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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.

Dr Xiangming Xu
East Malling Research

Signature .... Date 18 March 2010

Report authorised by:

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Grower Summary

Headline

A prototype computer prediction model for rose powdery mildew has been developed. Field data showed that the model accurately predicted the overall trend of epidemic development. The model needs to be refined in consultation with rose growers, especially in user interface and in the formulation of practical strategies to use the predictions.

Food-grade potassium bicarbonate was as effective as Systhane 20EW or Nimrod against powdery mildew when applied repeatedly on mature plants but less so on young fast growing plants. Potassium bicarbonate was the only product that showed a significant effect in reducing conidia viability when applied to sporulating lesions.

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. They do 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 alternative control agents, including naturally occurring products and biocontrol agents (BCA), when treatment is justified. This is called a 'supervised control strategy'. This requires knowledge of when there is a risk of disease. For rose powdery mildew, key information needed for a predictive disease risk model can be obtained from published information.

Operating a supervised control strategy also requires better understanding of the physical mode of action of available products: protectant (i.e. the ability to prevent mildew spores from infecting healthy leaves), curative (i.e. the ability to kill the young developing, but symptomless, colonies preventing them from forming visible lesions) anti-sporulant (i.e. the ability to reduce spore production), so the right products can be selected at the right time to control mildew. Recently, several alternative products have been shown to be effective against powdery mildew on other crop species.

There are two expected deliverables from this project:

1. A model forecasting 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

Model development

A prototype prediction model has been developed and implemented for rose powdery mildew as a stand-alone computer programme, which can use weather data (text) files of various formats generated by common data loggers. The model accurately predicted the overall trend of epidemic development under tunnels for two years. In consultation with rose growers, the model will be developed and will also enable supervised management of downy mildew. This development will take place under HNS 173, and should be available for use by the industry by summer 2012. Rose growers with a particular interest in the development of this model are encouraged to contact the HDC.

Testing alternative products

Several 'alternative' products were tested alongside 'standard' powdery mildew control products (Table 1).

Table 1 Summary of the products tested in 2008 and 2009 against rose powdery mildew

Trade name	Active ingredient	Application rate	Approval status	Protective	Curative	Anti-sporulant	Repeated	Notes
Serenade ASO®	*Bacillus subtilis* (strain QST 713)	10 ml L^{-1}	Approved	✓*	✓	✓	✓	
Farm-Fos-44®	Potassium phosphite	10 ml L^{-1}	Not approved	✓	✓	✓	✓	
Potassium bicarbonate	Potassium bicarbonate	10 g + 0.5 ml Silwet® L-77	Approved (commodity substance)	✓	✓	✓	✓	
Enzicur	Main ingredient is lactoperoxydase, an antimicrobial agent from milk	At a rate specified by the manufacturer	Not approved (currently under registration process in UK)	✓	✓			Only tested in 2008
Chitoplants®	Chitosan	0.5 g L^{-1}	Not approved (approved in Germany)	✓	✓	✓	✓	
Systhane™ 20EW	Myclobutanil	115 μl L^{-1}	Approved	✓	✓	✓	✓	
Nimrod™	Bupirimate	350 μl L^{-1}	Approved			✓	✓	Only tested in 2009
Milsana®	Extract of *Reynoutria sachalinensis and other ingredients*	12 ml L^{-1}	Not approved (but approved in several other countries)				✓	Only tested in 2009
Adjuvants								
Wetcit™	N/A	3 ml L^{-1}	Adjuvant	✓	✓			Only tested in 2008
Silwet® L-77	N/A	3 ml L^{-1}	Adjuvant	✓	✓	✓	✓	Only tested in 2009

*: ✓ indicates that the product was included in the specific test and does not necessarily indicate its physical mode of action.

The results from the product trial showed that 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, either as a protectant or curative application, none of them showed consistent effects against the disease. In contrast, several treatments had significantly reduced powdery mildew development in the repeated application trials, these included the alternative products potassium bicarbonate and Farm-Fos-44® and, to a lesser degree, Milsana® and Silwet™ L-77. Serenade® ASO did not show any significant controlling effect against rose powdery mildew in this trial

When plant growth was fastest (leaf emergence and expansion), powdery mildew was best controlled by standard fungicides, such as Systhane™ 20EW and Nimrod™. Thus, the emphasis of powdery mildew control should focus on correct timing of fungicides in the early stages, gradually integrating other approved alternative products such as food-grade potassium bicarbonate possibly at reduced rates and/or increased intervals depending on disease pressure on the basis of model predictions.

Financial benefits

Growers can benefit from the project results in the following ways (exact financial benefits are difficult to quantify because it depends on many other factors such as variety susceptibility, growing environment, management practices, products used):

1) Using approved alternative products for effective integrated control of rose powdery mildew with the view to reducing reliance on fungicides but at the same time maintain plant quality
2) Controlling mildew stringently when plants are very young and gradually integrating fungicides (possibly at reduced rate or increased interval) with approved alternative products such as food-grade potassium bicarbonate.
3) Using the prediction model to partly determine the dose and application timing. We need to conduct further trials at growers' sites to investigate the control need (threshold) in relation to model predictions.

Action points for growers

- Request a copy of the model and the full report (which contains advice on Installing and running the prediction software within Appendix 2) from the HDC.
- Trial the model to assist in the control of rose powdery mildew (initially on a small scale).
- Once you have more confidence in the model prediction, gradually make decisions of mildew control based on the model predictions
- When available, use the combined model (being developed under HNS 173), for powdery and down mildew management.

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, and does not always control the disease satisfactorily due to poor timing or choice of fungicides; it may accelerate the selection of mildew strains that are resistant to fungicides.

On susceptible plants, an appropriate strategy is to intervene with approved fungicides or alternative control agents, including natural products and biocontrol agents (BCA), only when treatment is justified. Such a 'supervised' disease control approach requires knowledge of when there is a risk of disease. This knowledge can be provided by computerised disease prediction systems. 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 (Berrie & Xu, 2003).

Much information on rose powdery mildew epidemiology is available from published studies; a considerable amount of qualitative information was published in 1970-80s (Price, 1970; Tammen, 1973; Leu & Kao, 1975; Frinking, 1977; Cobb *et al.*, 1978). While, more recently, a Defra-funded project at EMR investigated quantitative aspects of rose powdery mildew (Xu, 1999a; Xu, 1999b). 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 (Xu, 1999c), 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 that the right products can be selected at the right time to control mildew. Several alternative products, including the 'commodity substance' potassium bicarbonate, have been shown to be effective against powdery mildew in other crop species. However, only one study reported testing of other alternative products against rose powdery mildew (Pasini *et al.*, 1997). *Bacillus subtilis* (strain QST 713) is a biological bactericide (BCA) sold as 'Serenade® ASO' and has shown activity against many pathogens. Serenade® ASO is claimed to have 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 and currently has a Specific Off-Label Approval (SOLA) for use in ornamental plant production.

