Project title:	Outdoor celery: development of integrated strategies for the management of Septoria leaf spot (*Septoria apiicola*) and other diseases
Project number:	FV 237
Project leader:	Dr T M O'Neill, ADAS Arthur Rickwood
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Key worker:	Dr K R Green, ADAS Arthur Rickwood
Location of project:	ADAS Arthur Rickwood Mepal, Ely Cambs. CB6 2BA
Project co-ordinator:	Mr D Norman
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Key words:	Celery, leaf spot, *Septoria apiicola*, leaf wetness duration, temperature, fungicides, Bravo 500 (chlorothalonil). Cuprokylt (copper oxychloride), Croptex Fungex (copper ammonium carbonate), Amistar (azoxystrobin), Folicur (tebuconazole), Plover (difenoconazole), Alto 240EC (cyproconazole), BAS 516F

The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and 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.

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PRACTICAL SECTION FOR GROWERS

Commercial benefits of the project

As a result of the first year's work on this project, growers will be better able to select individual fungicide treatments and to devise effective programmes for control of celery leaf spot, through an improved knowledge of the protectant and eradicant activities of fungicides approved for use on the crop.

Background and objectives

Septoria leaf spot (*S. apiicola*) of celery, also known as 'late blight', is the most destructive disease of field-grown crops. Initially seen as small brown spots on leaves, the disease can progress rapidly to cause extensive defoliation and render the whole crop unmarketable if left unchecked. Under optimum conditions, the life-cycle for the fungus is as short as 10 days. In 1999, the disease was epidemic in East Anglia, the major production area in the UK, with losses estimated at well in excess of £100,000 in crops in Cambridgeshire & Norfolk. In 2000 and 2001, the disease did not occur until late in the season and was primarily a problem on crops grown to organic standards.

A seminar to review the disease and identify ways to reduce future losses was held in Cambridgeshire on 16 December 1999. This involved representatives from all involved in celery production, from seed producer, through propagators to growers and consultants. Principal agreed outcomes of this meeting were:

(i) to seek improvements in seed health by reviewing seed production in Italy (infected seeds are the usual original source of the disease when outbreaks occur)

(ii) to develop a research proposal to consider alternative seed treatments

(iii) to develop a research proposal to optimise fungicide use as a component of integrated management of the disease in field crops.

This project is the result of a successful proposal developed to address item (iii). The aim was to complement efforts at improving seed health and alternative seed treatments by optimising fungicide use as a component of integrated management of the disease in field crops. Fungicide treatments are of particular importance because although growers may be aware of cultural practices that can reduce the risk of Septoria infection and spread, these measures alone would be insufficient to control the disease and are not always practical to implement.

Septoria leaf spot can be effectively controlled through frequent fungicide applications but sprays may need to be applied at 10-day intervals for the duration of the growing season to ensure a marketable crop (up to 14 sprays per crop). Even were this acceptable as a practice, it was not possible in the UK at the beginning of this project, because of the recent loss of Bavistin DF (carbendazim) for use on field celery, and a restriction on the number of Bravo 500 (chlorothalonil) sprays to a maximum of three. Cuprokylt (copper oxychloride) and Croptex Fungex (cupric

ammonium carbonate) are still permitted but their effectiveness is probably limited and, with regular use, they leave a visible blue deposit on plants.

There are two potential ways in which to seek effective control of Septoria using a limited number of spray treatments. Firstly, to evaluate newer fungicides representative of different chemical groups, including products with known curative activity and shown to give good control of Septoria in other crops (e.g. winter wheat). Secondly, to target sprays when infection risk is high or predicted to be high. Leaf wetness duration and temperature are strongly influential in the development of celery leaf spot and are obvious parameters to consider to aid spray decisions.

Although the work focuses on the control of celery leaf spot disease caused by *S. apiicola*, experiments also incorporate monitoring the effects of treatments on other important celery diseases such as *Sclerotinia* and *Rhizoctonia*.

The commercial objective of the work is to develop an integrated strategy for improved control of leaf spot in field crops of celery by: (1) evaluating newer fungicides representative of different chemical groups; (2) spraying only when conditions are conducive to infection; (3) highlighting cultural practices that minimise the risk of disease spread.

Summary of results and conclusions

Conditions favouring infection

Experiments on celery plants in controlled environment (CE) cabinets were successfully used to quantify the relationship between infection by *S. apiicola* and leaf wetness duration at different temperatures. For temperatures ≥ 20^0 C and leaf wetness durations ≥ 24 hours, symptom development was generally rapid, with leaf lesions evident in as few as 10 days after inoculation and present on all plants after 15 days. Although infection occurred at most other combinations tested (in the range 5-30^0 C and 1-96 h leaf wetness), first symptoms were rarely seen before 15 days and the disease incidence at 28 days was often less than 100%. There was a trend for disease severity to increase with both temperature (5 to 25^0 C) and leaf wetness duration (1 to 96 h). At temperatures less than 10^0 C, considerable periods of leaf wetness duration (> 72 h) were required before disease severity exceeded 5%. Slight infection was recorded at low temperatures (5^0 C) and short wetness durations (1 h), which has not been reported previously. The wetness duration and temperature combinations that resulted in maximum disease severity (i.e. high risk conditions) are summarised as 2-dimensional contour plots and 3-dimensional response surfaces (Appendix 2).

Fungicide activity

Fungicides effective when applied prior to infection have a 'protectant' mode of action. 'Curative' fungicides are effective when applied after spore infection but before symptom development, while 'eradicant' fungicides can delay disease development when applied after symptoms are visible. The relative protectant and curative activity of eight fungicides on celery plants, cv. Celebrity was determined in a glasshouse trial. Fungicides (Bravo 500, Cuprokylt, Croptex Fungex, Amistar, Folicur, Plover, Alto 240EC and BAS 516F) applied once, 10 days prior to inoculation, all significantly reduced disease severity. Highly effective protective activity was shown by BAS 516F and Plover (< 1% leaf area affected), and good

activity by Amistar, Folicur and Bravo 500 (< 5%), compared with the untreated plants (16%). When applied 4 days prior to inoculation, all fungicides reduced disease severity and three (Bravo 500, Plover and BAS 516F) also reduced disease incidence.

When fungicides were applied 3 days after inoculation with *S. apiicola,* disease severity was reduced by all fungicides except the two copper products. Plover again reduced disease incidence. When fungicides were applied 10 days after inoculation with *S. apiicola*, only Plover and Folicur showed notable curative activity, reducing disease severity from 16% to < 5%.

Twenty-four hours after application of fungicides to leaves with Septoria lesions, spore germination was significantly reduced following treatment with Amistar and BAS 516F (eradicant activity), but not with the other fungicides. The triazole fungicides (Alto 240 EC, Folicur and Plover) act on fungal growth after germination and so would not be expected to reduce germination. None of the 8 fungicides affected spore release from pycnidia (spore cases) or the appearance of germ tubes developing from germinating spores.

Field trial comparing fungicide programmes

In an inoculated field trial on cv. Celebrity, conducted at ADAS Arthur Rickwood from 18 July to 31 October 2001, seven fungicide programmes significantly reduced disease severity. Alternating programmes of Amistar/Plover and BAS 516F/Plover, with sprays applied every 14 days (6 in total), reduced disease severity at harvest from 33% to less than 0.05%. A programme of Bravo 500/Plover was only slightly less effective (0.4% leaf area affected). Effectiveness of disease control was reflected in increased marketable yield. None of the treatments adversely affected the appearance of the harvested crop. Levels of *Rhizoctonia* and *Sclerotinia* were low and no treatment effects were detected.

Predicting Septoria risk

A simple celery Septoria risk-prediction scheme was devised, based on data generated in this project from infection studies and in-crop monitoring of leaf wetness and temperature. Further work is required to determine if this will be useful in predicting periods of low risk in a celery crop (and therefore no need to apply fungicide spray treatments), and/or periods of high risk (indicating a fungicide treatment is required).

Action points for growers

1. There is a high risk of infection by *Septoria apiicola* when leaf wetness duration exceeds 24 hours at temperatures around 20^0 C. *Consider applying a fungicide spray immediately after such an event, especially if a treatment has not been applied in the previous 7 days*.

2. At temperatures less than 10^0 C, leaf wetness duration in excess of 72 h was required before significant infection (> 5%) occurred. *Fungicide treatment during a cold spell (temperatures less than 10^0 C) is unlikely to be warranted, unless there is prolonged leaf wetness (e.g. more than 48 h)*

3. A simple disease risk assessment scheme, based on observed disease severity at 28 days after incubation at different leaf wetness durations and temperatures, is shown in the table below. *It may prove helpful as a decision tool to help you determine whether or not to spray during a particular weather period*.

