Contract report for the Horticulture Development Council

Blackberry: evaluation of fungicides for improved control of downy mildew and purple blotch

SF 85

March 2008

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AUTHENTICATION

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

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Grower Summary

Headline

- Fungicides for control of blackberry purple blotch were evaluated in a field experiment; initial results (January 2008) indicate significant control with Folicur and Signum. There is potential for spore release by purple blotch over many months of the year during wet weather.

Background and expected deliverables

UK production of high quality blackberries for fresh fruit sales is increasing rapidly to satisfy the growing consumer desire for health-giving foods and a strong supermarket demand. Some of the major varieties being planted (e.g. Loch Ness, Chester Thornless, Loch Tay) are susceptible to downy mildew (*Peronospora sparsa*). Purple blotch is common on the older, established varieties Bedford Giant and Silvan, and lesions suggestive of purple blotch have been reported on some new spined varieties (e.g. Carmel, Karaka Black, Obsidian). Both diseases have the potential to devastate crop production. Damaging attacks of downy mildew have occurred in some Spanish tunnel crops. This project aims to:

- evaluate a range of fungicides for control of downy mildew and purple blotch.
- determine the duration of wetness that favours epidemic development of downy mildew.
- identify the period in the year when purple blotch spores are released.
- devise and test sustainable spray programmes.

The industry will benefit through more reliable production of high quality fruit.

Summary of the project and main conclusions to date

Evaluation of fungicides for control of downy mildew – autumn 2007

Ten fungicides were evaluated for control of downy mildew on the susceptible blackberry cv. Loch Ness. At 7 days after the final spray application, downy mildew affected less than 1% leaf area of untreated plants and there were no significant differences between treatments.

Effect of leaf wetness duration on downy mildew

Five leaf wetness durations (0-48 h) were applied weekly for seven weeks to pot-grown plants of cv. Loch Ness. Downy mildew severity remained low with no consistent effect from increasing leaf wetness duration. At one week after the final wetness application, the mean leaf area affected ranged from 0.2% (2 h wetness) to 1.9% (16 h wetness). No effect of leaf wetness duration on the incidence or intensity of sporulation was detected in this experiment.

Effect of fungicides on germination of Septocyta ruborum spores (cause of purple blotch)

Six fungicides were each tested at 2 and 20 ppm a.i. for their effect on germination of *S. ruborum* spores in an agar plate assay. Germination on unamended agar was 88%. Spore germination was reduced by Bravo 500, Signum, Amistar and Folicur, but not by Cleancrop Curve or Cuprokylt Fl, at the concentrations tested.

Evaluation of fungicide treatments for control of purple blotch

A field trial was established in Norfolk in a crop of cv. Silvan showing widespread infection by purple blotch. The training system used on the site was designed to allow primocanes to trail on the ground and tie them in during winter, after the old fruiting canes had been cut out. Five fungicides (Amistar, Bravo 500, Cuprokylt Fl, Folicur and Signum) each applied as programmes of three sprays were compared with an untreated control. The aim was to protect the primocanes developing in 2007. Sprays were applied when primocanes were 15-30 cm, 60-90 cm and post-harvest. Additionally, three programmes of Folicur applied at different spray timings were evaluated in crops where primocanes were tied-in as they grew. In one further

treatment, floricanes were cut out in spring and a three-spray Folicur programme was applied to tied-in primocanes (ie a biennial cropping system).

In May 2007 at the start of the experiment, purple blotch lesions were obvious on floricanes in all plots. In January 2008, the disease affected 62% surface area of untreated new season floricanes. In areas of crop where primocanes had trailed on the ground, the severity of purple blotch was significantly reduced by Signum and appeared to be reduced by Amistar, Bravo 500 and Cuprokylt Fl. Folicur significantly reduced purple blotch when applied to primocanes tied onto wires as they grew, and in a biennial cropping system, but not when applied to the crop where primocanes had trailed on the ground.

Occurrence of S. ruborum spore production

The effect of temperature and moisture on release of spores from fruiting bodies (pycnidia) of *S. ruborum* was examined in May 2007. Abundant spores were exuded within 3 days when blackberry canes bearing pycnidia of *S. ruborum* were maintained moist, irrespective of temperature (5-20°). No spores were produced when pycnidia were maintained dry for 18 days at 5, 10, 15 or 20°C.

Cane sections affected by purple blotch were collected from a naturally infected crop at intervals from 1 May to 1 August 2007. No spores were visible on any samples at the time of the collections. After moist incubation for 5 days, abundant spore exudation was observed from all lesions during May and June, and from some in July and August. When new season floricanes were examined in January 2008, spore exudation was observed after damp incubation. These results indicate a potential for spore release by *S. ruborum* over many months (January – August).