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 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 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. We may also 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 within 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 (1999a). 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.

Validating forecasting models

Initially, we were planning to monitor mildew development and conduct a supervised mildew control on a commercial site. However, having discussed this with consultants it was felt unlikely that any growers would be willing to leave rose plants unsprayed for powdery mildew. Instead, we conducted trials on repeated applications of alternative products (as described below in the section entitled 'Testing Alternative Products') and assessed mildew development on untreated plants in a polytunnel at EMR.

In 2009, we placed six plants in a polytunnel to monitor powdery mildew development over time where a data logger was also placed to record temperature and relative humidity. After several assessments, it became apparent that the tunnel environment during 2009 was just too ideal for mildew development to such an extent that virtually all emerging young leaves were covered with powdery mildew within a very short period of time. Thus, the data had no practical values for validating the model since it did not show much variation in disease development over time. Fortunately, we had historical data of powdery mildew epidemics for two years (1996 and 1997). The data collection protocols are briefly summarised here.

A plantation of 128 rose 'Renaissance' plants was established in a polythene tunnel at Efford during the growing seasons in 1996 and 1997. Half-hourly weather variables (temperature, relative humidity, wind speed and direction and leaf surface wetness) were recorded using an automatic Skye logger sited in the polythene tunnel. Vapour pressure deficit was calculated from temperature and relative humidity. Disease severity (percentage area with powdery mildew) was assessed regularly (at least weekly) on 64 selected plants of this cultivar. On each of the 64 plants, three stems were tagged and assessed for mildew over time. Mildew development was monitored for about 40 days in each year.

Testing alternative products

General experimental protocols. A number of products were tested for their protective, curative and anti-sporulant effects as well as for their efficacies when used in a repeated spray programme. Summary of products tested, together with approval status, is given in Table 1. In all experiments, an untreated control was also included.

Table 1 Summary of the products tested in 2008 and 2009 against rose powdery mildew

Trade name	Active ingredient	Application rate	Approval status	Protective	Curative	Anti-sporulant	Repeated	Notes
Serenade ASO®	*Bacillus subtilis* (strain QST 713)	10 ml L^{-1}	Approved	✓	✓	✓	✓	
Farm-Fos-44®	Potassium phosphite	10 ml L^{-1}	Not approved	✓	✓	✓	✓	
Potassium bicarbonate	Potassium bicarbonate	10 g + 0.5 ml Silwet® L-77	Approved (commodity substance)	✓	✓	✓	✓	
Enzicur	Main ingredient is lactoperoxydase, an antimicrobial agent from milk	At a rate specified by the manufacturer	Not approved (currently under registration process in UK)	✓	✓			Only tested in 2008
Chitoplants®	Chitosan	0.5 g L^{-1}	Not approved (approved in Germany)	✓	✓	✓	✓	
Systhane™ 20EW	Myclobutanil	115 μl L^{-1}	Approved	✓	✓	✓	✓	
Nimrod™	Bupirimate	350 μl L^{-1}	Approved			✓	✓	Only tested in 2009
Milsana®	Extract of *Reynoutria sachalinensis and other ingredients*	12 ml L^{-1}	Not approved (but approved in several other countries)				✓	Only tested in 2009
Adjuvants								
Wetcit™	N/A	3 ml L^{-1}	Adjuvant	✓	✓			Only tested in 2008
Silwet® L-77	N/A	3 ml L^{-1}	Adjuvant	✓	✓	✓	✓	Only tested in 2009

*: ✓ indicates that the product was included in the specific test and does not necessarily indicate its physical mode of action.

The choice of fungicides for this purpose was made after consultation with Dove Associates. The application rate is the full label-recommended rate. In 2008 it was not possible to include Milsana® (a plant resistance inducer, extracted from *Reynoutria sachalinensis*) because it was out of stock due to the shortage of its ingredients; but it was included in the repeated application trial in 2009 at a rate of 12 ml L^{-1}. Enzicur was only tested once in 2008 because its use-by-date was late July. In addition, an untreated (but inoculated) control was included. Since its effects were not significant, hence Enzicur was not tested in 2009.

Each product was applied to the plants (shoot/leaves) until run-off using a 500 ml hand-held sprayer. Two or three 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 (‘Zéphirine Drouhin’). However, powdery mildew did not develop quickly enough on cv. ‘Prima Ballerina’. All subsequent tests were thus done on ‘Zéphirine Drouhin’, except the trial on repeated applications in early 2009, which were done on both cultivars. 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. ‘Zéphirine Drouhin’ were maintained in a glasshouse compartment (set to 20°C), 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. Mildew was assessed 8-14 days later after the commencing of inoculation. This experiment was carried out twice.

1) Products applied on 13 August 2008 and inoculated on 15 August 2008
2) Products applied on 19 February 2009 and inoculated on 23 February 2009 when newly potted plants had start to produce new shoots in the glasshouse (at the time of treatment, there were 3-4 leaves on a shoot)

Curative activity, i.e. the ability to kill the young developing, but symptomless, colonies thereby preventing them from forming visible lesions. Healthy plants (‘Zéphirine 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 8-14 days later. This experiment was repeated three times.

1) Inoculated on 7 July 2008 and products applied on 9 July 2008
2) Inoculated on 1 September 2008 and products applied on 4 September 2008

3) Inoculated on 18 February 2009 and products applied on 19 February 2009, when newly potted plants had start to produce new shoots in glasshouse (at the time of treatment, there were 3-4 leaves on a shoot)

Antisporulant activity, i.e. the ability to reduce spore production. Plants of 'Zéphirine Drouhin' were first inoculated with mildew conidia and left for mildew to develop. About 14 days later, two plants were randomly allocated per 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. Two experiments were conducted: one during October-November 2008 and the other in May 2009. In 2009, only one plant was available for each treatment, each with three lesions randomly sampled for assessment.

Repeated applications. An additional experiment was conducted to evaluate the effect of repeated applications of each individual product on the development of powdery mildew. Five products were used for this trial: food-grade potassium bicarbonate, potassium phosphite (Farm-Fos-44® – 10 ml/L), Chitoplants®, Systhane™ 20EW and Serenade® ASO. In addition, an untreated control was included. Two plants of 'Zéphirine Drouhin' were randomly allocated to each treatment; this experiment was conducted in a glasshouse compartment. The first application was made on 31 October 2008, the second on 12 November, the third on 27 November 2008 and the final one on 8 December 2008. Powdery mildew on leaflets was assessed twice: first on 26 November 2008 and the second one on 12 January 2009. A repeat trial one was initiated in March 2009 on both cultivars: the first spray on 2 March 2009, the second on 12 March 2009, the third on 23 March 2009, and the fourth on 3 April 2009. Mildew was assessed twice, on 20 March and 23 April 2009.