4. All five fungicides approved for use on field celery (Amistar, Bravo 500, Croptex Fungex, Cuprokylt and Plover) show good protective activity against leaf spot when applied 4 days before spore inoculation. Bravo 500 was outstanding, significantly reducing disease incidence as well as severity. Amistar and Plover showed excellent protectant ability when applied as much as 10 days before inoculation with *S. apiicola. In a high disease risk situation based on weather criteria and with no disease established in the crop, it is recommended that one or more of Amistar, Bravo 500 and Plover are used in a spray programme.*

5. Bravo 500 showed good curative activity when applied 3 days after inoculation with *S. apiicola,* and likewise Plover when applied 7 days after inoculation. *It is recommended that one of these fungicides be used immediately after a prolonged wet period, when it has not been possible to spray at the planned time (subject to remaining within the SOLA conditions of use). Plover has recently received approval to permit use on field celery (SOLA 1320/02).*

6. Programmes consisting of three sprays of Bravo 500 alternating with three of Amistar at approximately 14 day intervals, or Bravo 500 alternating with Plover, or Amistar alternating with Plover, can provide very good control of celery leaf spot. *Consider following one of these programmes if you are not using leaf wetness duration to assess infection risk. Note that disease control may be reduced if Septoria leaf spot is evident in the crop before the first spray is applied.*

Relative risk of Septoria leaf spot (disease severity after 28 days) according to weather parameters

Temperature (^{0} C)	Leaf wetness duration (hours)					
	Less than 1	1 – 6	6.1 - 24	24.1 – 48	48.1 – 72	72.1 - 96
5	Low	Moderate	Moderate	Moderate	Moderate	High
10	Low	Low	Low	Moderate	Very high	Very high
15	Low	Low	Low	High	Very high	Very high
20	Low	Moderate	High	Very high	Very high	Very high
25	Low	Low	Very high	Very high	Very high	Very high
30	Low	Low	Low	High	Very high	Very high

Disease severity
Low: < 1% High: 5-20%
Moderate: 1-5% Very high: > 20%

Anticipated practical and financial benefits

This project has improved growers' knowledge base by providing pragmatic guidelines for controlling field outbreaks of celery leaf spot other than by frequent, routine applications of preventative sprays. Progress has been made towards developing a more rational, integrated disease management strategy based on the biology of the causal fungus, and by using fungicides with known protectant and curative activity. It is probable that results from this work will also find application by protected celery growers, and by parsley growers (where leaf spot caused by the related species, *Septoria petroselini*, is a major problem). Making greater use of in-crop wetness sensors may enhance the technology base of the industry. There may be future potential for the development of decision support software based on the findings of this research. There is an economic benefit to the industry in preventing widescale crop loss, which can exceed £100 k per annum, and in maintaining continuity of supply. There is potential for improved control of other diseases of celery from some of the new fungicides we have evaluated; for example, work on other crops has indicated that azoxystrobin (Amistar) has activity against *Rhizoctonia, Sclerotinia* and *Botrytis*, all of which can attack field celery.

SCIENCE SECTION

Introduction

There has been no work on control of Septoria on celery in the UK for over 20 years. Work in the 1960s and 1970s clearly demonstrated that Septoria can survive in celery debris in soil over one winter but not two; and led to the development of the now standard warm thiram soak seed treatment.

In the USA and Canada, three models have been developed for Septoria on celery which predict disease severity as a function of leaf wetness and temperature. For example, Lacy (1994) identified 12 hour leaf wetness as a useful threshold above which there is a significantly increased likelihood of Septoria spore germination and leaf infection. Prolonged leaf wetness (24 and 36 hours) was highly conducive to infection. In three successive seasons, spray timing based on a 12 hour wetness threshold reduced by two (from seven to five) the number of sprays required to maintain control in an inoculated crop in the USA. These models represent a useful starting point for identifying weather periods associated with high risk of disease development, although it cannot be assumed that a model validated for a region of the USA will be directly applicable to the UK.

Work on celery leaf spot in Australia has confirmed that chlorothalonil has good protectant activity and also revealed that some triazole fungicides (e.g. propiconazole and tebuconazole) have curative activity (of 3-5 days), while chlorothalonil has none. Recent work on winter wheat in the UK has investigated the relative protectant and curative activities of new fungicides against *Septoria tritici*, and identified marked differences. In ADAS consultancy work, resistance of *S. apiicola* to carbendazim has been identified in isolates from some celery crops. In crops where resistant isolates are present, Bavistin DF and related fungicides will no longer control leaf spot.

There are two recently completed HDC-funded projects on other diseases of celery. Project PC/FV 173 investigated crater spot caused by *Rhizoctonia solani*; Amistar applied to the soil surface resulted in a significant disease reduction. Project PC 131 investigated fungicides for control of *Sclerotinia* (pink rot) in protected crops; Amistar and Bavistin DF applied as sprays gave significant control.

This report describes a series of trials conducted since October 2000. Controlled-environment experiments were conducted to quantify the effect of temperature and leaf wetness duration on disease development, as a preliminary step towards a spray-timing decision tool. Glasshouse trials evaluated both the protectant, curative and eradicant activity of approved and novel fungicides applied at specific intervals in relation to infection events. In addition, an artificially-inoculated field trial was conducted to determine the efficacy of fungicide programmes for the control of celery leaf spot. The project focuses on the control of celery leaf spot caused by *S. apiicola*, but incorporates monitoring of other important celery diseases such as *Sclerotinia* and *Rhizoctonia* to determine treatment effects on these diseases.

When this project commenced, Bravo 500, Croptex Fungex and Cuprokylt had full approval for use on field celery and Amistar had recently gained a specific off-label

approval for use on the crop. The triazole fungicides Plover (difenoconazole), Folicur (tebuconazole) and Alto (cyproconazole) had approval for use on other minor vegetable crops and were evaluated together with BAS 516F (experimental product) under Administrative Experimental Approvals.

Materials and methods

Experiment 1: Controlled environment experiment to quantify the relationship between temperature, leaf wetness and infection by (*Septoria apiicola*)

Experiment design

Celery plants artificially inoculated with *apiicola* were incubated at six temperatures (5, 10, 15, 20, 25 and 30^{o}C). At each incubation temperature, six leaf wetness periods were tested (1, 6, 24, 48, 72 and 96 h). The experiment was conducted over time, with one inoculation per temperature. At each temperature, 60 plants were inoculated, with ten plants per temperature-wetness combination. Leaf wetness treatments were in a randomised block design within a controlled environment (CE) cabinet and the order of temperature treatments in sequential experiments was randomised.

As temperatures were tested over time, their effect could have been confounded with that of inoculum. To minimise confounding, percentage spore germination was determined to ensure that spore viability remained uniform for all temperatures (>95 % germination). In addition, the experiment was repeated at a single temperature (20^{o}C) to provide data to use for concordance or otherwise of the experimental technique.

Plant material

For each temperature tested, young celery plants cv. Celebrity, unsprayed with fungicide were obtained from Delflands Nurseries, Cambs. The plants had 3-4 true leaves when the trial commenced.

Preparation of inoculum

10-30 g dried celery leaves infected with *S. apiicola* were immersed into 150-400 ml distilled water, left for 30 min and agitated. The suspension was strained through four layers of cheesecloth and adjusted to approximately 10^{6} conidia/ml using a haemocytometer. The actual spore concentration was recorded for each experiment.

A sample of spore suspension (20 ul) prepared for each temperature treatment was pipetted and spread on to each of three plates of PDA+S. Percentage spore germination was determined after incubation for 24 h at 20^{o}C.

Plant inoculation

The plants to be inoculated were sprayed to run-off using a spray bottle with atomiser. The plants were placed within a misting chamber in a controlled environment cabinet. For each run, six un-inoculated control plants were also placed in the misting chamber but separated from the inoculated plants. In order to maintain leaf wetness, intermittent mist was provided by a timer-operated cold mister. At the lower temperatures, misting for 1 min every 3 h was sufficient to maintain continual leaf wetness, while at higher temperatures the spraying frequency was increased to 2 min

every 1 h. Temperature and relative humidity within the mist chamber were monitored with a data logger. The plants received a 12 h day/12 h night light regime.

At the end of each wetness period, ten plants and the uninoculated control plants were removed and gently dried for approximately 30 min with an electrical fan until water droplets were no longer visible on the leaves. Plants for the 1 h leaf wetness treatments were dried immediately after inoculation. For early runs, the 2nd and 3rd true leaves were marked to allow subsequent disease assessments on these leaves. Later runs on older plants used the 3rd and 4th true leaves or 4th and 5th true leaves, as appropriate, to ensure that inoculation was to leaves of a common age. After drying, plants were potted on using 9 cm diameter pots and placed on capillary matting. Temperatures and relative humidity in the glasshouse were monitored with a data logger. The plants were grown on for 4 weeks to allow symptom development. The plants were watered around the base, avoiding leaf wetting and were spaced so that there was no contact between plants.

Assessments

Twice weekly from the time of inoculation, the incidence of Septoria lesions was scored on each plant. At 14 and 28 days after inoculation, percentage leaflet area affected by Septoria lesions was estimated for each of the 3 leaflets of the two previously marked leaves.

Statistical analyses

This was conducted using response surface methodology available in Statistica (Anon, 2000). The two factors of interest were incubation temperature and the duration of wetness. These were not linear in their effect on the percentage of area covered by lesions and therefore a response surface design was employed for analysis. The regression surfaces, using distance weighted least squares, were prepared for disease severity data at 14 and 28 days after inoculation.

Experiment 2: Glasshouse trial to determine the relative protectant and curative activity of fungicide products applied for the control of celery leaf spot (*Septoria apiicola*)

Site and crop details

The experiment was conducted in Glasshouse compartment number 4 at ADAS Arthur Rickwood. Peat-block raised celery plants (cv. Celebrity) unsprayed with fungicide (3-4 true leaves) were transplanted into 9-cm diameter plant pots containing Levington's M2 compost and placed on capillary matting, which was watered as required. Glasshouse vents were left open and screens were used to avoid temperature extremes. A data logger was used to record temperature and relative humidity within the glasshouse for the duration of the experiment and ambient lighting was used.

Experiment design

The experiment comprised a two-way factorial design with six plants per plot and four replicate blocks. There were eight fungicide treatments applied at four different timings, with a full replication of the inoculated untreated control for each timing, to give a total of 36 fungicide timing treatment combinations and 144 plots. There was a spacing of at least 30 cm between plots because of the possible effect of fungicide volatiles on the unsprayed controls. Uninoculated untreated control plants (20) were

positioned in the same glasshouse at least 1 m from the main experiment, to avoid infection via spore splash from neighbouring plants.