Occurrence of stem purpling and purple blotch on different varieties

Seven blocks of blackberry comprising six varieties on a farm in Norfolk were assessed for stem purpling and purple blotch in June 2007. Canes with sections discoloured purple and bearing fungal spore cases were found at a high incidence in

cvs. Silvan (99%), Karaka Black (32%) and Kotata (31%). These symptoms were absent on cv. Loch Tay and at a low incidence on cvs Chester Thornless (1%) and Loch Ness (3-15%). Purple blotch (*S. ruborum*) was confirmed only on cv. Silvan; pycnidia containing a different fungal spore were found in both blocks of cv. Loch Ness. Stem purpling with no evidence of fungal spore cases was common on cvs. Chester Thornless, Kotata and Karaka Black. No canes with wilted shoots were found except on cv. Silvan affected by purple blotch. These results suggest that stem purpling requires microscopic identification of associated fungi before concluding the purple blotch (*S. ruborum*) is the cause of purple lesions.

Development methods for production of purple blotch

In May 2007, pot grown plants of cv. Silvan were inoculated with *S. ruborum* by four methods. In September, plants were treated with half-rate paraquat or stored cold (4°C) for 48h in an attempt to advance the development or purple blotch symptoms. Neither the paraquat nor the cold-shock stress treatment resulted in early development of purple blotch symptoms; the first symptoms appeared in February 2008. By March 2008, purple blotch lesions were confirmed on many green canes, especially those inoculated with mycelium of *S. ruborum*, or where cane sections affected by purple blotch were suspended above developing primocanes.

Financial benefits

Production of high quality blackberries in protected Spanish tunnels for fresh fruit sales, has increased markedly to 60 ha in the last 2 years, and is expected to exceed 100 ha by 2008/09 in order to cope with supermarket demand. The two major diseases of blackberry are purple blotch and downy mildew and both have the potential to devastate production if not adequately controlled.

- For a tunnel-protected crop of cv. Loch Ness, assuming a yield of 20 tonnes/ha and returns of £6,500/tonne, the fruit value is around £130,000/ha.

- Assuming an average annual yield loss of 5% to the combined effects of these diseases, this equates to £6,500 / ha, or £650,000 p.a. for the UK

industry (100 ha); losses for individual; nurseries could be significantly greater than 5% (e.g. 50%).

- The potential benefit to growers from this project is the identification of treatments that provide effective control of downy mildew and purple blotch with minimum use of fungicides during fruiting, and thereby maintain high quality production without a significant yield loss to these diseases.

Action points for growers

- Purpling on blackberry canes can be caused by factors other than the purple blotch fungus, *Septocyta ruborum*. If purple lesions develop on canes, consult an expert to determine if purple blotch is the cause.

- In a blackberry variety highly susceptible to purple blotch (e.g. Silvan), seek to protect the primocanes from infection by treatment with Folicur, Amistar and/or Cuprokylt Fl during the period of rapid cane growth.

- Tying-in primocanes as they develop, rather than allowing them to trail on the ground until autumn, combined with fungicide treatment (see above) is likely to improve disease control.

- Alternate fungicide groups for sequential spray treatments in order to reduce the risk of selecting fungicide resistant pathotypes of *S. ruborum*.

Note

The new fungicide Signum showed good activity against purple blotch. Use of Signum on blackberry is not currently permitted. In March 2008, HDC submitted an application to PSD for an Off Label Approval for use of Signum on blackberry to control purple blotch.

Science Section

Introduction

Production of Spanish tunnel protected high quality blackberries for fresh fruit sales has markedly increased in the last two years to 60 ha, and this area is expected to exceed 100 ha by 2008/09 in order to cope with the demand from the major UK supermarkets. A range of new varieties are being planted including Loch Ness, Chester Thornless, Karaka Black, Carmel and Obsidian; also Helen and Loch Tay for early harvest. The major varieties at present are Loch Ness (c. 60% of the production area), Chester Thornless (15%) and Loch Tay (15%). Once planted, crops are often grown for 20 years or more. Crops are now generally produced in Spanish tunnels as required by the supermarkets and this allows for early production and harvesting in wet weather.

The two major diseases of blackberry are downy mildew *(Peronospora sparsa)* and purple blotch *(Septocyta ruborum)*. Both have the potential to devastate crop production if not adequately controlled. Downy mildew infects leaves, petioles, canes, flowers and fruit, while purple blotch is a disease of the stems and lateral shoots. In spring, auxiliary buds in sections of cane affected by purple blotch begin to grow normally but later stop growth and die. Also, the presence of purple blotch in a crop can markedly increase winter-kill of floricane.