Data analysis

Total number of leaflets inoculated and infected per shoot as well as average leaflet area infected per shoot was analysed. Generalised non-linear mixed modelling was used to analyse 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) were used to compare treatments. Finally, a linear mixed model approach was used to analyse data over several experiments where several common treatments were present.

Results

Model development

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

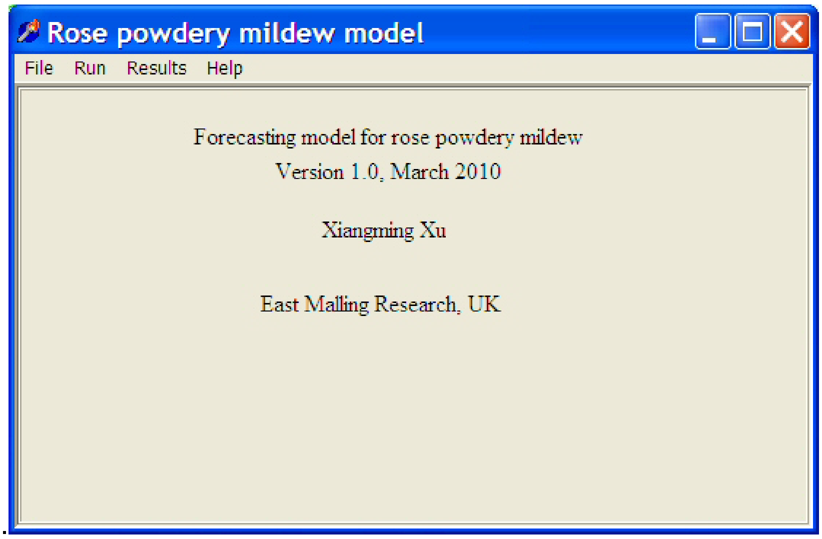

Figure 1. A screen shot of the main window of the rose mildew prediction model

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.

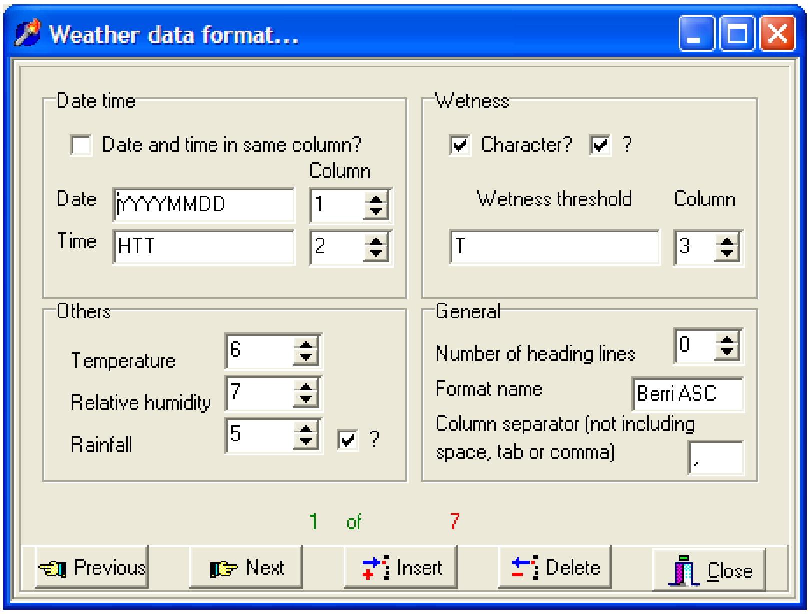

Figure 2. A screen shot of the window used for defining weather data format

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 (currently being developed under a separated HDC-funded project, HNS 173). Thus, in the future, users can run models for rose downy and powdery mildews either separately or simultaneously.

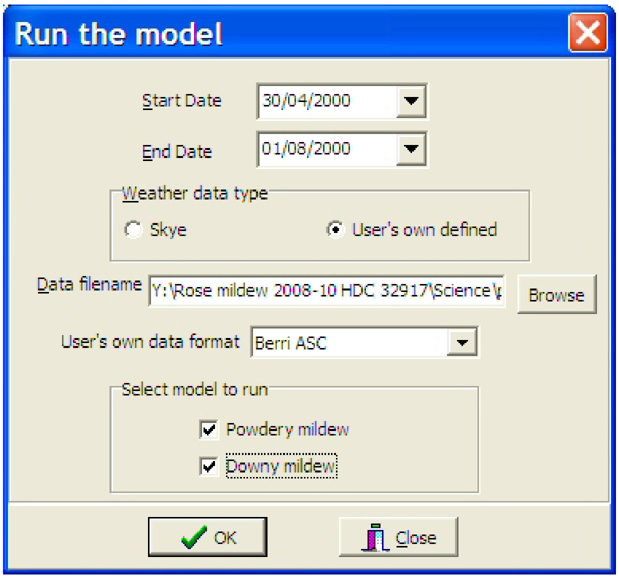

Figure 3. A screen shot of the window used for initiating the predictive model

The model will display predicted mildew development (Figure 4).

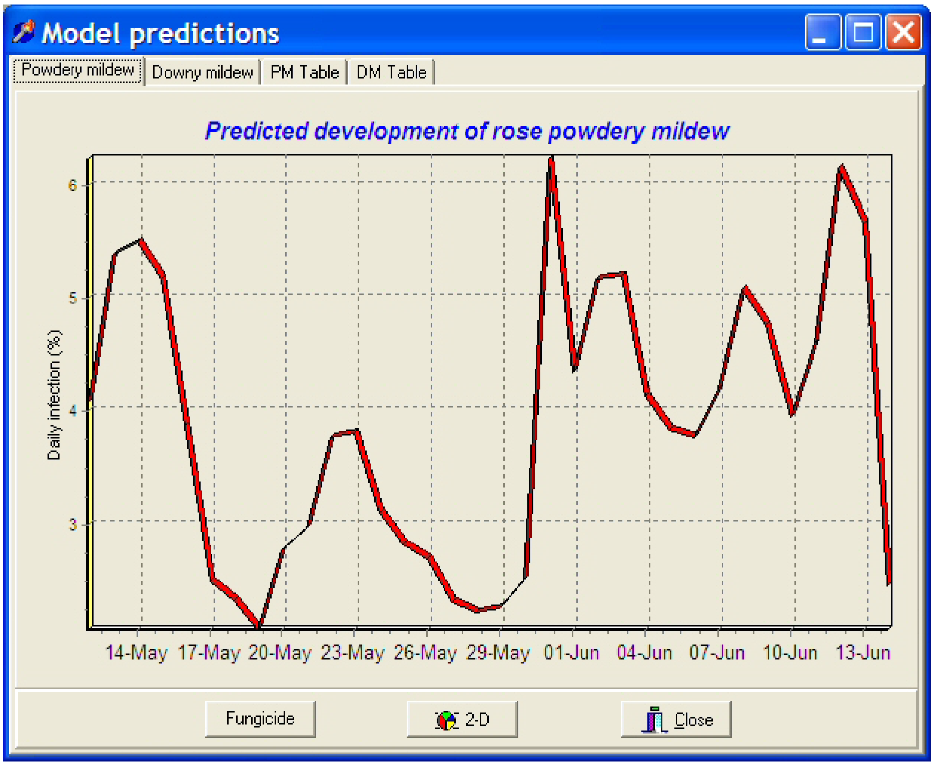

Figure 4. A screen shot of model predictions for illustration purpose only

The program also provides a context-sensitive help system. Thus whenever users press F1 key, a help screen will be displayed with relevant information. For example, Figure 5 is a screen shot of the help displayed when users press the F1 key when viewing the forecasting graph (Figure 4).

