Contract report for the Horticultural Development Council

Outdoor lettuce: evaluation of new fungicides for ringspot control

FV 289

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Project title:	Outdoor lettuce – evaluation of new fungicides for ringspot control
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The results and conclusions in this report are based on a series of experiments 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

I 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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1. GROWER SUMMARY

1.1 Headline

Four products were identified, from an initial selection of nine, for further testing of ringspot control on lettuce. These are Amistar (azoxystrobin), Signum (pyraclostrobin + boscalid), Switch (fludioxonil + cyprodinil) and an experimental fungicide, with Scotts Octave (prochloraz) used as the standard.

Fungicide efficacy was strongly influenced by the timing of application relative to inoculation of lettuce plants with ringspot spores. Treatments were most effective when applied two days before or just prior to inoculation. Further experiments are planned to confirm the activity of these candidate fungicides against ringspot.

Note – Growers need to be aware that not all the actives/products listed above are currently approved for use to control ringspot on lettuce

1.2 Background and expected deliverables

Ringspot (also known as anthracnose), caused by the fungus *Microdochium panattonianum,* is a significant problem in some crops of field grown lettuce, rather less so in recent years in protected lettuce. Endive and chicory may also be affected. Epidemiological and disease control studies on ringspot have been undertaken in the 1980s and 1990s. This work is now out of date with regard to fungicides. Recent work involving lettuce ringspot has been limited, though azoxystrobin (as Abound Flowable fungicide) now has a recommendation for lettuce ringspot control in the USA.

Typical symptoms are brown leaf spots that lose their centre and become "shot-holes" and sunken lesions on mid-ribs. Severely affected plants are stunted. Most affected plants are unmarketable. Losses are variable from crop to crop, and are higher after prolonged wet periods. Problems continue in intensive lettuce producing areas particularly in southern England and in Scotland. A national loss in production of 1% is worth £0.4 million.

The fungus survives on crop residues and produces micro-sclerotia that are capable of survival in soil for several months. Seed-borne transmission has proved difficult to verify. After initial infection from soil, ringspot can develop rapidly through splash dispersal. Free moisture from rain, dew or irrigation is required for spore production, germination and infection. Germination occurs over a wide range of temperatures within 24 hours and symptoms can appear in as little as three days.

Control of ringspot relies on crop rotation of lettuce with other crops and use of fungicides. There are some differences in cultivar susceptibility, but this has only been exploited to a limited extent in the UK. Soil sterilisation may provide control of soil-borne inoculum, but its cost is usually prohibitive.

Fungicides offer the opportunity to control the disease within selected crops as required. Previous work in the UK identified activity in prochloraz and thiram; prochloraz, propiconazole and chlorothalonil showed activity in Australian screening studies. More recently activity has been shown for Amistar in the USA. Information is not available for many currently used or new products. The activity of new fungicides has been investigated in cereals and various other arable crops, particularly in HGCA-funded projects. These enable potential new products for vegetables to be identified at an early stage.

The activity against ringspot of fungicides recommended for other diseases on lettuce is not known e.g. Rovral Flo (iprodione) and Amistar are currently used for control of *Sclerotinia* on lettuce (SOLA nos. 2004/0513 and 2001/1465 respectively) and Signum has full label approval for *Sclerotinia* control on outdoor lettuce. In additional other new products have been introduced on arable crops which have potential for use on vegetables (e.g. azole products; the product Switch may be available as a lettuce SOLA in 2006/07).

Testing and ranking of fungicides will enable growers to select the best products for the range of diseases they encounter. The current choice of fungicides is limited and growers will require a selection of products to minimise risks of selecting fungicide resistant strains.

The project will complement work on other lettuce diseases being considered by HDC so growers have up-to-date information for the control of the major lettuce pathogens.

Overall aim of the project is:

To evaluate the efficacy of current and novel fungicides for ring spot control.

Specific objectives:

1. To define the curative and protectant properties of fungicides.
2. To define the dose response activity in relation to timing of the most promising products.
3. To quantify the persistence of products to guide timing of treatments.