Fungicides

Fungicides were applied to the celery plants 4 and 10 days before inoculation, and 3 and 7 days after inoculation. A spray guard was used to ensure that fungicide spray did not drift between treatments. Fungicides were applied at a spray volume of 1,000 l/ha (100 ml/m^2) using an Oxford precision sprayer operating at 2 Bar pressure with a medium flat fan nozzle (02F80). Fungicide rates were as follows:

Fungicide	Active ingredient	Rate
Bravo 500	Chlorothalonil	3.0 l/ha
Cuprokylt	Copper oxychloride	5.0 kg/1000 l water
Croptex Fungex	Copper ammonium carbonate	9.5 l/1000 l water
Amistar	Azoxystrobin	0.5 l/ha
Folicur	Tebuconazole	1.0 l/ha
Plover	Difenoconazole	1.0 l/ha
Alto 240 EC	Cyproconazole	0.25 l/ha
BAS 516 F	Details not provided	1.0 l/ha

Inoculation

To prepare inoculum of *S. apiicola*, 100 g dried celery leaves infected with *S. apiicola* were immersed in 800 ml distilled water for 30 min, then agitated. The suspension was strained through four layers of cheesecloth and adjusted to 1 x 10^6 spores/ml giving a final volume of 1.2 l. The inoculum was applied to all of the plants in the main experiment using an automatic misting gun (1.5 sec per plot). Prior to inoculation, all plots were watered via the capillary matting. Following inoculation with *S. apiicola*, a polythene tent was constructed over the entire trial and the plants were misted at least three times per day for 72 hours to ensure continual leaf wetness. The polythene tent was removed after 72 h. To check inoculum viability, 20 ul spore suspension was pipetted onto each of three plates of PDA+S. Percentage germination was recorded after incubation at 20ºC for 24 h and was found to exceed 95 %.

Assessments and analyses

From one week after inoculation, the trial was observed daily to determine the day on which leaf spot symptoms were first visible. A disease severity assessment (percentage leaf area affected per plant) was conducted 19-20 days after inoculation. In addition, pycnidial counts were recorded 21 days after inoculation, using a lesion area of 0.2 x 0.2 cm per plant. Data were analysed using analysis of variance in GENSTAT.

Experiment 3: Glasshouse trial to determine the relative eradicant activity of fungicide products applied for the control of celery leaf spot (*Septoria apiicola*)

Site and crop details

The experiment was conducted in a glasshouse at ADAS Arthur Rickwood using unsprayed uninoculated plants remaining from Experiment 2. Twenty seven plants were inoculated with *S. apiicola* using the same technique as described in Experiment 2. The plants were then placed in a controlled environment cabinet at 15ºC and misted hourly for 6 h before transferring to the glasshouse, where they were maintained

(ambient temperature and light; watered by hand as necessary), until *Septoria* lesions had developed on all plants.

Experiment design
The experiment comprised three randomised blocks with eight fungicide treatments and an untreated control. Each plot contained a single plant, previously inoculated with *S. apiicola* and showing distinct *Septoria* lesions. Plants were positioned at least 30 cm apart. The fungicides used in the experiment were as listed for Experiment 2, applied at the same rates.

Pycnidial assessments
Twenty four hours after the fungicide application, six leaflets containing abundant lesions were excised from each plot. Pycnidia from each plant were placed in a droplet of sterile distilled water and observed microscopically to determine when spore release occurred. The six leaflets were placed in 2 ml SDW in a Sterilin tube and macerated with a glass rod for 30 sec. The resulting spore suspension was pipetted onto each of 3 plates of PDA+S (60 ul per plate) and incubated at 20ºC. After 24 h, percentage spore germination on each plate was assessed. The plots were processed in the same randomised order as they occurred in the glasshouse and processing was staggered to ensure that spore germination records took place 24 h later. Percentage spore germination results were subjected to analysis of variance following square root transformation. The germinating spores were also observed to determine whether germ tubes were distorted or abnormal in any way.

Experiment 4: Field trial to evaluate fungicide programmes for the control of celery leaf spot (*Septoria apiicola*)

Site and crop details
Crop: Celery plants cv. Celebrity were raised in modules from thiram-treated seed at Delflands Nursery, Cambs. Fungicides were not applied during the propagation stage. The plants were transplanted to the field by hand.

Site: House Ground, ADAS Arthur Rickwood.

Soil: Previously cropped with daffodils.
pH 6.3
P 48 mg/l (index = 4)
K 529 mg/l (index = 4)
Mg 190 mg/l (index =4)
Bo 4.37 mg/l

Fertilisers applied prior to transplanting:
N 75 kg/ha
P 125 kg/ha
K 300 kg/ha
Bo 0.5 kg Solubor /10 l water

Land preparation: The area was power harrowed before and after fertiliser application, followed by bed formation.

Weed control: Gesaguard was applied 2.5 weeks after transplanting (2.3 kg/ha in 200 l/ha water). Linuron was applied 5 weeks after transplanting (2.5 l/ha in 400 l/ha water).

Pest management: A prophylactic spray of Hallmark (Lambda-cyhalothrin) (SOLA 0289/00) was applied 1 week after transplanting (0.05 l in 300 l/ha water). Regular monitoring of sticky fly traps set up in the trial area at the time of planting, indicated that further insecticide sprays were not necessary.

Irrigation: Misting equipment (Rightrain 3" irrigation standpipes) was set up at the beginning of the trial and was used to ensure that precipitation from irrigation together with rainfall was at least 2.5 cm per week for the duration of the trial.

Logger: A DeltaT data logger (DL2e) was set up at the beginning of the trial to monitor the following variables: rainfall, soil temperature (15 cm), canopy temperature, relative humidity and leaf wetness (type SWS).

Experiment design

The experiment was laid out as a randomised block design with four replicate blocks. There were seven fungicide programmes and a double replication of the untreated control, giving a total of 36 plots. Each plot comprised a bed measuring 1.8 m (width) x 4.8 m (length) with five rows of celery per bed at a spacing of 30 cm between and along rows. Plots were separated by a bed width of 1.8 m to avoid spray drift between plots; these beds were not planted with celery.

Treatments

The following alternating fungicide programmes commenced 1 week after transplanting:

1. Untreated control
2. Bravo 500 / Cuprokylt
3. Bravo 500 / Amistar
4. Bravo 500 / Plover
5. Bravo 500 / Folicur
6. Amistar / Plover
7. Amistar / Folicur
8. BAS 516 F / Plover
9. Untreated control

Fungicides were applied in a water volume of 400 l/ha using an Oxford precision sprayer with a 1.5 m or a 2 m boom and medium flat fan nozzle (02F110) at 2 Bar pressure. Fungicide rates of use were Amistar l/ha; Bravo 500 3 l/ha; Cuprokylt 5 kg/1000 l water; Folicur 1.0 l/ha; Plover 0.5 l/ha; BAS 516F 1.0 l/ha.

Inoculation

The experiment was inoculated with *S. apiicola* 2 days after the first fungicide application. A spore suspension was prepared by soaking dried celery leaves infected with *S. apiicola* in distilled water. After 1 h, the leaf material was squeezed out and put aside and the spore suspension was decanted through a piece of muslin and a funnel into a 10 l water container. The final volume of the spore suspension was 18 l

at a concentration of 2×10^5 spores/ml. The spore suspension was applied to all plots (500 ml/plot) using a pump action hand-held mister. Leaf debris used for spore suspension preparation was distributed evenly between the middle three plant rows of each plot. The trial was irrigated three times on the day of inoculation and twice on the two following days.

Disease assessments

Approximately 1 and 2 months after crop inoculation, the percentage leaf area affected by leaf spot was recorded for the central 12 plants per row in the middle three rows of each plot. The incidence of disease symptoms due to other pathogens (e.g. *Rhizoctonia* and *Sclerotinia*) on these plants was also recorded. In addition, an assessment of percentage plot area affected by celery leaf spot was made by calculating the mean of four estimates of disease severity per plot. This assessment was repeated at approximately 1-2 weekly intervals until harvest (six assessments in total).

Harvest and yields

The trial was harvested 15 weeks after transplanting. From each plot, 24 plants were cut from the centre of the middle three rows (eight plants per row), using celery cutting knives. The total weight for the 24 harvested plants was recorded and used to calculate mean plant weight. The plants were then trimmed to market specifications (30 cm petiole, no blemishes) and weighed. Plants weighing >500 g were recorded as marketable. The incidence of plants affected with *Sclerotinia* or *Rhizoctonia* was recorded. Plants from plots that had received treatments with BAS 516 F, Plover or Folicur were disposed of according to the appropriate Administrative Experimental Approvals.

Statistical analyses

Yield data were subjected to analysis of variance. Disease incidence and severity data was analysed using Friedmans Test, since the data did not conform to the assumptions of ANOVA.

Experiment 5: In-crop monitoring of leaf wetness and temperature

In collaboration with G S Shropshire & Sons, daily wetness duration and temperature were logged within a commercial celery crop (Stretham, Cambs) from 24/7/2001 to 30/10/2001. Data were also collected in the crop grown at ADAS Arthur Rickwood, from 31/7/2001 to 31/10/2001. The aim was to monitor the frequency at which prolonged periods of leaf wetness (>24 h), favourable for rapid development of Septoria, occurred in crops. There is potential to use such information as the basis of a simple disease risk prediction scheme.

Data were recorded within the commercial celery crop on the farm using a DL2 logger fitted with a rainfall gauge, temperature probe and leaf wetness sensor. Unfortunately, malfunctions occurred and data were lost. At the ADAS Arthur Rickwood trial site, data were recorded within the celery crop using a DL2 logger fitted with a rainfall gauge, temperature probes, RH sensor and leaf wetness sensor. Leaf wetness sensors were placed at canopy level, within a bed of celery, the RH sensor at ground level, the temperature probe just under the top of the canopy, and the rain gauge at ground level. Leaf wetness duration was recorded every 30 minutes.

Results and discussion

Experiment 1: Controlled environment experiment to quantify the relationship between temperature, leaf wetness and infection by *Septoria apiicola*

Spore germination exceeded 90 % for each set of inoculum used in the experiment. There was no disease development on the untreated control plants.

For temperatures ≥20ºC and leaf wetness durations ≥24 h, the appearance of symptoms was generally rapid with Septoria lesions first observed after 10-11 days and 100 % incidence being reached 15 days after inoculation (Tables 1 and 2). Although lesions were seen on plants from the majority of other leaf wetness-temperature combinations, first symptoms were not generally observed before 15 days after inoculation, they continued to develop on individual plants until the end of the experiment (28 days after inoculation) and the final disease incidence was <100%.

Table 1. Effect of temperature and leaf wetness duration on the number of days between inoculation with *Septoria apiicola* and appearance of the first lesions of on celery.