Recent observations indicate that downy mildew and purple blotch continue to be the main diseases affecting modern varieties both grown in the open and under protection. Purple blotch has occurred on Bedford Giant and Silvan; downy mildew on Loch Ness, Chester Thornless and Loch Tay. Some growers have commented that downy mildew is worse on protected than open field crops. “Dryberry” symptoms caused by downy mildew can affect all the fruit on a lateral.

Until recently, control of downy mildew was largely based on use of Elvaron Multi (tolylfluanid), chlorothalonil products, copper based fungicides and the foliar fertiliser potassium phosphite, although none have specific approval for mildew control on blackberry. However, use of Elvaron Multi was suspended in early 2007 and use of chlorothalonil on blackberry was revoked in September 2007. Sprays for downy mildew are applied to protect new foliage, flowers and developing berries. Only copper fungicides and phosphite can be used during the period the crop is protected; unfortunately, use of the former is restricted to just three applications per year. Sprays of chlorothalonil and potassium phosphite at 14 d intervals did not provide adequate control of downy mildew on Loch Ness grown in Spanish tunnels (T. Chambers; pers. comm.). Control of purple blotch is usually sought using carbendazim. Use of carbendazim, however, is restricted to post-harvest applications,

permitted under SOLA for cane blight control and this approval expires on 30 June 2008. Isolates of *S. ruborum* resistant to carbendazim and copper fungicides have been reported in some plantings, increasing the difficulty in obtaining effective control of purple blotch.

There is an urgent need to identify alternative fungicides which provide effective control of downy mildew and purple blotch on blackberry with minimum risk of leaving pesticide residues in fruit.

The objectives of work in this first year of the project are primarily concerned with fungicide screening:

- To conduct a small-plot experiment to evaluate fungicides for downy mildew control;
- To set up a field experiment evaluating fungicide treatments for control of purple blotch;
- To investigate methods for evaluating fungicides against *S. ruborum* by a spore-germination or small *in planta* test.

Additionally, work is planned to investigate some aspects of the biology of each disease.

Evaluation of fungicides for control of downy mildew

Introduction

An experiment was devised to evaluate seven fungicides (two approved for use on blackberry and five novel products), the foliar fertiliser potassium phosphite (with and without wetter) and a wetter alone (Omex SW7) for control of downy mildew. There is evidence on rose that phosphate (O'Neill, 2008) and Omex SW7 combined with a foliar feed (G. Bahiri, pers. comm.) provide some control of downy mildew.

Materials and methods

Crop and site details

The experiment was located in a polytunnel at ADAS Arthur Rickwood, Cambs. Plants of the susceptible variety Loch Ness were potted in 1L containers on 22 August 2007 and placed on Mypex matting. Plants were irrigated by overhead sprinkler irrigation, supplemented with hand-watering. On 3 October, plants were wetted and then covered with polythene for 24 h in an attempt to encourage development of downy mildew.

Experimental design and statistical analysis

Treatments were arranged in four randomised blocks along the length of the tunnel, equidistant from overhead sprinklers. There was double replication of the untreated control in each block. There were 10 plants per plot, placed pot-thick, with a plant showing symptoms of downy mildew placed adjacent to each plot. Results were examined by ANOVA.

Treatments

Treatment details are given in Table 1.1. These were applied as high volume sprays at 1,000 L/ha using a knapsack sprayer. Sprays were applied at weekly intervals from 5 to 28 September, and then at 14 day intervals until 7 November, a total of seven applications.

Table 1.1: Details of fungicides, a foliar fertiliser and a wetter evaluated for control of blackberry downy mildew - 2007