1.3 Summary of the project and main conclusions

The project is testing new and existing fungicides for disease control activity against ringspot on outdoor lettuce. Tests are done under controlled conditions on lettuce plants grown in pots under glasshouse conditions and artificially inoculated with ringspot spores. Fungicides have been tested at full commercial rates and applied at four timings relative to inoculation (5 days before, 2 days before and just prior to inoculation and 2 days after inoculation). Disease assessments were done at weekly intervals for four weeks after inoculation. The percentage plants with ringspot and the percentage leaf area affected were recorded at each assessment. The data for the incidence of ringspot gave better separation of treatments than disease severity; the data for disease incidence are shown in Figure 1 and Figure 2.

All the fungicides gave a significant decrease in ringspot incidence compared with the untreated control (Fig. 1). There were some significant differences between products, with an experimental fungicide showing the lowest incidence overall and showing better control than all the products apart from Unicrop Thianosan DG (thiram).

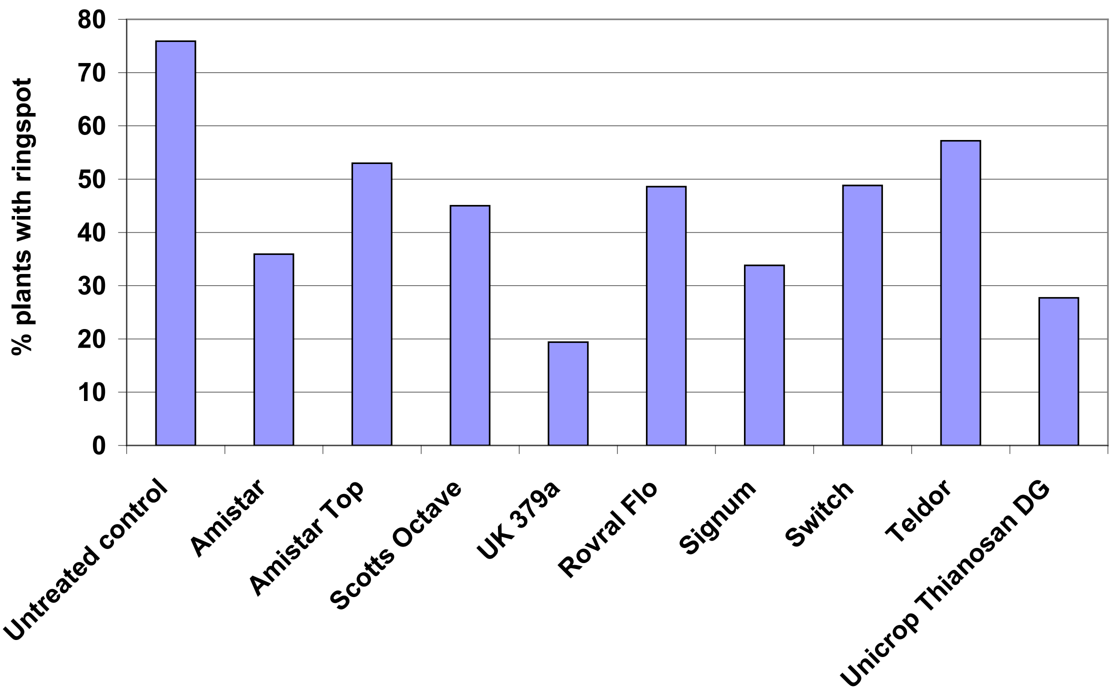

Figure 1. Comparison of fungicides for control of ringspot, 28 days after inoculation.

There was a significant effect of spray timing averaged over all products (Fig. 2). Applications made 5 days before inoculation or 2 days after inoculation were significantly less effective (P<0.01) than application just prior to inoculation. Treatment applications 2 days before inoculation gave similar results to those just prior to inoculation.

Treatments generally gave poor control when applied two days after inoculation and this suggests that there is likely to be limited curative (“kick-back”) activity against ringspot. This is not surprising given that symptoms can appear in 4 days under optimum conditions.

Interactions between fungicide product and spray timing were also significant. This suggests some differences in the mode of action of fungicides. For example, the experimental fungicide was very effective when applied 5 days before inoculation and Switch was more effective when used 2 or 5 days before inoculation than on the same day as inoculation.