	Wetness duration (h)					
Temp (ºC)	1	6	24	48	72	96
5	15-28[a]	15-28	18-28	11-28	15-28	15-28
10	-	25-28	-	18-28	15	15
15	11-28	11-28	15-28	11-28	11-28	15-28
20[b]	25	-	22-28	11-18	11-15	11
20[b]	15-28	18-28	10-15	10-15	10	10
25	22-28	15-28	15	11-15	11-15	11-15
30	18-28	18-28	15-28	15	11-15	15

[a]The range represents the period during which first lesions appeared on the 10 plants sampled
[b]Duplicate runs
Shaded areas represent combinations where all plants developed symptoms within 15 days.

Table 2. Effect of temperature and leaf wetness duration on incidence of Septoria lesions, 25 days after inoculation of celery plants with *Septoria apiicola.*

	% Disease incidence[a] for different leaf wetness durations					
Temp (ºC)	1 h	6 h	24 h	48 h	72 h	96 h
5	50	80	70	80	80	90
10	0	10	0	90	100	100
15	90	70	60	40	80	70
20[b]	10	0	30	100	100	100
20[b]	50	20	100	100	100	100
25	10	20	100	100	100	100
30	20	10	60	100	100	100

[a]% disease incidence for ten plants per treatment
[b]Duplicate runs
Shaded areas indicate combinations where all plants developed symptoms within 25 days.

Tables 3 and 4 show clearly that there was a trend for disease severity (assessed 14 days and 28 days after inoculation) to increase with temperature and leaf wetness. The exception to this trend was at 30ºC which was less conducive to disease development than 25ºC. Symptoms were observed at lower temperatures and after shorter periods of leaf wetness than has been previously reported. However, at temperatures <10ºC, considerable periods of continuous leaf wetness (>72 h) were required before disease severity >5 % were recorded. The disease severity data together with disease incidence and time to first symptom development could provide a simple basis for guiding spray timing during a field experiment in 2002.

Statistical analysis of the effect of temperature and leaf wetness duration on disease severity, with graphical presentation (response surfaces) of the results, is shown in Appendix 4. For the 28 day disease severity data, all the quadratic coefficients for temperature and leaf wetness duration, and their interactions, were statistically significant at P<0.001(see Appendix 4 for tabulated values).

There were slight differences in the data from the two runs at 20ºC. Also, it was surprising that infection at 10ºC seemed less consistent than at 5ºC. One possibility is that condensation in the glasshouse may have lead to periods of leaf wetness after the plants were removed from the CE cabinet. However, logger data from the glasshouse shows that relative humidity remained low for the duration of the experiment, so this seems improbable.

For practical reasons in the present work, leaf wetness durations between 6 and 24 h were not investigated. It is proposed that leaf wetness durations between 6 and 24 h (e.g. 6, 12, 18 and 24 h) are investigated at selected temperatures to determine more precisely the minimum leaf wetness duration which allows severe disease (> 5% leaf area after 28 days) at these temperatures.

Table 3. Effect of temperature and leaf wetness duration on % leaf spot severity 14 days after inoculation of celery plants with *Septoria apiicola*

	% Disease severity[a] for different leaf wetness durations					
Temp (ºC)	1 h	6 h	24 h	48 h	72 h	96 h
5	0.1	0.3	0.1	0.1	0.0	0.3
10	0.0	0.0	0.0	0.0	1.3	1.7
15	0.0	0.1	0.0	0.6	2.3	2.9
20[b]	0.0	0.0	0.0	2.2	4.9	5.8
20[b]	0.0	0.0	2.5	9.1	14.3	13.0
25	0.0	0.1	2.4	8.2	11.9	13.8
30	0.0	0.0	0.0	1.7	5.2	2.6

[a]Mean % disease severity for six leaflets per plant, ten plants per treatment
[b]Duplicate runs.
Shaded areas indicate disease severity >1%

Table 4. Effect of temperature and leaf wetness duration on % leaf spot severity 28 days after inoculation of celery plants with *Septoria apiicola*

	% Disease severity[a] for different leaf wetness durations					
Temp (ºC)	1 h	6 h	24 h	48 h	72 h	96 h
5	0.7	3.5	2.9	2.2	1.5	19.0
10	0.0	0.0	0.2	3.8	32.8	50.1
15	0.1	0.2	0.3	10.5	24.2	25.8
20[b]	0.0	3.3	0.3	20.2	34.7	45.2
20[b]	0.2	0.1	8.4	35.5	52.6	31.4
25	0.3	0.5	20.9	43.2	55.7	50.9
30	0.0	0.1	0.4	10.7	31.1	26.5

[a]Mean % disease severity for six leaflets per plant, ten plants per treatment
[b]Duplicate runs.
Shaded areas indicate disease severity >5%

Conclusions

1. The shortest time to symptom development (10 days) occurred when leaves were incubated at 20^0 C with 24 hours, or more, leaf wetness. When incubated at 20^0 C with only 6 h leaf wetness duration, it was 18-28 days before first symptoms occurred.
2. There was a statistically significant increase in disease severity with temperature (in the range 5-25^0 C) and leaf wetness duration (from 1 to 96 h).
3. Disease severity was greatest (over 50% leaf area affected after 28 days) when inoculated leaves were incubated at 20-25^0 C with leaf wetness for 72 hours.
4. Above 25^0 C, disease severity declined and at 30^0 C disease severity after 28 d was broadly similar to that at 15^0 C.
5. Infection occurred at temperatures as low as 5^0 C, although it required 96 h of leaf wetness at this temperature before the disease severity 28 days later exceeded 5% leaf area affected.
6. When leaf wetness duration was restricted to just 1 hour, there was a low incidence of infection at most temperatures. However, 28 days later this had resulted in a disease severity of only 0.1 - 0.7% leaf area affected.

Experiment 2: Glasshouse trial to determine the relative protectant and curative activity of fungicide products applied for the control of celery leaf spot (*Septoria apiicola*)

There was no symptom development for the duration of the trial on the uninoculated control plants. Symptoms of leaf spot were first visible 11 days after inoculation on the unsprayed control treatments, and with a mean daily glasshouse temperature of 15^{o} C, results were in agreement with those from Experiment 1. At 20 days after inoculation, there was 100 % disease incidence on the unsprayed control plants, with a mean disease severity of 15.5 % leaf area affected. There were highly significant effects (P<0.001) due to fungicides, timing and a fungicide timing interaction on disease incidence and severity assessed 20 days after inoculation (Table 5).

Fungicides applied 10 days prior to inoculation all significantly reduced disease severity compared with the untreated control. Highly effective protectant activity was shown by BAS 516 F and Plover, resulting in disease severity of less than 1%, while disease severity was also low (<5 %) for Amistar, Folicur and Bravo 500.

Fungicides applied 4 days prior to inoculation all provided effective protectant activity, reducing disease severity to less than 4 %. There was no significant difference between the fungicide treatments for disease severity, although it was notable that there was only a trace of disease on plants treated with Bravo 500, BAS 516 F and Plover (<0.5 %) and that disease incidence was significantly reduced for these treatments. Elsewhere it has been reported that chlorothalonil (e.g. Bravo 500) re-distributes on plants with rain or irrigation, helping to explain the good protectant activity with this chemical.

For treatments applied 3 days post-inoculation, disease severity was significantly reduced by all fungicides except for the copper-based products. All of the products except Amistar and the copper fungicides, resulted in disease severity of <5 % with Plover again giving a significant reduction in disease incidence. While several fungicides significantly reduced disease severity when applied 10 days post-inoculation, only the triazole fungicides Plover and Folicur showed notable curative activity (<5 % disease severity). The good curative activity of the triazole fungicides is in agreement with Wicks (1990), though in contrast to his results we found that chlorothalonil also has curative activity when applied 3 days after inoculation.

Pycnidia were first recorded in block 1 for all treatments applied 7 days post-inoculation. There were no significant differences between treatments in the number of pycnidia developing and the assessment was discontinued. These results are in contrast to studies on leaf spot (*Phoma* sp.) on oilseed rape and ring spot (*Mycosphaerella* sp.) on brassicas, where Folicur and Plover were reported to stop pycnidial development, leading to the occurrence of leaf spots without pycnidia.

In summary, BAS 516 F, Plover and Amistar were the only fungicides to provide effective long-term protectant activity. Bravo 500, Alto 240 EC, Folicur and the copper fungicides were effective short-term protectants. A slight short-term curative effect was demonstrated with BAS 516 F, Bravo 500, and Alto 240 EC, although Plover and Folicur were more effective. Plover was the only fungicide to provide a long-term (>7 day) curative effect.

Table 5. Effect of fungicides applied at different timings on the incidence and severity of leaf spot on celery plants 20 days after inoculation with *Septoria apiicola*

Fungicide	Timing pre/post inoculation	% disease severity[a]	Rank (disease severity)	Disease incidence[b]
Untreated control	10 days pre	16.4	9	6.0
Bravo 500		4.5	5	6.0
Cuprokylt		9.1	8	6.0
Croptex Fungex		7.5	7	6.0
Amistar		1.2	3	6.0
Folicur		3.7	4	6.0
Plover		0.8	2	5.5
Alto 240 EC		7.3	6	6.0
BASF 516 F		0.4	1	6.0
Untreated control	4 days pre	15.6	9	6.0
Bravo 500		0.0	1	1.0
Cuprokylt		2.5	7	6.0
Croptex Fungex		3.2	8	6.0
Amistar		1.4	6	6.0
Folicur		0.5	4	5.8
Plover		0.2	3	4.0
Alto 240 EC		0.8	5	6.0
BASF 516 F		0.1	2	4.8
Untreated control	3 days post	14.4	9	6.0
Bravo 500		2.1	4	5.8
Cuprokylt		11.7	7	6.0
Croptex Fungex		13.7	8	6.0
Amistar		10.7	6	6.0
Folicur		0.3	2	5.3
Plover		0.1	1	3.0
Alto 240 EC		1.9	3	6.0
BASF 516 F		3.3	5	6.0
Untreated control	7 days post	15.8	9	6.0
Bravo 500		15.3	8	6.0
Cuprokylt		10.9	5	6.0
Croptex Fungex		13.5	6	6.0
Amistar		13.6	7	6.0
Folicur		4.3	2	6.0
Plover		2.8	1	6.0
Alto 240 EC		7.0	3	6.0
BASF 516 F		9.7	4	6.0
SED (105 d.f.)		1.759		0.452

[a]% leaf area infected
[b]Mean no. of infected plants per plot of six plants

Experiment 3: Glasshouse trial to determine the relative eradicant activity of fungicide products applied for the control of celery leaf spot (*Septoria apiicola*)

There was no effect of fungicide treatment on spore release from pycnidia or appearance of germ tubes. There was, however, a treatment effect on percentage spore germination (Table 6). There was a significant (P<0.01) reduction in spore germination compared with the untreated control due to both Amistar and BAS 516 F (both strobilurin fungicides), with both treatments reducing percentage from 42% to less than 2%. These fungicides could therefore help to reduce secondary spread of the disease in a field situation.