This computer software package is available to HDC members via the HDC website. Please note that a rose downy mildew model is being developed (HNS 173) and will be incorporated into this software package. The current version of the rose downy mildew model as implemented in this package should not be used as it is incomplete and needs further development.

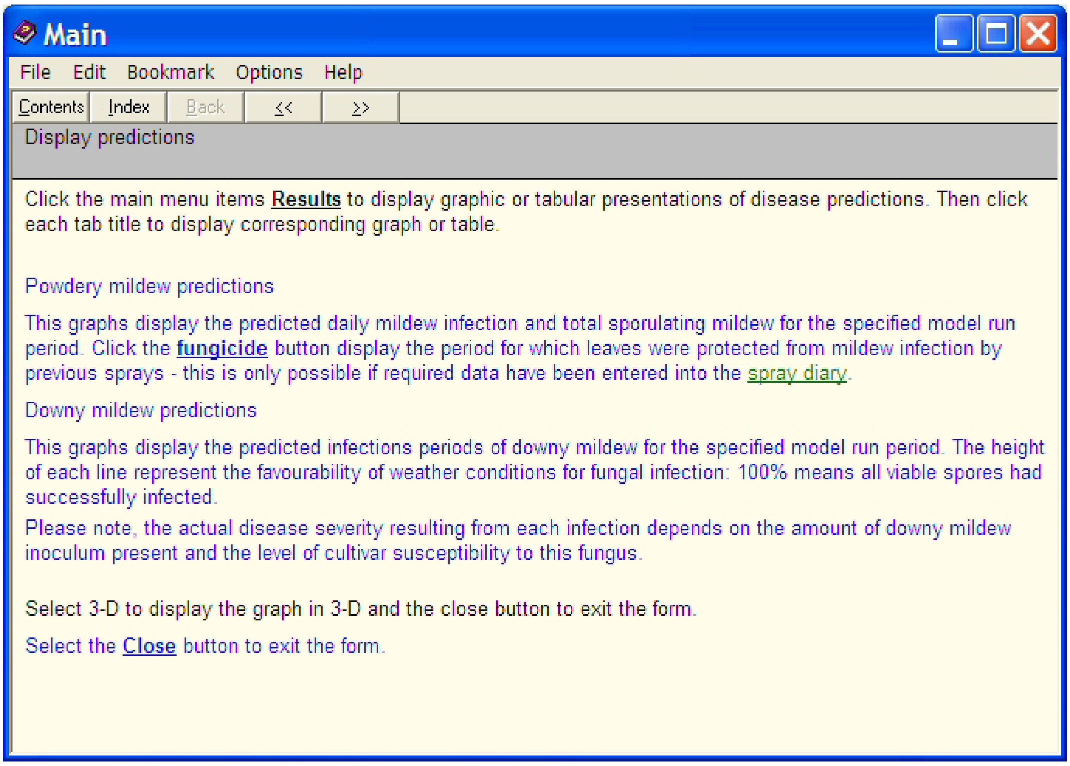

Figure 5. A screen shot of a context-sensitive help window

Model validation

Mildew development was rapid in both years. In 1996, plants were potted up late in the season (late July). In both years, the predictions were generally well matched with the observed epidemic development (Figure 6). From these data, it appears that a threshold of predicted daily infection of around 5% could be used as a starting point for further evaluation of the model in practice.

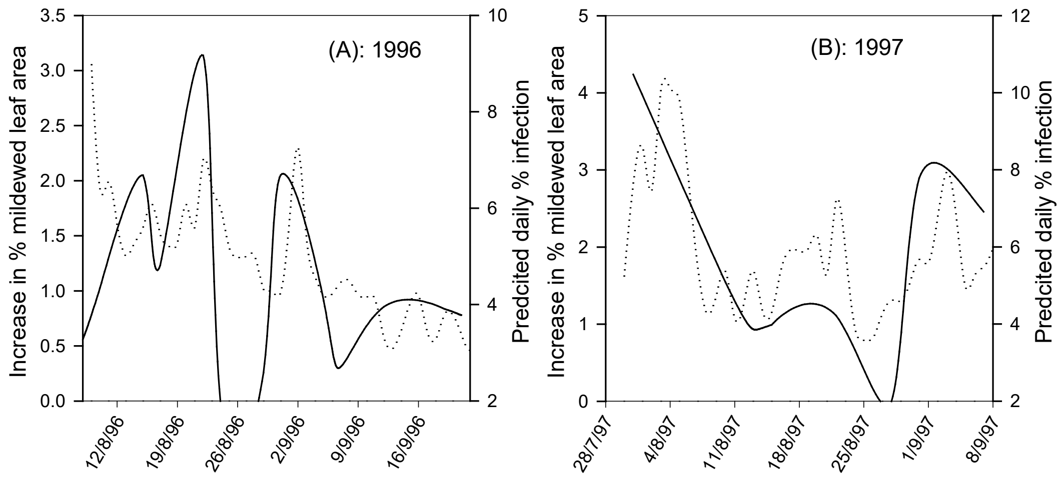

Figure 6. Model predicted percentage of daily mildew infection (dotted line) and the observed (solid line) in the observed percentage of mildew on the top five leaves between successive assessment dates over two years (1996 & 1997)

Testing alternative products

Protectant

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

In the second experiment, powdery mildew developed on all inoculated leaflets in all treatments. Treatment differences in mildew severity nearly reached the 5% significance ($P = 0.07$). Of all the treatments, only food-grade potassium bicarbonate (10%) led to significant less mildew than the control (21%); mildew severity for all other treatments ranged from 16% to 22%. However, when the two experiments were analysed together, differences among treatments were still statistically non-significant.

Curative

In the first experiment, there were no significant differences in the incidence of infected leaflets (Figure 7A) and the percentage of mildewed leaf area among the treatment. The incidence of leaflets infected ranged from 3% (Systhane™ 20EW and Chitoplant) to 14% (Serenade® ASO), with 10% for the untreated control. In the second experiment, again for the overall treatment, there were no significant differences in the incidence of infected leaflets (Figure 7A) and the percentage of mildewed leaflet area among the treatments. However, when comparing individual treatments, Systhane™ 20EW (1%) and Farm-Fos-44® (3%) had significantly ($P < 0.05$) lower incidences than the control (29%). Similarly there were no significant differences in percentage of leaf area mildewed among all treatments in both experiments (Figure 7B).