From this first experiment four products have been selected for further testing along with Scotts Octave as the standard. These are Amistar, Signum, Switch and an experimental fungicide. The results of the first experiment should be treated cautiously at this stage. Some phytotoxicity was noted with Switch treatments and appears to relate to the test conditions used. Further experiments will continue to evaluate fungicide timing and dose during the remainder of the project.

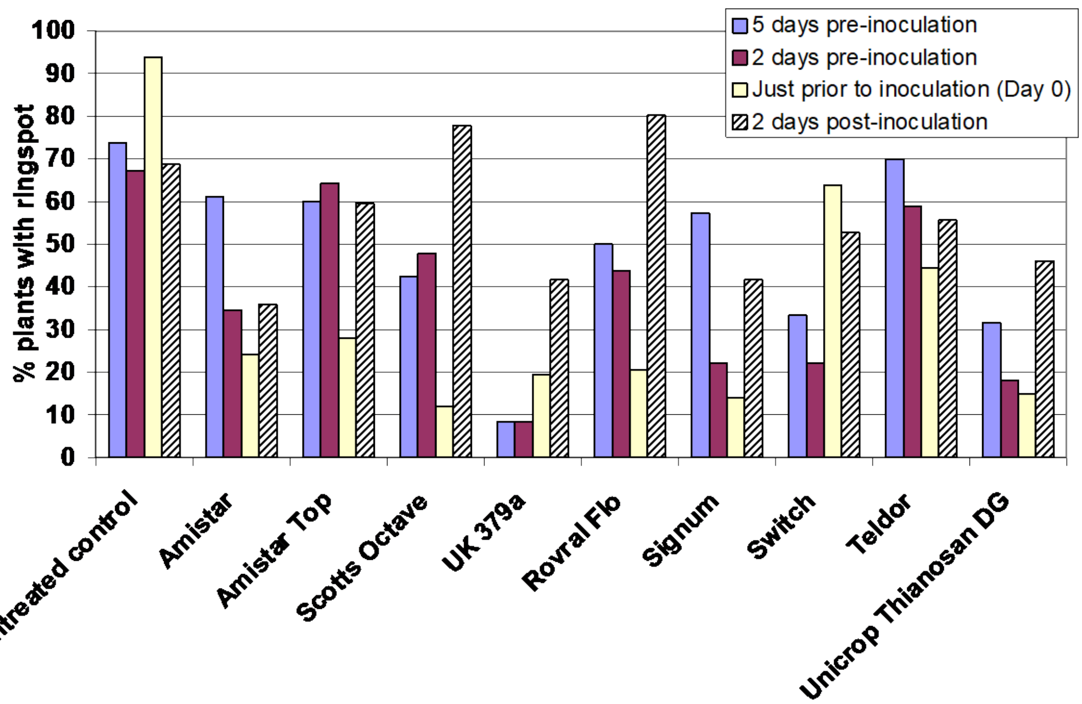

Figure 2. Effect of fungicide timing on ringspot control, 28 days after inoculation.

1.4 Financial benefits

Lettuce growers in England and Scotland continue to suffer losses from ringspot, particularly in Romaine types. An estimated national loss in production of 1% is worth £0.4 million.

The industry will benefit through reduced losses due to lettuce ringspot, achieved through:

- Increased knowledge of fungicides with activity against ringspot
- Information on fungicide efficacy in relation to dose and timing against ringspot
- Improved understanding of ringspot disease.

Project results will be of relevance to both home and overseas production. When linked to fungicide activity against other diseases (e.g. sclerotinia), more general disease control benefits may be produced when product choice is optimised.

1.5 Action points for the industry

- Ringspot symptoms develop very rapidly and fungicides show limited curative ("kick-back") activity. Protectant programmes will be required to achieve good control of ringspot.
- A range of fungicides available for use on lettuce show some activity against ringspot but only Scotts Octave carries a recommendation for ringspot control (see SOLA 0650/01 for outdoor lettuce).
- Further testing of candidate fungicides is required within the project to substantiate the initial promising results.