Table 6. Effect of fungicide treatment applied to celery leaves affected by late blight on germination of *Septoria apiicola* spores obtained from excised pycnidia[a]

Fungicide	% spore germination	% spore germination (square-root transformed)
Untreated control	42.2	6.46
Bravo 500	24.6	4.04
Cuprokylt	49.0	6.70
Croptex Fungex	18.7	4.15
Amistar	0.9	0.76
Folicur	19.8	4.40
Plover	26.7	4.98
Alto 240 EC	42.0	6.29
BAS 516 F	1.4	0.96
SED (16 d.f.)		1.562

[a] Lesions excised 24 h after fungicide treatment.

Experiment 4: Field trial to evaluate fungicide programmes for the control of celery leaf spot (*Septoria apiicola*)

Septoria leaf spot was first observed 18 days after inoculation and increased rapidly to affect all untreated plants within 2 months (Fig 1). Disease severity on untreated plants was 2.5% leaf area affected at 1 month after inoculation and had increased to 33% by 2 months (Table 7).

Fungicide treatment resulted in significant reductions in both disease incidence and severity. Amistar/Plover and BAS 516F/Plover programmes were notably more effective than other treatments, with disease incidences of less than 25% two months after inoculation, while all other treatments were greater than 75%. All treatments significantly reduced disease severity, from 33% to less than 7% (Table 7). The Amistar/Plover and BAS 516F/Plover programmes were highly effective, reducing disease severity to < 0.05%. The Bravo/Plover programme (0.4% leaf area affected) was only slightly less effective.

The effectiveness of treatments in controlling Septoria was reflected in the marketable yield (Table 8). There was no obvious phytotoxic effect from any of the fungicide treatments (i.e. no scorch and no alteration in colour or shape of the treated plants).

Sclerotinia and *Rhizoctonia* were not observed during the growing season. On harvested plants, *Sclerotinia* was observed on one plant only (Amistar/Folicur treatment). The mean incidence of *Rhizoctonia* crater spot ranged from 0-12% for individual treatments, with no significant effect of fungicide treatment on disease incidence.

Table 7. Effect of fungicide programmes on the incidence and severity of celery leaf spot (*Septoria apiicola*), assessed twice during the growing season

Treatment	1 month after inoculation		2 months after inoculation	
	% disease incidence[a]	% disease severity[b]	% disease incidence[a]	% disease severity[b]
Untreated control	97.2	2.0	100.0	33.1
Bravo 500/Cuprokylt	82.6	0.4	100.0	6.8
Bravo 500/Amistar	77.1	0.5	100.0	3.6
Bravo 500/Plover	14.6	0.0	79.2	0.4
Bravo 500/Folicur	28.5	0.0	100.0	5.8
Amistar/Plover[c]	0.0	0.0	15.3	0.0
Amistar/Folicur	0.0	0.0	81.9	1.3
BAS 516 F/Plover[c]	0.0	0.0	24.3	0.0
Untreated control	99.3	2.9	100.0	33.5
Significance (Friedman's test)	<0.001	<0.001	<0.001	<0.001

[a]Mean % of plants (out of 36) with leaf spot symptoms
[b]Mean % plant area affected with leaf spot symptoms
[c]Mean disease severity <0.05 %

Table 8. Effect of fungicide programmes on yield and marketability of celery, ADAS Arthur Rickwood, 2001

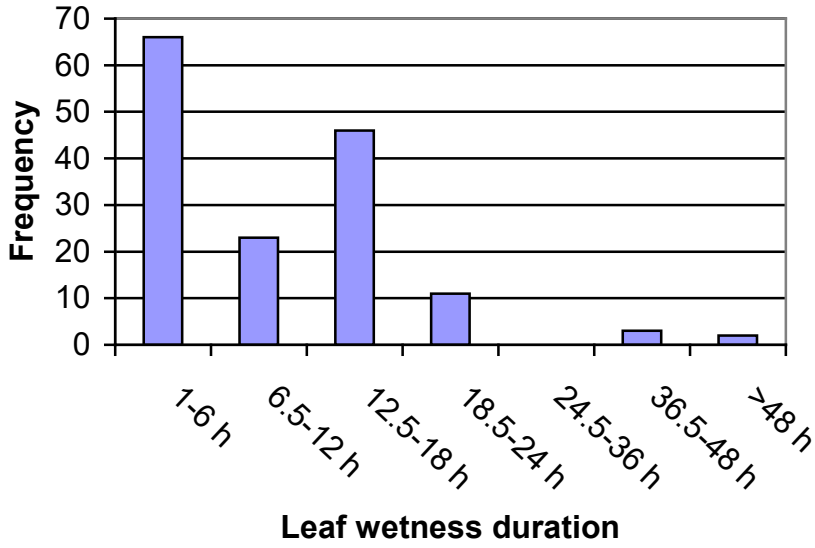
Treatment	Yield at harvest (kg per plot)	Marketable yield (kg per plot)	Mean weight of marketable sticks (g)	% Marketable sticks[a]
Untreated control	4.80	0.00	0	0
Bravo 500/Cuprokylt	17.90	0.84	478	8.3
Bravo 500/Amistar	20.67	7.01	533	54.2
Bravo 500/Plover	24.00	12.30	608	83.3
Bravo 500/Folicur	18.74	2.91	375	25.0
Amistar/Plover	22.89	11.55	575	83.3
Amistar/Folicur	22.51	7.89	540	62.5
BAS 516 F/Plover	25.13	14.08	647	91.7
Untreated control	4.45	0.00	0	0
Significance (24 df)	< 0.001	< 0.001	<0.001	< 0.001
SED	1.159	1.725	0.067	2.718

[a]Above minimum weight of 450 g and minimum height of 28-30 cm, with no *Septoria* present.

Experiment 5. In-crop monitoring of leaf wetness and temperature

Figure 2 shows the frequency of different leaf wetness durations in the celery trial at ADAS Arthur Rickwood, from the end of July until the end of October 2001. While wetness durations of 1-6 h were the most common, durations of 12.5 – 18 h were also frequent. From the logger data (not shown), it was interesting to note that 19 h leaf wetness and a mean temperature of 16.5ºC on 2-3 August 2001, may have represented a high risk infection period, resulting in symptom development approximately 14 days later. Five leaf wetness durations exceeding 24 h occurred during the monitoring period, all in the second half of the growing season (from 17/9/01). The high rate of disease development that occurred from 10 weeks after planting (Figure 1) was probably due to the longest leaf wetness duration (64 h) which commenced on 17/9/01 (9 weeks after transplanting).

Figure 2. Frequency of leaf wetness durations (h) occurring in a celery field trial, ADAS Arthur Rickwood, (31.07.01 – 31.10.01)



Examination of the logger data showed that it was usual for a period of leaf wetness to occur during the night, probably resulting from dew, sometimes in combination with rainfall. Typical timings and mean temperatures for these leaf wetness periods are shown in Table 8. It is clear from both Table 8 and Figure 2 that leaf wetness durations of 6 - 24 h with mean temperatures of 10-15ºC were common at night during the growing season. It should be noted that similar conditions in the controlled environment experiment (Experiment 1) resulted in low levels of infection (<1 % disease severity) (Table 3).

Based on the available data, preliminary risk thresholds for leaf wetness and temperature to aid spray decisions in a field trial to be conducted in 2002, will be set as follows:

Spray treatments timed according to leaf wetness only:

< 12 h	No spray
≥ 12 h	Spray (low risk threshold)
≥ 18 h	Spray (medium risk threshold)
≥ 24 h	Spray (high risk threshold)

Spray treatments timed according to mean night-time temperature only:

< 5ºC	No spray
5-10ºC	Spray (low risk threshold)
11-15ºC	Spray (medium risk threshold)
16-20ºC	Spray (high risk threshold)

Table 8. Leaf wetness durations and mean temperatures on selected dates during the celery field experiment, ADAS Arthur Rickwood, 2001

Date (2001)	Leaf wetness duration (h)	Time	Mean temperature (ºC)
01 – 02 Aug	10.5	22:12 – 08:42	12.0
08 – 09 Aug	14.0	19:42 – 09:12	14.3
15 – 16 Aug	11.5	20:42 – 07:42	16.2
24 – 25 Aug	13.5	20:42 – 09:42	14.7
01 – 02 Sept	3.5	04:42 – 07:42	15.4
08 – 09 Sept	5.0	03:12 – 07:42	13.1
15 – 16 Sept	14.5	19:42 – 09:42	8.6
24 – 25 Sept	20.0	17:42 – 13:12	9.5
01 – 02 Oct	10.0	03:12 – 12:42	14.2
08 – 09 Oct	20.0	14:42 – 10:12	11.3
15-16 Oct	21.0	15:42 – 12:12	10.4
24 –25 Oct	16.0	18:12 – 09:42	10.5

Overall conclusions

1. There was a consistent trend for a combination of temperatures >20ºC and leaf wetness durations >24 h to result in rapid and severe development of celery leaf spot on inoculated plants. The results from the experiment could be used to guide spray timing in the next field trial. However, repetition of the experiment using a narrower range of critical temperatures/leaf wetness durations, selected based on this year's results, would provide a useful clarification of the data.

2. The glasshouse experiments provided detailed information regarding the relative protectant and curative activity of the fungicides tested. The results were closely supported by disease assessments in the field. The results highlighted the superior performance of Plover which showed long term control when applied both pre- and post-infection but also demonstrated the efficacy of Amistar and Bravo 500, and the potential for BAS 516 F to be used as a protectant fungicide for celery production.