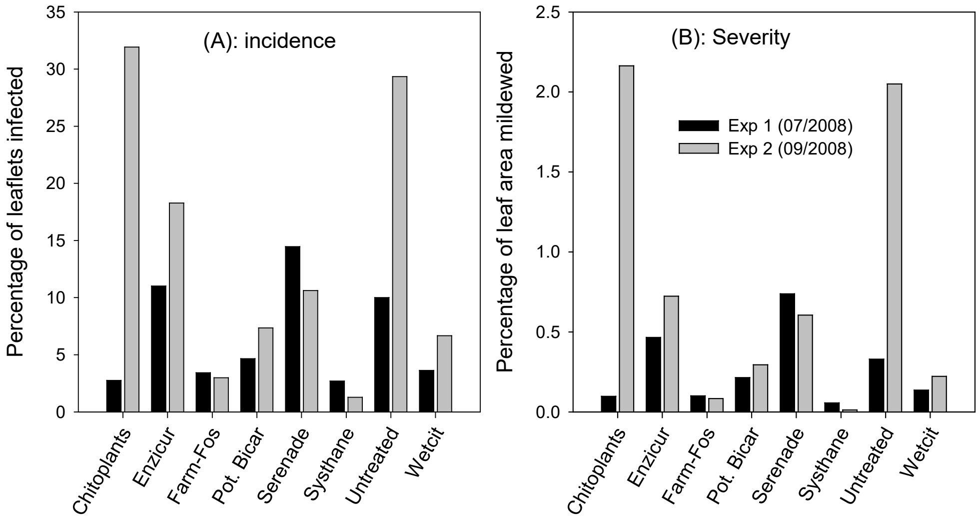

Figure 7. Incidence of rose leaflets infected with powdery mildew and its severity (affected area) in the first two curative experiments

In the third experiment, all leaflets developed powdery mildew symptoms in all treatments. However, there were significant ($P < 0.05$) differences in the disease severity among treatments. Disease severity was significantly lower on plants treated with food-grade potassium bicarbonate (13%), Systhane™ 20EW (10%) and Silwet® L-77 (17%) than those untreated plants (38%). All other treatments (Chitoplants - 33%, Farm-Fos-44® – 21% and Serenade® ASO – 29%) did not differ significantly from the control.

Data for common treatments in all three experiments were analysed together via a generalised non-linear mixed model. This analysis showed that the six treatments differed significantly ($P < 0.001$) from each other. Treatments with food-grade potassium bicarbonate, Farm-Fos-44® and Systhane™ 20EW all significantly reduced mildew severities compared to the control; these three products did not significantly differ among themselves.

Antisporulant

There were significant differences ($P < 0.05$) among treatments in the first trial (Figure 8A). Of the five treatments, only food-grade potassium bicarbonate led to significantly more deformed spores (58%) than the untreated control (19%). For the remaining four treatments, percentages of deformed conidia were all greater than the untreated, but the differences were not statistically significant. In the second trial, again the percentage of deformed conidia was greatest on lesions treated with food-grade potassium bicarbonate (Figure 8B); but treatment differences were not statistically different.

When the two experiments were analysed together for their common treatments, there were significant ($P < 0.05$) treatment effects. The food-grade potassium bicarbonate treatment led to the greatest percentage of deformed spores (61%) than all other treatments (including the untreated), which were not statistically different from each other (ranging from 26% to 42%).

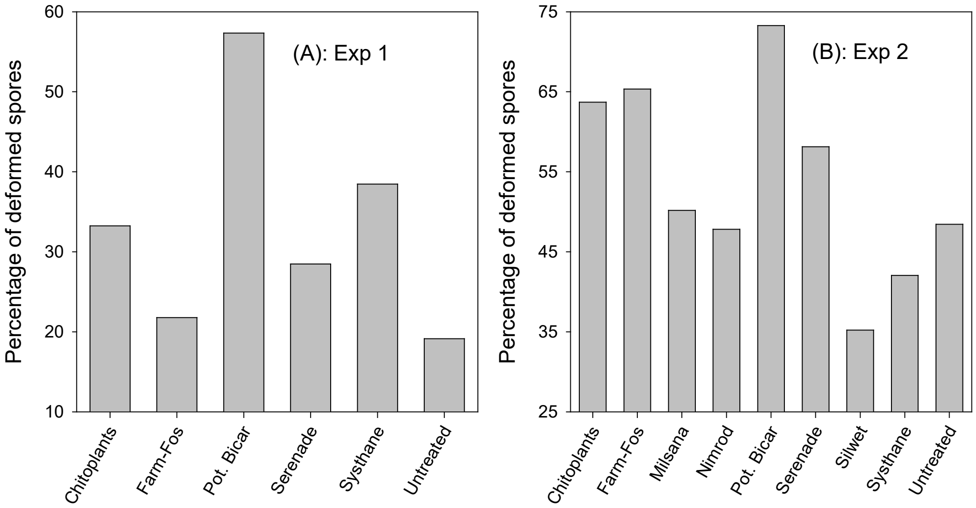

Figure 8. Percentage of deformed conidia in lesions subjected to different treatments

Repeated applications

For the first assessment, after two applications in the first trial, 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 food-grade potassium bicarbonate significantly reduced the incidence of leaflet infection (Figure 9); about 66% of leaflets treated with food-grade potassium bicarbonate were infected, compared to nearly 97% for the untreated control. All treatments, except the Chitoplants, significantly reduced the percentage of leaf area infected compared to the control; among them food-grade potassium bicarbonate and Farm-Fos-44® had significantly less diseased area than Systhane™ 20EW (Figure 9).

In the second assessment (three weeks after the last applications), there were significant differences in the incidence of leaflets infected ($P < 0.05$) and in the percentage of leaf area infected ($P < 0.01$) among the treatments. Both Farm-Fos-44® and food-grade potassium bicarbonate led to significantly less incidence of infected leaflets (41% and 26%) and infected leaf area (< 2%) than the other four treatments (Figure 9).

All leaflets were infected in the second trial. Two out of the three plants of 'Zéphirine Drouhin' allocated to the Farm-Fos-44® treatment died soon after the first treatment. In the first assessment, there were significant ($P < 0.001$) differences in the leaf area infected among the treatments, which was consistent between the two cultivars. Two fungicides (Nimrod™ and Systhane™ 20EW) led to the lowest disease severity (ca. 10%, compared to the control > 45%). Next best treatments are Farm-Fos-44®, Milsana® and food-grade potassium bicarbonate. SIlwet® L-77 also led to significant reductions in disease severity. The level of control achieved by Chitoplants and Serenade® ASO was very small (Figure 10).