2. SCIENCE SECTION

2.1 Introduction

Ringspot (also known as anthracnose), caused by the fungus *Microdochium panattonianum,* is a significant problem in some crops of field grown lettuce, rather less so in recent years in protected lettuce. Endive and chicory may also be affected (Koike *et al*., 2007). Epidemiological and disease control studies on ringspot have been undertaken in the 1980s and 1990s (Galea *et al*., 1986; Galea and Price, 1988a & b). This work is now out of date with regard to fungicides (Wickes *et al*., 1994). Recent work involving lettuce ringspot has been limited, though azoxystrobin (as Abound Flowable fungicide) now has a recommendation for lettuce ringspot control in the USA.

Typical symptoms are brown leaf spots that lose their centre and become “shot-holes” and sunken lesions on mid-ribs. Severely affected plants are stunted. Most affected plants are unmarketable. Losses are variable from crop to crop, and are higher after prolonged wet periods. Problems continue in intensive lettuce producing areas particularly in southern England and in Scotland. A national loss in production of 1% is worth £0.4 million.

The fungus survives on crop residues and produces micro-sclerotia that are capable of survival in soil for several months. Seed-borne transmission has proved difficult to verify. After initial infection from soil, ringspot can develop rapidly through splash dispersal. Free moisture from rain, dew or irrigation is required for spore production, germination and infection. Germination occurs over a wide range of temperatures within 24 hours and symptoms can appear in as little as three days.

Control of ringspot relies on crop rotation of lettuce with other crops and use of fungicides. There are some differences in cultivar susceptibility, but this has only been exploited to a limited extent in the UK. Soil sterilisation may provide control of soil-borne inoculum, but its cost is usually prohibitive.

The activity of new fungicides has been investigated in cereals and various arable crops, particularly in HGCA-funded projects. These enable potential new products for vegetables to be identified at an early stage. Fungicides offer opportunity to control the disease within selected crops as required. Previous work in the UK identified activity in prochloraz and thiram; prochloraz, propiconazole and chlorothalonil showed activity in Australian screening studies. More recently activity has been shown for Amistar in the USA. Information is not available for many currently used or new products.

The activity of fungicides, against ringspot, which are recommended for other diseases on lettuce is not known e.g. Rovral Flo and Amistar are currently used for control of *Sclerotinia* on lettuce (SOLA nos. 2004/0513 and 2001/1465 respectively) and Signum has full label approval for *Sclerotinia* control on outdoor lettuce. In addition other new products have been introduced on arable crops which have potential for use on vegetables (e.g. azole products; the product Switch may be available as a lettuce SOLA in 2006/07).

Testing and ranking of fungicides will enable growers to select the best products for the range of diseases they encounter. The current choice of fungicides is limited and growers will require a selection of products to minimise risks of selecting fungicide resistant strains.

The project will complement work on other lettuce diseases being considered by HDC so growers have up-to-date information for the control of the major lettuce pathogens.

Results from this project may also be useful for product selection on other horticultural crops affected by similar leaf spots (e.g. *Ramularia*, *Marssonina*).

2.1.1 Overall aim of the project

To evaluate the efficacy of current and novel fungicides for ringspot control.

2.1.2 Specific objectives

1. To define the curative and protectant properties of fungicides
2. To define the dose response activity in relation to timing of the most promising products
3. To quantify the persistence of products to guide timing of treatments

2.2 Materials and Methods

The fungicide screening experiments were done on young lettuce plants grown in pots and artificially inoculated under glasshouse conditions. Lettuce seedlings (var. Frisco) were obtained from a commercial propagator (2-3 true leaf stage) and transplanted into F1 compost in 13 cm pots, four plants per pot. A total of 360 pots were used for the main experiment, with a further 9 pots used for the uninoculated control. A plot consisted of a group of three pots (each containing four plants) in a tray. Pots for the uninoculated control were placed 3-5 m away from the inoculated experiment in the same glasshouse. The plots were at least 0.5 m apart and arranged in a randomised complete block design on the glasshouse floor, with three replicate blocks. The plants were allowed to establish in the pots after transplanting for approximately 2 weeks and used in the experiment at the 3-4 true leaf stage. Watering was to the trays rather than overhead, to achieve moist but not waterlogged compost. The glasshouse temperature was set at a minimum of 10^{o}C, with venting at 20^{o}C. A data logger was set up to record air temperature and relative humidity for the duration of the trial.