3. The effective control demonstrated by Plover in both glasshouse and field trials provides strong support for the recently successful SOLA application (SOLA 1320/02).

4. In-crop monitoring of leaf wetness duration and temperature provided useful information on the frequency of different leaf wetness durations throughout a growing season and mean temperatures during these periods. Data has been used to help devise risk thresholds to guide spray decisions during a field trial in 2002.

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Wicks T (1990). Glasshouse and field evaluation of fungicides for the control of *Septoria apiicola* on celery. *Crop Protection* **9**, 433-438.

Technology transfer

Publications

K Green & T O'Neill (2001). Management of celery leaf spot. HDC Factsheet 06/01. East Malling, UK: HDC.

K Green & T O'Neill TM (2002). Management of celery leaf spot. HDC Factsheet 06/01 (update). East Malling, UK: HDC.

Visits to ADAS Arthur Rickwood to see glasshouse and field trials for FV 237

15 May 2001, E. Garrod, P. Hooker (Project progress meeting)
16 May 2001, D. Norman
28 September 2001, HDC representatives
8 October 2001, D. Norman
15 October 2001, P. Hooker

Review meeting

17 April 2002, D. Norman, P. Hooker, T. O'Neill and K. Green

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APPENDIX 1

Experiment diaries

Experiment 1: Controlled environment experiment to quantify the relationship between infection and leaf wetness at different temperatures, for celery leaf spot (*Septoria apiicola*)

Date	Activity
12.02.01	Run at 5ºC set up
17.02.01	Run at 25ºC set up
05.03.01	Run at 20ºC set up
12.03.01	Run at 10ºC set up
19.03.01	Run at 15ºC set up
02.04.01	Repeat run at 20ºC set up
09.04.01	Run at 30ºC set up

Experiment 2: Glasshouse trial to determine the relative protectant, curative and eradicant activity of fungicide products applied for the control of celery leaf spot (*Septoria apiicola*)

Date	Activity
09.04.01	Pot-up celery transplants and maintain in glasshouse
12.04.01	Set up trial design in glasshouse
17.04.01	Fungicide spray 1 (10 days pre-inoculation)
23.04.01	Fungicide spray 2 (4 days pre-inoculation)
27.04.01	Artificial inoculation of celery plants with *S. apiicola* Spore suspension plated onto PDA+S to check spore viability
28.04.01	Spore germination counts
30.04.01	Fungicide spray 3 (3 days post-inoculation)
04.05.01	Fungicide spray 4 (7 days post-inoculation)
08.05.01	Leaf spot development first observed
16.05.01	Disease severity assessment - 19 days post-inoculation (block1)
17.05.01	Disease severity assessment - 20 days post-inoculation (blocks 2-4)
18.05.01	Pycnidial count recorded for Block 1, 7 day post-inoculation treatments only

Experiment 3: Glasshouse trial to determine the relative eradicant activity of fungicide products applied for the control of celery leaf spot (*Septoria apiicola*)

Date	**Activity**
09.04.01	Pot-up celery transplants and maintain in glasshouse
01.06.01	Artificial inoculation of celery plants with *S. apiicola*
03.07.01	Apply fungicide treatments to celery plants with well-developed leaf spot symptoms
04.07.01	Leaf lesions collected from all treatments to check spore release from pycnidia and set up spore viability tests
05.07.01	Spore germination counts

Experiment 4: - Field trial to evaluate fungicide programmes for the control of celery leaf spot (*Septoria apiicola*)

Date	Activity
09.07.01	Soil samples collected from trial site and sent for analysis
17.07.01	N, P, K and Bo applied to trial site before power harrowing and bed formation
18.07.01	Celery transplanted
19.07.01	Data logger installed
24.07.01	Irrigation equipment installed. Gaps filled where plants had died
25.07.01	Fungicide application 1 Insecticide (Hallmark) applied
27.07.01	All plots inoculated with *Septoria apiicola*
28-29.07.01	Plots irrigated twice per day
09.08.01	Fungicide application 2
10.08.01	Herbicide (Gesaguard) applied
14.08.01	Septoria symptoms first observed
21.08.01	Disease assessment 1 (% plant severity) Disease assessment 1 (% plot severity)
22.08.01	Fungicide application 3
31.08.01	Herbicide (Linuron) applied
04.09.01	Disease assessment 2 (% plot severity)
06.09.01	Fungicide application 4
20.09.01	Disease assessment 3 (% plot severity)
21.09.01	Fungicide application 5
28.09.01	Disease assessment 4 (% plot severity)
03.10.01	Fungicide application 6
05.10.01	Disease assessment 2 (% plant severity)
12.10.01	Disease assessment 5 (% plot severity)
25.10.01	Disease assessment 6 (% plot severity)
30.10.01	Blocks 1 and 2 harvested for yield assessments
31.10.01	Blocks 3 and 4 harvested for yield assessments

APPENDIX 2:

Statistical analysis of the effect of leaf wetness duration and temperature on infection of celery by *S. apiicola*

Severity at 14 days

Data are average area affected per leaflet (2 leaves – 3 leaflets each.)

STAT.	PERAREA - Parameter estimates (ton3.sta)					
VISUAL	Distribution : NORMAL					
GLZ	Link function: LOG					
Effect	Level of Effect	Column	Estimate	Standard Error	Wald Stat.	p
Interc		1	-15.2089*	2.205236*	47.5646*	.000000*
TEMP		2	1.3072*	.167653*	60.7919*	.000000*
TEMP^2		3	-.0294*	.003528*	69.2188*	.000000*
WETNESSD		4	.0812*	.021165*	14.7204*	.000125*
WETNESSD^2		5	-.0005*	.000096*	28.9953*	.000000*
TEMP*WETNESSD		6	.0002	.000727	.0594	.807473

Table 1. Regression analysis for response surface at 14 days severity. Response variable is percentage area of lesion per leaflet. All the regression coefficients are statistically significant.

STAT.	PERAREA - Confidence Intervals of Estimates (ton3.sta)			
VISUAL	Distribution : NORMAL			
GLZ	Link function: LOG			
Effect	Level of Effect	Column	Lower CL 95. %	Upper CL 95. %
Interc		1	-19.5311	-10.8867
TEMP		2	.9786	1.6358
TEMP^2		3	-.0363	-.0224
WETNESSD		4	.0397	.1227
WETNESSD^2		5	-.0007	-.0003
TEMP*WETNESSD		6	-.0012	.0016

Table 2. The 95% confidence intervals for the regression analysis for response surface at 14 days severity.

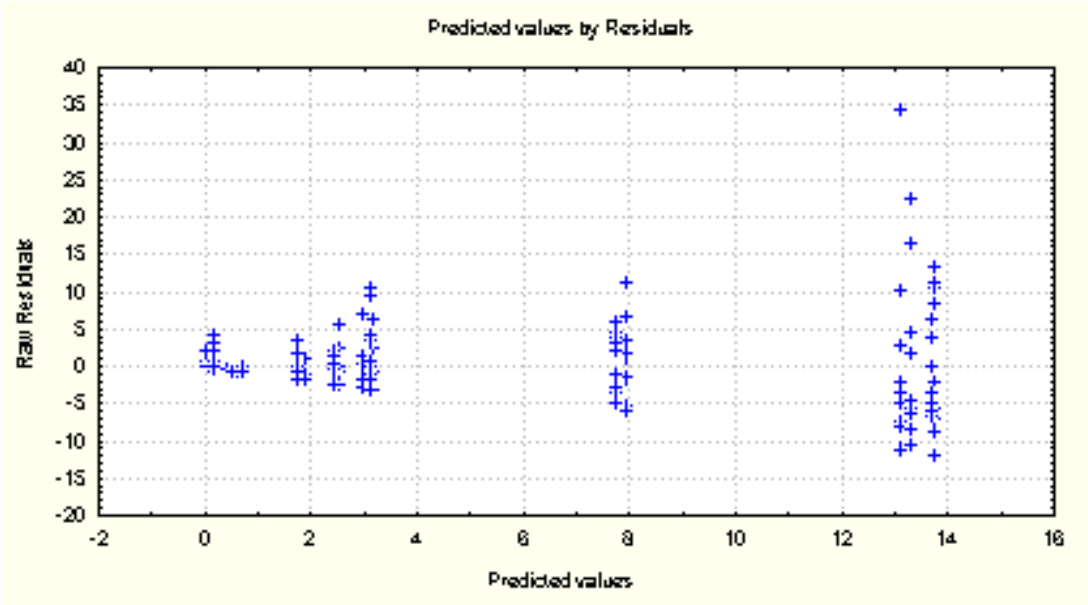

Figure 1. Residual errors plotted as a function of predicted values for severity at 14 days.

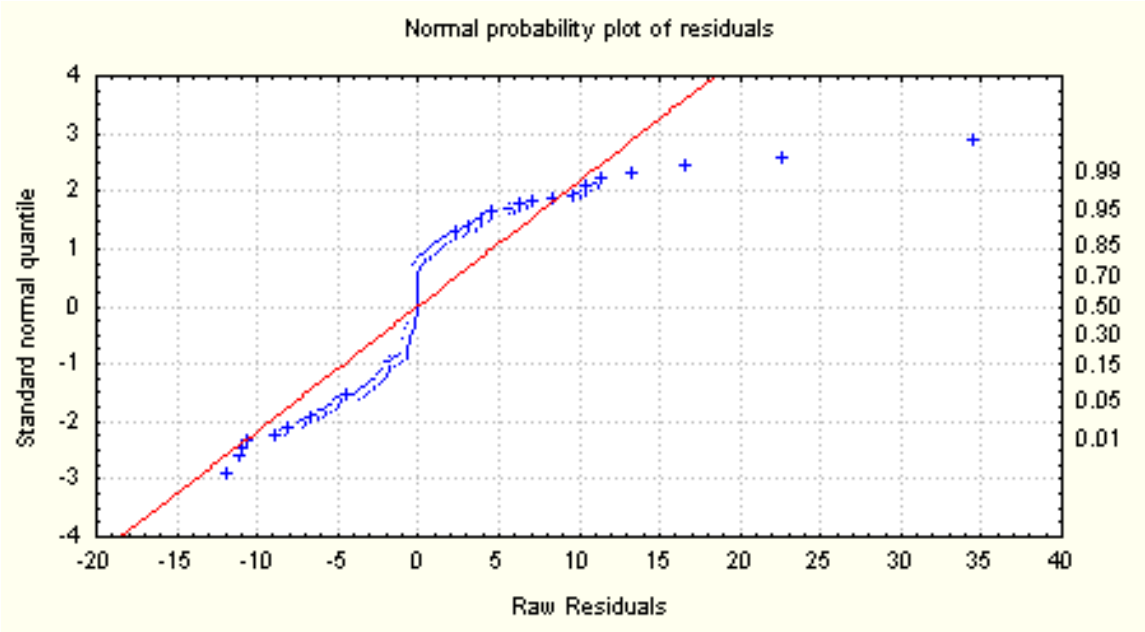

Figure 2. Normal probability plot for residuals which indicate systematic bias of modelling data due to large number of zeroes and sparse data at 14 days severity.

