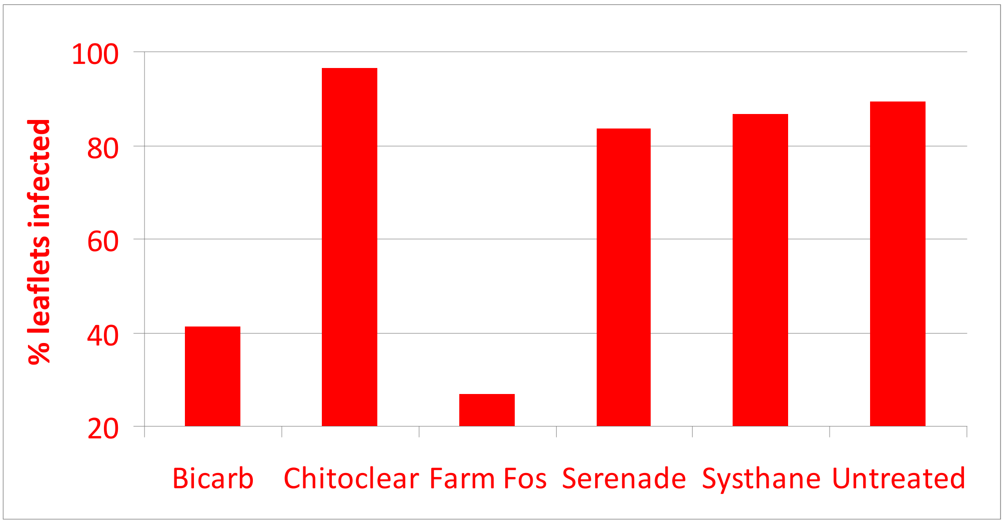

Figure 10. Percentage of leaf area infected with mildew of the second assessment after two applications of each product in the first repeated-application study

Discussion

The treatment effects against rose powdery mildew varied greatly between the single application and multi-application studies. When the products were tested for their effects against powdery mildew in a single application, none of them showed any consistent effects against the disease. In contrast, several treatments had significantly reduced powdery mildew development in the repeated application trial, particularly potassium bicarbonate and Farm fos. This difference may be attributable to two reasons. First, the infection (inoculum) pressure in the single-application studies is likely to be much higher than in the multi-application trials since plants in the former were artificially inoculated but inoculated naturally in the latter. Thus, the high inoculum pressure may have overwhelmed these products. Second, the effect of each product may be small and hence difficult to detect in the single application trials given the large variation in disease development. Such small effects are more likely to be detectable in multi-application trials. New experiments in 2009 will shed more light on this.

We have developed a prediction model for rose powdery mildew that is implemented through a computer programme. This predictive model needs to be validated first before being evaluated for its practical usefulness. It was originally planned to collect epidemics in 2008 and 2009 (i.e. two data sets). For various reasons, we could not achieve this mainly because of the shortage of plants – Prima Ballerina plants hardly produced any new leaves after July and many Zephirine Drouhin failed to grow. To make up for this, we shall collect two sets of epidemic data in 2009 – one under a tunnel and one in open field (sand-bed). It is planned to hold a grower workshop in 2009 to demonstrate this prediction system, possibly in conjunction with the rose downy mildew project.

In the original proposal, we planned to conduct a product trial on a commercial site in 2009. However, having discussed with the industry, we decided to conduct the trial at EMR. This is because it now appears very unlikely that any grower wishes to have plants at their nursery which are untreated for powdery mildew. Without the untreated control, it is difficult to explain trial data in terms of product control efficacy.

Conclusions

- We have developed a predictive model for rose powdery mildew and we will collect data to validate this model in 2009. This model is implemented as a stand-alone computer programme and can use weather data text files of various formats generated by common data loggers
- We have conducted trials to investigate the protectant, curative and anti-sporulant effects of several alternative products against powdery mildew. Only potassium bicarbonate and Farm fos showed consistent effects in reducing powdery mildew development. Further experiments will be carried out in 2009 to confirm these results
- Following discussions with the industry, we shall include Nimrod as a control as well as Systhane

Technology transfer

- We presented the talk on the project progress to British Rose Grower Association on 4 December 2008
- We held in-depth discussions on the project results so far with the grower project co-ordinator (Ms Abi Rayment of Dove Associates) on 25 February 2009

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