Ringspot was obtained from a natural field outbreak of field lettuce in spring 2006. On the day of inoculation, a spore suspension of *M. panattonianum* was prepared from cultures on potato dextrose agar amended with streptomycin (PDA+S). Plates were flooded with sterile distilled water and a sterile scalpel or loop used to scrape the surface of 6-week old cultures of *M. panattonianum* to prepare a spore suspension. In addition, spores of *M. panattonianum* were collected from lettuce leaves with typical spores of ringspot that had been incubated for 24 h at approximately 20^{o}C in a plastic tray sealed in a polythene bag. The spore suspension was filtered through muslin and adjusted to a concentration of 1 x 10^{5} spores/ml. A 2 L volume of the spore suspension was prepared and applied to the point of run-off to each of 360 pots using a hand-held mister. Immediately after spore inoculation, the trial area was covered with a 'tent' of polythene sheeting (avoiding contact with plants) that was kept over the trays for approximately 48 h (until the last spray) to prolong leaf wetness duration and produce high relative humidity. Uninoculated control plants sprayed with water only were covered with separate sheeting.

Fungicide treatments (Table 1) were applied either 5 days before, 2 days before, immediately prior to artificial inoculation (Day 0), or 2 days after inoculation. The fungicides were applied in 1000 L water/ha (100 ml/m^{2}) using an Oxford precision sprayer with single nozzle (plus guard to prevent spray drift) at 2 bar pressure. Product rates are shown in Table 1.

The plants were monitored for disease development daily from five days after inoculation. The number of plants with ringspot symptoms (out of 4) and the severity of symptoms (percentage leaf area affected) were recorded for each plant 7, 15, 22 and 28 days after inoculation. Observations were made on phytotoxic symptoms or growth effects at each assessment.

Table 1: Fungicides used in the first fungicide evaluation (Experiment 1).

Product	Active ingredient	Product rate
Amistar	Azoxystrobin	1 L/ha
Amistar Top	Azoxystrobin + difenoconazole	1 L/ha
Scotts Octave	Prochloraz	0.2 kg/ha
UK 379a	Tolylfluanid + trifloxystrobin	1.44 kg/ha
Rovral Flo	Iprodione	2.3 L/ha
Signum	Boscalid + pyraclostrobin	1.5 kg/ha
Switch	Cyprodinil + fludioxonil	0.8 kg/ha
Teldor	Fenhexamid	1.5 kg/ha
Unicrop Thianosan DG	Thiram	4 kg in 1000 L water

Notes:

Amistar	SOLA 1465/01 for outdoor lettuce
Amistar Top	Administrative Experimental Approval
Octave	SOLA 0650/01 for outdoor lettuce
UK 379a	Administrative Experimental Approval
Rovral Flo	SOLA 0513/04 for outdoor lettuce
Signum	Outdoor lettuce on label
Switch	Administrative Experimental Approval (rate based on expected outdoor lettuce rate, B. Hall, Syngenta, pers. comm.)
Teldor	SOLA 0026/05 for outdoor lettuce
Unicrop Thianosan DG	Outdoor lettuce on label

Data for disease severity (% plant area affected by symptoms) was analysed by analysis of variance (ANOVA) using Genstat.

2.3 Results and Discussion

When the polythene sheet was taken off approximately 48 hours after inoculation, plants that had developed symptoms of grey mould due to *Botrytis cinerea* were removed to prevent subsequent spread of the disease (1-2 plants from each of 13 plots). These were treated as missing values in statistical analyses.

All the fungicide products except Rovral Flo gave significant decreases in the percentage of plants which developed ringspot after inoculation (Table 2). The differences in ringspot incidence between Rovral Flo and other fungicides was significant for Scotts Octave, UK379a, Signum, Switch and Unicrop Thianosan DG. The application of fungicides 2 days after inoculation gave significantly poorer control than applications 0, 2 and 5 days before inoculation. There was no significant interaction between fungicide and timing. UK379a, Switch, Signum and Scotts Octave reduced disease incidence to less than 10% when applied 5 days before inoculation. These products generally gave low disease incidence when used at the pre-inoculation timings. Control with Amistar Top and Unicrop Thianosan DG showed encouraging trends with activity improving as spray timing was closer to inoculation. Amistar and Switch showed the lowest disease incidence at the 2 days post-inoculation timing.