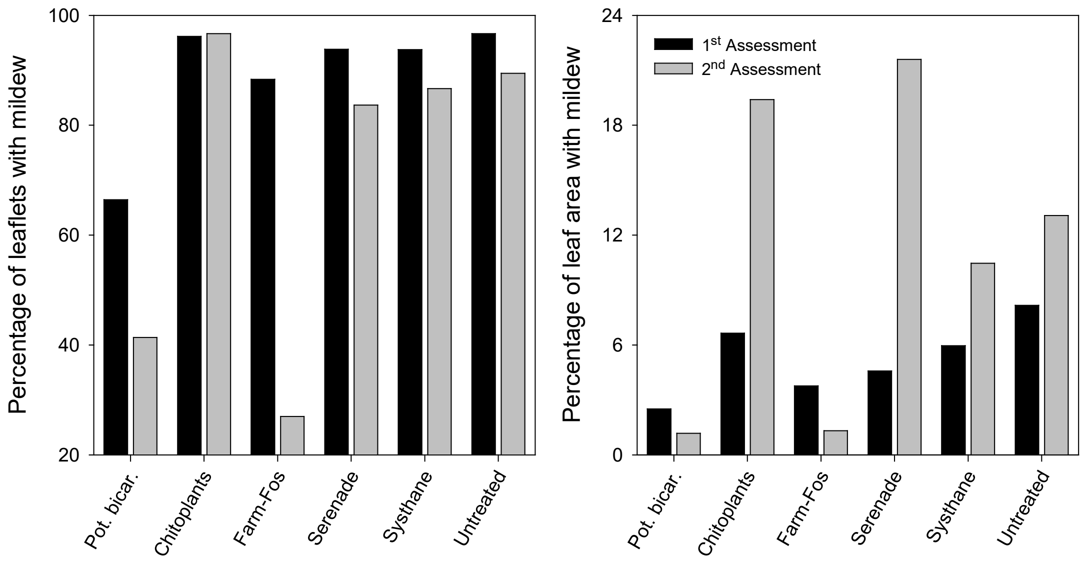

Figure 9. Percentage of leaflets infected and leaf area infected with powdery mildew in the first repeated application trial; the first assessment was made after two applications of each product and the second assessment after four applications

By the time of the second assessment, most buds/shoots inoculated initially on plants of 'Prima Ballerina' were already dead. On 'Zéphirine Drouhin', there were significant ($P < 0.01$) differences in infected leaf area among the treatments (Figure 10). The best treatments include Milsana®, food-grade potassium bicarbonate, Nimrod™, Silwet® L-77 and Systhane™ 20EW (≤ 20%). There were no significant differences between Chitoplants (43%) and the control (50%).

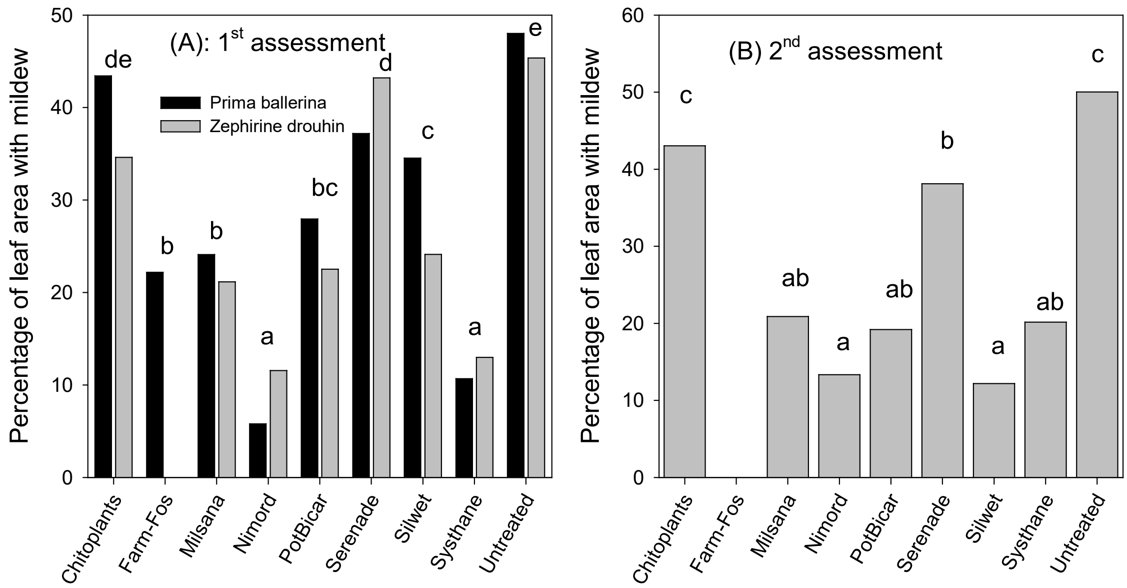

Figure 10. Percentage of leaf area infected with powdery mildew in the second repeated application trial; the first assessment was made after two applications of each product and the second assessment after four applications. Two out the three

plants of 'Zéphirine Drouhin' allocated to the Farm-Fos-44® treatment died soon after the first treatment. Treatments with the same letter (above the bar) are not statistically different from each other

Discussion

Model development

We have developed a prediction of a computer-based model for rose powdery mildew. The predictions of daily mildew infection risks correctly identified the overall trend in mildew development in two unsprayed tunnels. This suggests that the model captured the major influences of key environmental factors on powdery mildew development on rose. Of course, the risk is conditioned on the degree of cultivar susceptibility, which has to be taken into consideration in any practical disease management. Because of the nature of rapid increase of rose mildew epidemics, it is ill-advised to rely only on models for disease management particularly in areas with severe disease pressure. However, the model is intended to assist growers in adjusting the dose (and possibly products) during periods of rose growth where shoot growth is not that rapid or weather conditions are not favourable for mildew development. It should be noted that using model predictions for disease management is a process rather than an instantaneous event. It should also be noted that this model represents a first version which will be further developed in consultation with rose growers and will also enable supervised management of downy mildew in addition to powdery mildew. The further development will occur under HDC project HNS 173.

Testing alternative products

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, either as a protectant or curative application, none of them showed consistent effects against the disease. In contrast, several treatments with alternative products had significantly reduced powdery mildew development in the repeated application trials, particularly potassium bicarbonate and Farm-Fos-44® and, to a lesser degree, Milsana® and Silwet™ L-77. This difference may be attributable to two reasons. First, the infection (inoculum) pressure in the single-application studies is expected 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 artifical inoculum pressure may have overwhelmed these products. Second, the effect of each product may be relatively small and hence it may be 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.

Interestingly, the efficacy achieved by fungicides (Systhane™ 20EW and Nimrod™) was much higher in the second repeated trial than those of alternative products in the first. This difference was consistent on two cultivars with totally differently growth characteristics. Two possible explanations are likely for this difference. In the second trial, leaf emergence was at a much faster rate (at most there were only five leaves on a shoot) than in the first trial where at the time of inoculation there were already at least 20 leaves per shoot. This faster leaf emerging rate in the second trial may present much more susceptible tissue that needs to be protected between two sprays. Second, the inoculum pressure was higher in the second trial as there were many plants with heavy sporulating lesions on young leaves at the time of the trial. Furthermore, because of the sparse canopy in the second trial, more conidia may have landed on young leaves. Under this high disease pressure, fungicides may perform better than alternative products.