Response surface.

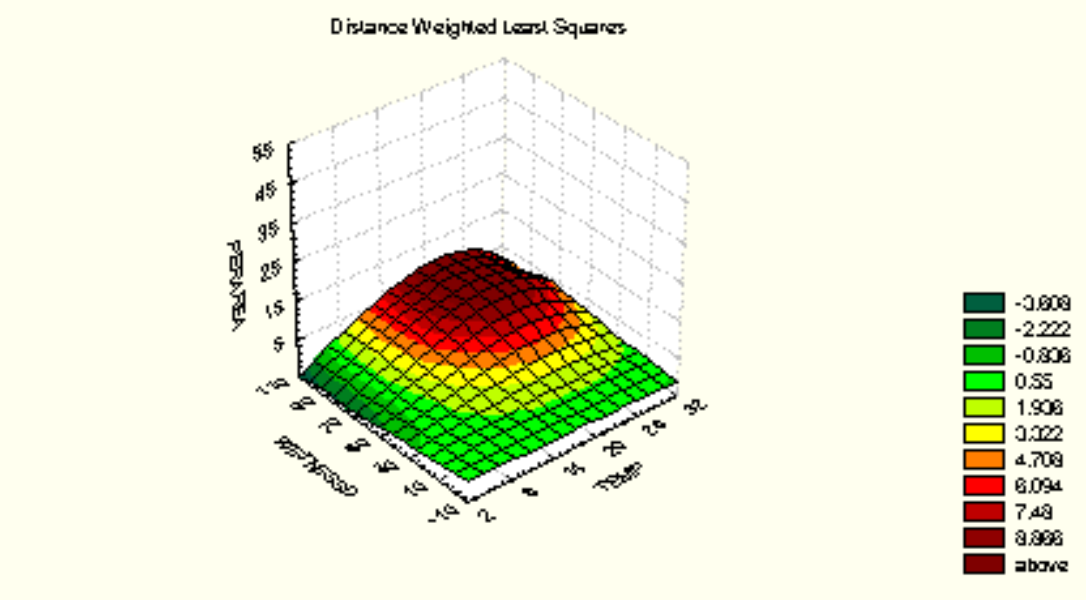

Figure 3. Response surface plot of percent area of lesions per leaflet versus temperature and wetness duration, for 14 days severity. Distance weighted least squares are used in this response surface generation.

Contour map.

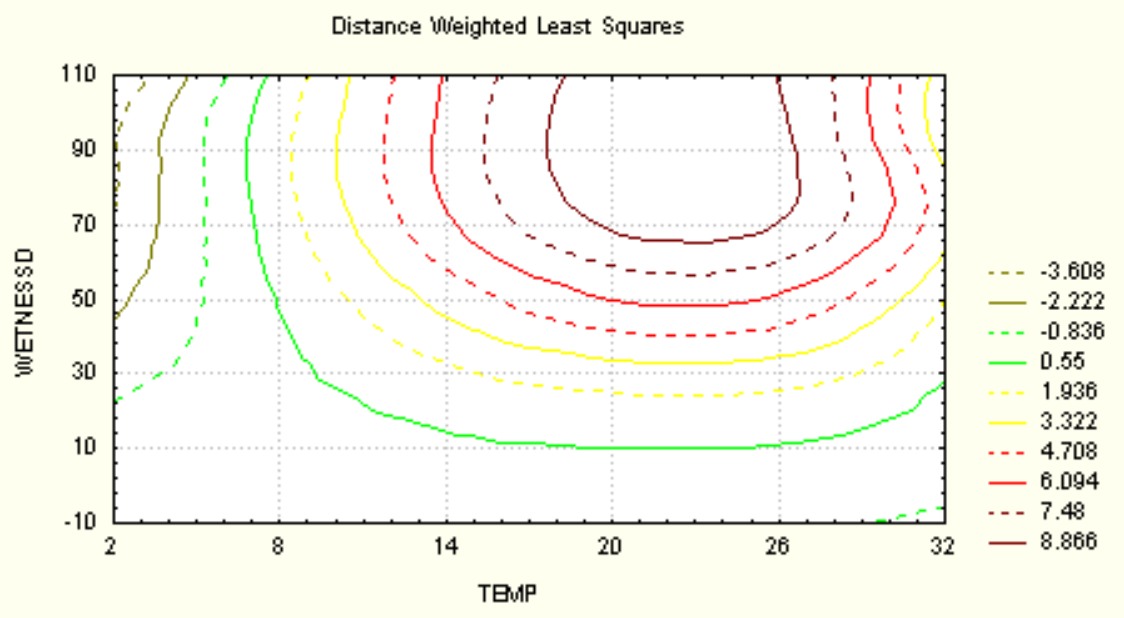

Figure 4. Contour plot of percent area of lesions per leaflet versus temperature and wetness duration using least squares analysis for 14 days severity.

Severity at 28 days.

STAT. VISUAL GLZ	PERAREA - Parameter estimates (ton3.sta) Distribution : NORMAL Link function: LOG					
Effect	Level of Effect	Column	Estimate	Standard Error	Wald Stat.	p
Interc		1	-4.19113*	.977161*	18.3963*	.000018*
TEMP		2	.30653*	.047837*	41.0592*	.000000*
TEMP^2		3	-.00505*	.000804*	39.4628*	.000000*
WETNESSD		4	.12226*	.017428*	49.2107*	.000000*
WETNESSD^2		5	-.00061*	.000095*	42.0558*	.000000*
TEMP*WETNESSD		6	-.00115*	.000355*	10.4854*	.001203*

Table 3. Regression analysis for response surface at 28 days severity. Response variable is percentage area of lesion per leaflet. All the regression coefficients are statistically significant.

STAT. VISUAL GLZ	PERAREA - Confidence Intervals of Estimates (ton3.sta) Distribution : NORMAL Link function: LOG			
Effect	Level of Effect	Column	Lower CL 95. %	Upper CL 95. %
Interc		1	-6.10633	-2.27593
TEMP		2	.21277	.40028
TEMP^2		3	-.00663	-.00348
WETNESSD		4	.08810	.15642
WETNESSD^2		5	-.00080	-.00043
TEMP*WETNESSD		6	-.00185	-.00045

Table 4. The 95% confidence intervals for the regression analysis for response surface at 28 days severity.

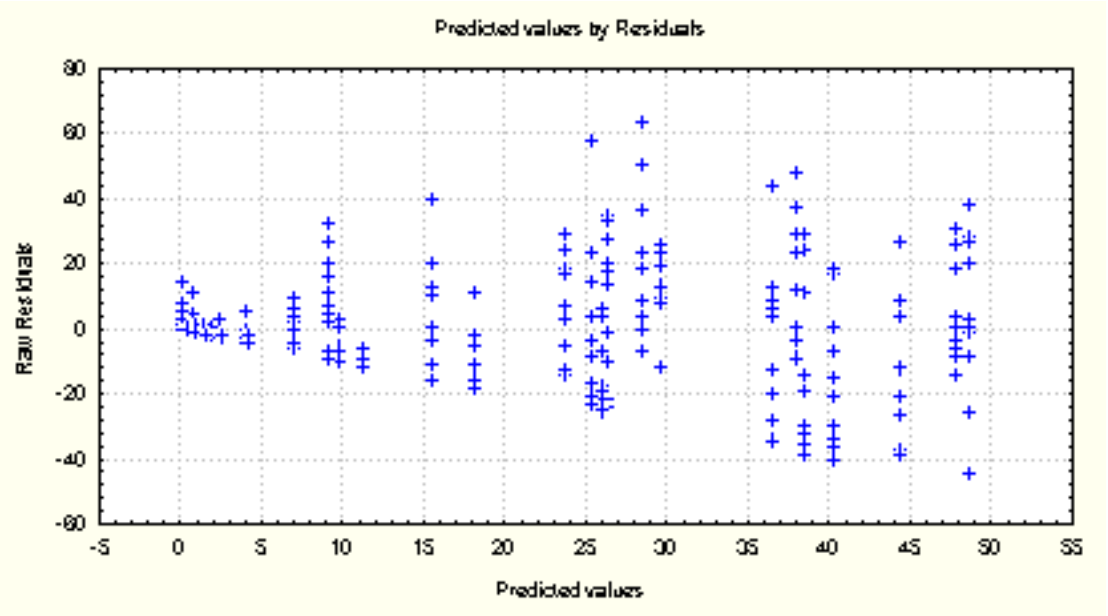

Figure 5. Residual errors plotted as a function of predicted values for severity at 28 days.