Table 2: Incidence of ringspot in relation to fungicide product and timing in Experiment 1 - 15 days after inoculation.

Product	Ringspot (% plants affected)				
	5 days pre-inoculation	2 days pre-inoculation	Just prior to inoculation	2 days post-inoculation	Mean
Untreated control	46.1	52.4	69.4	51.7	54.9
Amistar	33.3	3.0	21.0	11.1	17.1
Amistar Top	30.6	8.8	2.8	44.4	21.7
Scotts Octave	8.3	11.1	2.8	44.4	16.7
UK 379a	0.0	0.0	2.8	30.6	8.3
Rovral Flo	36.4	27.3	11.9	74.0	37.4
Signum	3.0	9.4	0.0	41.7	13.5
Switch	2.8	0.0	27.8	5.6	9.0
Teldor	23.3	30.6	27.8	22.2	26.0
Unicrop Thianosan DG	24.2	8.3	2.8	28.3	15.9
Mean	20.8	15.1	16.9	35.4	22.0
SED (df)	5.83 (timing) 18.45 (interaction)				9.22
P	0.003 (timing) NS (interaction)				<0.001

Table 3: Severity of ringspot in relation to fungicide product and timing, in Experiment 1 - 15 days after inoculation.

Product	Ringspot (% leaf area affected)				
	5 days pre-inoculation	2 days pre-inoculation	Just prior to inoculation	2 days post-inoculation	Mean
Untreated control	0.49	0.77	4.00	0.33	1.40
Amistar	0.27	0.01	0.11	0.04	0.11
Amistar Top	0.06	0.02	0.01	0.29	0.11
Scotts Octave	0.02	0.06	0.01	0.24	0.08
UK 379a	0.00*	0.00*	0.01	0.17	0.04
Rovral Flo	0.15	0.23	0.03	1.75	0.54
Signum	0.01	0.02	0.00*	0.58	0.15
Switch	0.01	0.00*	0.10	0.02	0.03
Teldor	1.57	0.16	0.23	0.07	0.51
Unicrop Thianosan DG	0.13	0.02	0.01	0.30	0.11
Mean	0.27	0.13	0.45	0.38	0.31
SED (df)	0.292 (timing) 0.924 (interaction)				0.462
P	NS (timing) NS (interaction)				NS

*trace only

The severity of ringspot lesions was low at the first assessment and no significant treatment differences were identified (Table 3). Ringspot was particularly severe in the untreated control for day 0.

Table 4: Incidence of ringspot in relation to fungicide product and timing in Experiment 1 - 28 days after inoculation.

Product	Ringspot (% plants affected)				
	5 days pre-inoculation	2 days pre-inoculation	Just prior to inoculation (Day 0)	2 days post-inoculation	Mean
Untreated control	73.7	67.3	93.9	68.7	75.9
Amistar	61.1	34.6	24.2	35.9	35.9***
Amistar Top	60.1	64.3	28.0	59.6	53.0**
Scotts Octave	42.4	47.9	12.0	77.8	45.0***
UK 379a	8.3	8.3	19.4	41.7	19.4***
Rovral Flo	50.0	43.8	20.5	80.3	48.6**
Signum	57.3	22.1	14.1	41.7	33.8***
Switch	33.3	22.2	63.9	52.8	48.8**
Teldor	70.0	58.8	44.4	55.6	57.2*
Unicrop Thianosan DG	31.6	18.1	15.0	46.1	27.7***
Mean	48.8**	38.7	33.6	56.0***	44.3
SED (df)	5.15 (timing) 16.30 (interaction)				8.15
P	<0.001 (timing) 0.014 (interaction)				<0.001

*= Significant at 5% level ; ** = Significant at 1% level; ***= Significant at 0.1% level

Table 5: Incidence of ringspot in relation to fungicide product and timing, in Experiment 1 - 28 days after inoculation