Only potassium bicarbonate showed significantly reduced conidia viability, which was consistent with the observation that it tends to dry leaf surface to cause loss of conidia viability. During disease assessment, it was clear that colonies on leaves treated with potassium bicarbonate did not have the typical fluffy presence of mildew lesions compared to other treatments. Thus, disease control by potassium bicarbonate may be even greater if these plants were relatively isolated from external inoculum sources. Of all the alternative products tested, the two best were potassium bicarbonate and Farm-Fos-44®. The effect of potassium bicarbonate on powdery mildew is well demonstrated in many other cases (Leeson & Crisp, 2004; Mitchell & Walters, 2004; Matheron & Porchas, 2007). However, its effect might be slightly exaggerated by the fact that it was applied together with the adjuvant Silwet L-77, (as recommended commercially) which when applied on its own showed the ability to reduce mildew development in the present study. The inhibitory effect of Silwet L-77 on powdery mildew was also observed on muskmelon (Matheron & Porchas, 2007).

Serenade ASO is currently one of only a few biocontrol products reported to have some control on powdery mildew of several other species (Crisp *et al.*, 2006; Romero *et al.*, 2007; Gilardi *et al.*, 2008; Pertot *et al.*, 2008). Serenade ASO did not result in any significant effects on rose powdery mildew in this trial

As reported for other species (Daayf *et al.*, 2000; Fofana *et al.*, 2002; Petsikos-Panayotarou *et al.*, 2002; Carlen *et al.*, 2004; Konstantinidou-Doltsinis *et al.*, 2006; Randoux *et al.*, 2006; Konstantinidou-Doltsinis *et al.*, 2007; Bokshi *et al.*, 2008; Su *et al.*, 2009), Milsana® (a plant resistance inducer, extracted from *Reynoutria sachalinensis*) also significantly reduced powdery mildew development on rose when applied repeatedly.

Conclusions

Model development

- We have developed a predictive model for rose powdery mildew; validation results suggested that the model accurately predicted overall trends of epidemic development
- 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
- A first version of the model is available from the HDC. Rose growers are encouraged to critically assess the model and recommend ways to improve it.

Testing alternative products

- Controlled trials demonstrated that potassium bicarbonate and Farm-Fos-44® (approved for use as a liquid fertiliser) are the most efficacious alternative products against rose powdery mildew although their effects on very young plants were much less than those achieved by conventional fungicides
- Milsana® and Silwet® L-77 also had significant inhibitory effects on rose powdery mildew but to a lesser degree than food-grade potassium bicarbonate and Farm-Fos-44®
- Present results suggest that relying purely on alternative products, particularly in early seasons, is not advisable. However, approved products (see Table 1) could be integrated with conventional fungicides under high disease pressure, which can be predicted by the forecasting model

Technology transfer

- We presented a talk on the project progress to British Rose Grower Association on 4 December 2008
- We held in-depth discussions on the project results with the grower project co-ordinator (February 2009 & March 2010)
- It was originally planned to have demonstration workshop coincided with BRGA annual meeting in December 2009. Unfortunately, this meeting did not take place in 2009 for various reasons. It has been decided to hold a workshop in 2010-2011 to demonstrate both powdery and downy mildew models (the latter is currently under development with HDC funding – HNS 173).

- We have developed a questionnaire (Appendix 1.) on rose diseases in general; the questionnaire was sent out in early 2010 and we currently are collecting responses. So far we had 10 responses and we shall report the summary of these replies in the downy mildew project (HNS 173) later this year.
- The powdery mildew prediction software is currently being piloted by rose growers, but will be available from the HDC. Currently, downy mildew model is being developed (HNS 173) and to be incorporated into this software package (the current version of downy mildew in the package should not be used since it is incomplete).

References

Berrie AM, Xu X-M, 2003. Managing apple scab and powdery mildew using Adem™. *International Journal of Pest Management* **49**, 243-250.

Bokshi AI, Jobling J, McConchie R, 2008. A single application of Milsana (R) followed by Bion (R) assists in the control of powdery mildew in cucumber and helps overcome yield losses. *Journal of Horticultural Science & Biotechnology* **83**, 701-706.

Carlen C, Faby R, Karjalainen R, Pommier JJ, Steffek R, 2004. Control of air borne diseases in strawberries with natural and synthetic elicitors. 237-240 in: Simpson DW, ed. *Proceedings of the Euro Berry Symposium - Cost 836 Final Worskhop.*

Cobb GS, Hanan JJ, Baker R, 1978. Environmental factors affecting rose powdery mildew in greenhouses. *HortScience* **13**, 464-466.

Crisp P, Wicks TJ, Lorimer M, Scott ES, 2006. An evaluation of biological and abiotic controls for grapevine powdery mildew. 1. Greenhouse studies. *Australian Journal of Grape and Wine Research* **12**, 192-202.

Daayf F, Ongena M, Boulanger R, El Hadrami I, Belanger RR, 2000. Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew-infected plants with extracts of *Reynoutria sachalinensis*. *Journal of Chemical Ecology* **26**, 1579-1593.

Fofana B, McNally DJ, Labbe C, Boulanger R, Benhamou N, Seguin A, Belanger RR, 2002. Milsana-induced resistance in powdery mildew-infected cucumber plants correlates with the induction of chalcone synthase and chalcone isomerase. *Physiological and Molecular Plant Pathology* **61**, 121-132.

Frinking HD, 1977. Research on wind dispersion of rose-mildew spores (*Sphaerotheca pannosa*) in field, glasshouse and climate room. *Grana* **16**, 155-158.

Gilardi G, Manker DC, Garibaldi A, Gullino ML, 2008. Efficacy of the biocontrol agents *Bacillus subtilis* and *Ampelomyces quisqualis* applied in combination with fungicides against powdery mildew of zucchini. *Journal of Plant Diseases and Protection* **115**, 208-213.

Konstantinidou-Doltsinis S, Markellou E, Kasselaki AM, Fanouraki MN, Koumaki CM, Schmitt A, Liopa-Tsakalidis A, Malathrakis NE, 2006. Efficacy of Milsana (R), a formulated plant extract from *Reynoutria sachalinensis*, against powdery mildew of tomato (*Leveillula taurica*). *Biocontrol* **51**, 375-392.

Konstantinidou-Doltsinis S, Markellou E, Kasselaki AM, Siranidou E, Kalamarakis A, Tzembelikou K, Schmitt A, Koumakis C, Malathrakis N, 2007. Control of powdery mildew of grape in Greece using Sporodex (R) L and Milsana (R). *Journal of Plant Diseases and Protection* **114**, 256-262.