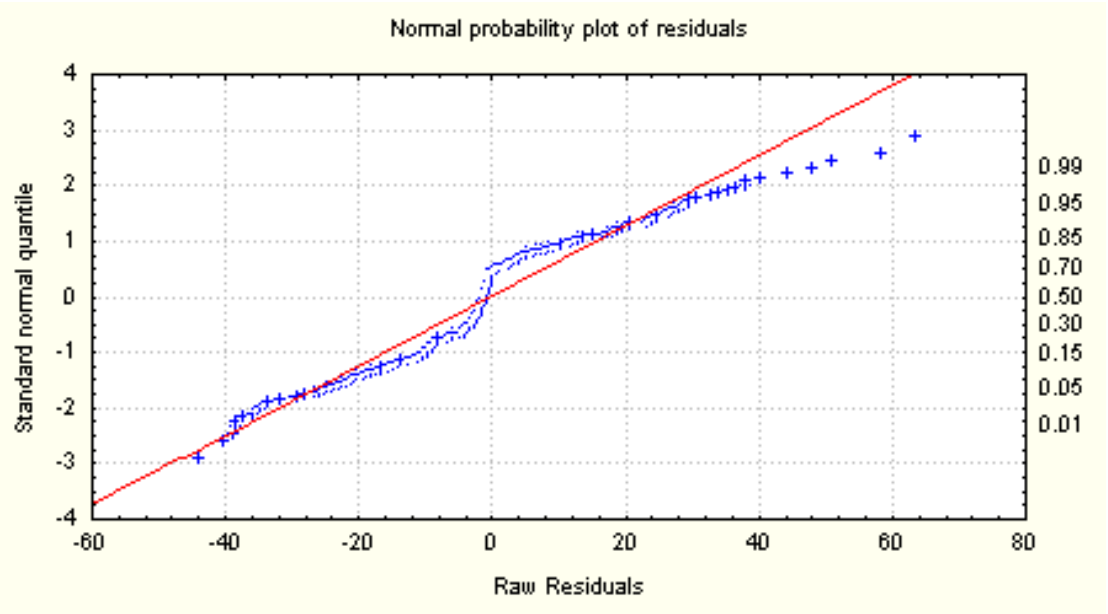

Figure 6. Normal probability plot for residuals which indicate relatively only a small amount of systematic bias in modelling data at 28 days severity.

Response surface.

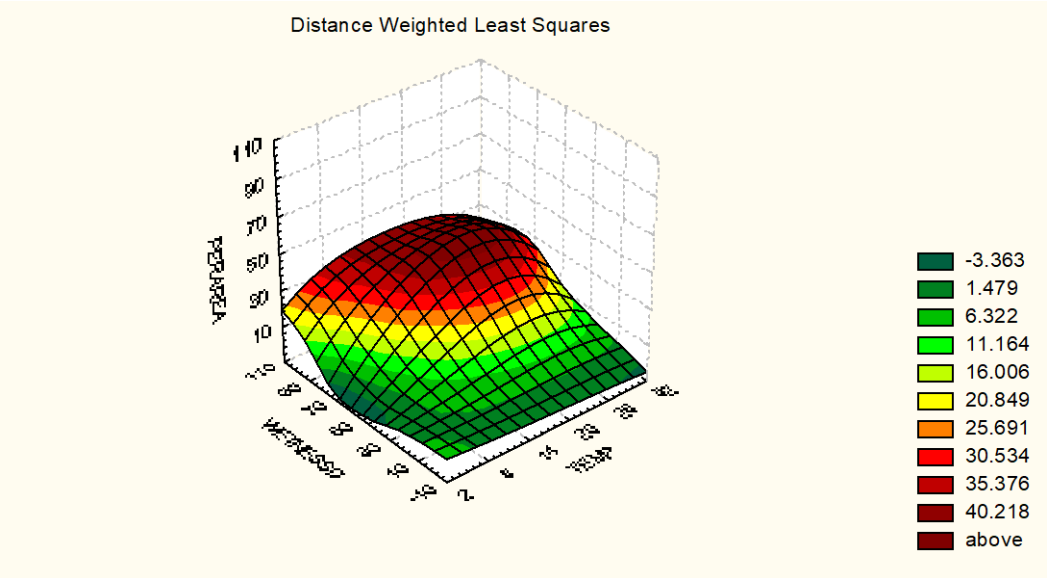

Figure 7. Response surface plot of percent area of lesions per leaflet versus temperature and wetness duration, for 28 days severity. Distance weighted least squares are used in this response surface generation.

Contour plot.

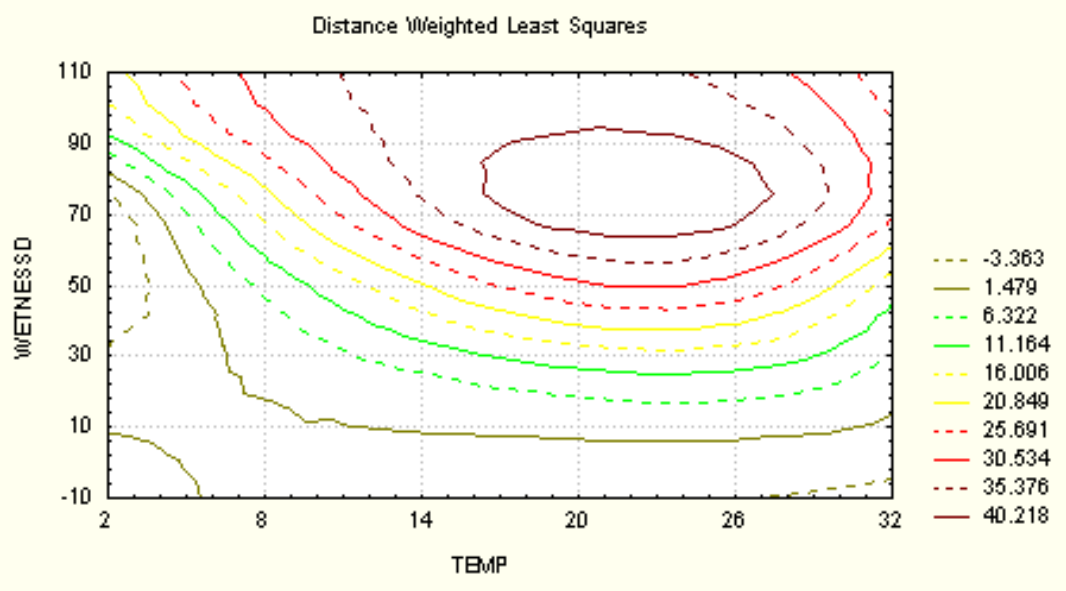

Figure 4. Contour plot of percent area of lesions per leaflet versus temperature and wetness duration using least squares analysis for 28 days severity.

Comments on the statistical analyses

For the 14 days disease severity data, the percentage area of leaflets affected by lesions generally increases with increasing wetness duration and temperature, and reaches a maximum at approximately 20ºC and 72 h of wetness duration. This was modelled by regression, using a quadratic model for each predictor and an interaction term. The coefficients and their 95% confidence intervals are shown in Tables 1 and 2 respectively and an indication of data and model compatibility is given by the residual vs predicted plot (Figure 1) and the probability plot for the residuals (Figure 2). For the 14 day data, systematic bias does occur due to large number of zeroes and sparse data generally. However, the 3-dimensional plot, smoothed by distance weighted least squares fit, shows an optimal ridge for wetness duration and temperature (Figure 3) perhaps better visualised as a contour plot (Figure 4.). After 72 h duration and >20ºC the percent area affected tends to decline.

For the 28 days disease severity data, the percentage area of leaflets affected by lesions generally increases with increasing wetness duration and temperature and reaches a maximum at approximately 25ºC and 72 h of wetness duration, which can be modelled by regression using the same form of equation. The coefficients and their 95% confidence intervals are shown in Tables 3 and 4 respectively and an indication of data and model compatibility is given by the residual vs predicted plot (Figure 5) and the probability plot for the residuals (Figure 6). For the 28-day data, systematic bias is much reduced and so data and model appear to be satisfactory. The 3-dimensional plot, smoothed by distance weighted least squares fit, also shows an optimal ridge for wetness duration and temperature (Figure 7) and in the corresponding contour plot (Figure 8). After 72 h duration and >25ºC, the percent area affected tends to decline. However, at lower temperatures (5-15ºC) wetness continues to have an increasing effect on lesion development beyond 72 hours.

Tables of means.

Tabulated Statistics

Control: SEVCODE = 1
Rows: Temp Columns: WETDUR

	0	6	24	48	72	96
5	0.050	0.333	0.117	0.100	0.000	0.317
10	0.000	0.000	0.000	0.000	1.283	1.717
15	0.000	0.067	0.000	0.583	2.317	2.900
20	0.000	0.000	2.450	9.117	14.267	13.000
25	0.000	0.083	2.417	8.167	11.933	13.833
30	0.000	0.000	0.000	1.717	5.200	2.600

Control: SEVCODE = 2
Rows: Temp Columns: WETDUR

	0	6	24	48	72	96
5	0.733	3.517	2.883	2.233	1.467	19.000
10	0.000	0.000	0.217	3.800	32.833	50.117
15	0.117	0.233	0.250	10.500	24.233	25.750
20	0.167	0.083	8.350	35.517	52.567	31.450
25	0.333	0.500	20.933	43.200	55.683	50.933
30	0.000	0.083	0.417	10.650	31.117	26.467

Cell Contents --
perarea:Mean

Tabulated Statistics

Control: SEVCODE = 1
Rows: Temp Columns: WETDUR

	0	6	24	48	72	96
5	0.158	0.385	0.249	0.225	0.000	0.687
10	0.000	0.000	0.000	0.000	1.212	1.618
15	0.000	0.141	0.000	0.934	3.029	2.955
20	0.000	0.000	2.514	5.218	9.370	13.726
25	0.000	0.264	1.230	3.917	4.808	11.036
30	0.000	0.000	0.000	1.755	4.738	2.509

Control: SEVCODE = 2
Rows: Temp Columns: WETDUR

	0	6	24	48	72	96
5	1.296	5.015	3.728	2.740	1.531	17.668
10	0.000	0.000	0.685	4.359	16.059	22.491
15	0.369	0.630	0.791	11.400	26.283	21.511
20	0.351	0.264	5.793	22.028	25.732	20.870
25	0.805	1.581	12.349	11.191	17.067	20.461
30	0.000	0.264	0.810	12.045	25.104	25.267

Cell Contents --
perarea:StDev