Product	Ringspot (% leaf area affected)				
	5 days pre-inoculation	2 days pre-inoculation	Just prior to inoculation	2 days post-inoculation	Mean
Untreated control	0.82	1.98	2.94	1.29	1.76
Amistar	0.98	0.17	0.17	0.18	0.37
Amistar Top	0.36	0.27	0.26	0.92	0.45
Scotts Octave	0.22	0.34	0.06	1.21	0.46
UK 379a	0.03	0.79	0.13	0.30	0.31
Rovral Flo	0.59	0.80	0.22	2.23	0.96
Signum	0.14	0.13	0.04	1.62	0.49
Switch	0.12	0.11	0.52	0.22	0.24
Teldor	1.42	0.16	0.23	0.07	0.51
Unicrop Thianosan DG	0.13	0.49	1.06	0.32	0.82
Mean	0.79	0.43	0.09	0.32	0.41
SED (df)	0.248 (timing) 0.784 (interaction)				0.392
P	NS (timing) NS (interaction)				0.009

* trace only

The overall mean incidence of ringspot increased from 22% of plants affected 15 days after inoculation to 44% plants affected 28 days after inoculation. Amistar, UK379a, Signum and Unicrop Thianosan DG gave significant decreases in ringspot compared with the untreated control. There were significant differences between individual fungicides (LSD = 16.23), with UK379a, Unicrop Thianosan DG, Signum and Amistar being more effective than Teldor and

Amistar Top (Table 4). There was a significant effect of fungicide timing with the lowest incidence of disease resulting from fungicide application just prior to inoculation (day 0). This gave significantly better control than either 5 days pre-inoculation or 2 days post inoculation. There was a significant interaction between fungicide and timing (LSD = 32.45) indicating differences in fungicide activity. UK379a was very effective when applied 5 days before inoculation and up to day 0, whilst Switch was more effective when applied 2 and 5 days before inoculation than at day 0 or 2 days after inoculation. Signum was most effective when used 2 and 0 days before inoculation than at day 5 before inoculation; Amistar showed a similar trend.

The severity of ringspot increased from 0.3% leaf area affected to 0.4% between 15 and 28 days after inoculation. This represented a considerable increase in the number of lesions as the leaf area of the lettuce plants also increased as new leaves were produced. All fungicides significantly decreased ringspot severity compared with the untreated control. The largest treatment difference between Rovral Flo and Switch did not quite reach significance (LSD = 0.78). There were no significant effects of fungicide timing or fungicide x timing interactions. There were a few leaves where the disease developed strongly and this has influenced severity data and treatment trends compared with ringspot incidence data. This was most apparent for UK379a applied 2 days before inoculation and Unicrop Thianosan DG applied on day 0 (Table 5).

There were some symptoms of phytotoxicity in the Switch treatments that resulted in cupping and reduced growth of the developing leaves. This is associated with the rate of product used in the experiment (the field lettuce rate is higher than the rate for protected crops) combined with the use of high humidity conditions at inoculation.

2.4 Conclusions

All the test fungicides showed some activity against ringspot. Disease incidence data (% plants affected) separated treatments rather more effectively than disease severity (% leaf area affected). As ringspot symptoms can develop in 4-8 days, there is limited opportunity to exploit curative activity against ringspot. There were significant effects of fungicide timing with the most effective sprays being applied 2 days before or just prior to inoculation. Some products were effective when applied up to 5 days before inoculation. Treatments applied 2 days after inoculation are unlikely to be very successful when the disease is developing rapidly. Commercially, it is appropriate to maintain protectant spray programmes when ringspot is present.

Scotts Octave (prochloraz) is currently available for ringspot treatment and performed well. Amistar, UK379a, Signum and Switch are showing promising activity against ringspot though caution is required given that this finding is based on a single experiment. Unicrop Thianosan DG performed well, but Scotts Octave has been given preference as a standard for the next experiment as it is the only fungicide with off-label approval for lettuce ringspot control. These four fungicides together with Octave as standard have been selected for the next phase of the project which will examine a restricted range of products at full and half dose.

2.5 Technology transfer

Telephone responses to growers, consultants and fungicide manufacturers requesting information on fungicide effects.

2.6 References

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2.7 Acknowledgements

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