Leeson GR, Crisp P, 2004. Food additive as an organic fungicide for powdery mildew - Ecocarb (R). 205-208 in: Nichols MA, ed. *Proceedings of the South Pacific Soilless Culture Conference.*

Leu LS, Kao CW, 1975. Conidial liberation and germination of the rose powdery mildew fungus, *Sphaerotheca pannosa*. *Plant Protection Bulletin, Taiwan* **17**, 311-318.

Matheron ME, Porchas M, 2007. Comparative performance and preservation of chemical management tools for powdery mildew on muskmelon. 357-361 in: McConchie R and Rogers G, eds. *Proceedings of the IIIrd International Symposium on Cucurbits.*

Mitchell AF, Walters DR, 2004. Potassium phosphate induces systemic protection in barley to powdery mildew infection. *Pest Management Science* **60**, 126-134.

Pasini C, Daquila F, Curir P, Gullino ML, 1997. Effectiveness of antifungal compounds against rose powdery mildew (*Sphaerotheca pannosa* var. *rosae*) in glasshouses. *Crop Protection* **16**, 251-256.

Pertot I, Zasso R, Amsalem L, Baldessari M, Angeli G, Elad Y, 2008. Integrating biocontrol agents in strawberry powdery mildew control strategies in high tunnel growing systems. *Crop Protection* **27**, 622-631.

Petsikos-Panayotarou N, Schmitt A, Markellou E, Kalamarakis AE, Tzempelikou K, Siranidou E, Konstantinidou-Doltsinis S, 2002. Management of cucumber powdery mildew by new formulations of *Reynoutria sachalinensis* (F. Schmidt) Nakai extract. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* **109**, 478-490.

Price TV, 1970. Epidemiology and control of powdery mildew (*Sphaerotheca pannosa*) on roses. *Annals of Applied Biology* **65**, 231-248.

Randoux B, Renard D, Nowak E, Sanssene J, Courtois J, Durand R, Reignault P, 2006. Inhibition of *Blumeria graminis* f. sp *tritici* germination and partial enhancement of wheat defenses by Milsana. *Phytopathology* **96**, 1278-1286.

Romero D, de Vicente A, Zeriouh H, Cazorla FM, Fernandez-Ortuno D, Tores JA, Perez-Garcia A, 2007. Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon. *Plant Pathology* **56**, 976-986.

Su H, Morgan C, Campbell B, Huang H, Hernandez J, Koivunen ME, Marrone PG, 2009. Efficacy of new formulations of Milsana (R), conventional and organic Regalia (TM), in controlling cucumber powdery mildew (*Sphaerotheca fuliginea*). *Phytopathology* **99**, S125-S125.

Tammen JF, 1973. Rose powdery mildew studied for epidemics. *Science in Agriculture* **20**, 10.

Xu X-M, 1999a. Effects of temperature on the length of the incubation period of rose powdery mildew (*Sphaerotheca pannosa* var. *rosae*). *European Journal of Plant Pathology* **105**, 13-21.

Xu X-M, 1999b. Effects of temperature on the latent period of the rose powdery mildew pathogen, *Sphaerotheca pannosa*. *Plant Pathology* **48**, 662-667.

Xu X-M, 1999c. Modelling and forecasting epidemics of apple powdery mildew (*Podosphaera leucotricha*). *Plant Pathology* **48**, 462-471.

Appendix 1. **Questionnaire**

How successful are the controls of rose diseases on your nursery?

1 Identification

Nursery name and location: ______________________

2 Do you grow roses

Under protection (glasshouse/tunnel) ☐ **Open field** ☐

3 If you grow roses in open field, please briefly describe the climatic characteristics (e.g. temperature, wind, humidity) of the site

....

....

....

....

4 Do you have weather logging instruments

Under protection (glasshouse/tunnel) ☐ **Open field** ☐

5 If you have weather logging instruments, what is their primary usage?

....

....

....

....

6 If you do not have weather logging instruments, do you plan to purchase one in the near future?

Yes ☐ **or No** ☐

7 What do you think are the major disease problems on roses?

Powdery mildew ☐
Downy mildew ☐
Black spot ☐
Rust ☐

Other (please specify)

....

....

....

....

8 Please list the cultivars that you believe in your experience are most susceptible to

Powdery mildew

....

....

....

Downy mildew

....

....

....

Blackspot

....

....

9 At what stage do you find powdery mildew becomes problematic on your nursery?

Primary mildew ☐ Secondary mildew (extension shoots) ☐

if at the primary mildew stage, is the primary mildew on

Blossom trusses ☐ Vegetative mildew ☐

10 What are your management strategies?

Powdery mildew

Routine spray ☐

Using other information (e.g. predictions) to adjust routine spray ☐

Downy mildew

Routine spray ☐

Using other information (e.g. predictions) to adjust routine spray ☐

Blackspot

Routine spray ☐

Using other information (e.g. predictions) to adjust routine spray ☐

11 If you use prediction systems, please list which ones:

....

....

....

....

12 Typical annual spray programme

Start:

End:

Product name	Powdery mildew	Downy mildew	Blackspot	Others	Number of applications p.a.

13 Are you willing to use predictive systems to assist in decision making?

Yes ☐ **No** ☐

14 If no, please give your main reasons:

....

....

....

15 If there is a training session on using predictive systems, would you prepared to attend?

Yes ☐ **or No** ☐

16 Are there any other factors in influencing your pest and disease control on roses, such as other HNS species present?

....

....

....

....

....

....

....

Thank you for completing this questionnaire. Please return this form to Dr. Xiangming Xu, East Malling Research, East Malling, West Malling, Kent, ME19 6BJ by the end of February 2010.

Appendix 2
Installing and running the prediction software

Installation

1) First create a directory in your computer system to store program files

2) Copy all the files from the CD or from the website to the newly created directory: there should be six files: rosemildew.exe, rosemodel.cfg, rosemodel.hlp, installation.txt, help.txt and rh_temp.txt.

Running the program

First you need to define the weather data formats are defined (only needed to be done once); the program comes with several weather data format already defined. Once this is done, you can run the model

1) Now you can run the program by double clicking 'rosemildew.exe' file

2) A weather data file (rh_temp.txt) is included for test runs - this file was from USB502 temperature and humidity duo sensors

3) Select the Run Model from the main menu item 'RUN'

4) Specifying the start date and end date for the weather data set. For the test data specify the start date as 01/05/2008 and end date as 30/06/2008.

5) Use the 'Browse' button to select the data filename. For test runs select 'rh_temp.txt' as the input weather data file

6) Select data format as 'Temp and RH only' from the drop down menu (by clicking the down triangle symbol)

7) Check the 'Powdery mildew'

8) Now click OK to run the model.

9) That's it - now you can view model predictions by clicking Results in the main menu.

[Note: further guidance on how to interpret the results will be developed under HNS 173]