Fungicide		Active ingredient	Rate of product	Based on :
1.	Untreated	-	-	-
2.	Amistar	Azoxystrobin	1.0 L/ha	SOLA 0511/94, protected blackberry.
3.	Cuprokylt FL	Copper	5.0 L/ha	SOLA 3132/06 Outdoor blackberry and 3139/06 protected blackberry.
4.	FarmFos 44	Potassium phosphite	2.5 ml/L	Nutrient. Outdoor and protected.
5.	FarmFos 44 + Silwett L77 wetter	Potassium phosphite + wetter at 0.05%	2.5 ml/L + 0.5 ml/L	Nutrient + adjuvant. Outdoor and protected.
6.	FarmFos 44 + Omex SW7 wetter	Potassium phosphite + wetter at 0.25%	2.5 ml/L + 0.25 ml/L	Nutrient + adjuvant. Outdoor and protected.
7.	Omex SW7 wetter	Silicon-based wetter	2.5 ml/L	Nutrient adjuvant.
8.	Signum	Boscalid + pyraclostrobin	1.8 g/L	Strawberry outdoor (label) and protected (SOLA 1673/04) 3 d hi
9.	Experimental	-	2.5 g/L	Company seeking to register for use on grapevine. Some control of rose DM in Columbia.
10.	Paraat	Dimethomorph	0.1 g/plant in 100 ml water per plant	Strawberry outdoor and protected, SOLA 1751/06. 1 application immediately after planting/potting
11.	Consento	Fenamidone+ propamocarb	2 ml/L	Blight fungicide. Max rate is 2 L/ha.
12.	Experimental	UKA 387	3 g/L	Good results on grape DM in trials. Max rate is 3 Kg/ha.

Disease assessments

Plants were individually assessed for the percentage leaf area affected by purple spots and blotches characteristic of downy mildew on three occasions during the experiment and at one week after the final spray application. The number of plants affected by downy mildew was also recorded.

Results and discussion

Downy mildew symptoms were visible on around 7% of plants at the first assessment (6 September), and this had increased to around 40% of plants at 1 week after the final spray (25 October). The disease was confirmed on a sample of leaves following microscopic examination. There were no statistically significant differences between treatments or blocks in the incidence of plants with downy mildew at any of the assessments (Table 1.2).

The disease remained at a low severity throughout the experiment, affecting less than 1% leaf area in all treatments (Table 1.3). None of the treatments significantly affected disease severity.

The reason for the disease failing to develop to a significant extent is unknown. Infector plants were introduced into each plot, and downy mildew sporulation was observed on leaves. Possibly the environment was insufficiently conducive for abundant sporulation or infection by the fungus, but this is considered unlikely as plants sporulation was observed and plants were watered by overhead irrigation at least once a week, generally late in the day to favour prolonged leaf wetness and infection. Possibly there was residual fungicide on plants sufficient to control downy mildew under the conditions of the experiment, although this also seems unlikely. Also, the mean daily temperature was generally in the range considered most favourable to infection by downy mildew (9-18°C).

Another possible explanation is that leaves were mostly fully expanded, the experiment being done relatively late in the season, and were less susceptible than recently emerged leaves produced in spring.

Table 1.2: Effect of fungicides, a foliar fertiliser and a wetter on the incidence of blackberry plants affected by downy mildew – autumn 2007

Treatment	Mean number plants affected (of 10)			
	6 Sep	19 Sep	01 Oct	25 Oct
1. Untreated	0.3	2.8	3.5	3.5
2. Amistar	0.0	1.5	2.5	3.0
3. Cuprokylt FL	0.5	2.5	3.3	3.5
4. FarmFos 44	1.0	4.0	4.3	5.0
5. FarmFos 44 + Silwet 77 wetter	1.0	2.5	3.3	3.3
6. FarmFos 44 + Omex SW7 wetter	0.8	3.0	3.8	3.8
7. Omex SW7 wetter	1.0	2.5	3.8	4.5
8. Signum	1.3	2.8	3.3	3.3
9. Experimental	0.0	3.5	4.5	4.5
10. Paraat	1.5	2.3	3.0	3.5
11. Consento	0.5	2.5	5.0	5.0
12. Experimental UKA 387	1.3	2.0	2.8	3.0
Significance (33 df)	NS	NS	NS	NS
LSD	1.32	2.19	2.67	2.78

Table 1.3: Effect of fungicides, a foliar fertiliser and a wetter on the severity of blackberry downy mildew – autumn 2007

Treatment	Mean % leaf area affected			
	6 Sep	19 Sep	01 Oct	25 Oct
1. Untreated	0.01	0.10	0.23	0.33
2. Amistar	0.00	0.05	0.09	0.25
3. Cuprokylt FL	0.04	0.18	0.29	0.52
4. FarmFos 44	0.11	0.32	0.46	0.59
5. FarmFos 44 + Silwet 77 wetter	0.05	0.20	0.45	0.64
6. FarmFos 44 + Omex SW7 wetter	0.05	0.24	0.43	0.62
7. Omex SW7 wetter	0.05	0.18	0.41	0.76
8. Signum	0.06	0.14	0.25	0.25
9. Experimental	0.00	0.20	0.35	0.37
10. Paraat	0.07	0.32	0.48	0.88
11. Consento	0.04	0.07	0.35	0.74
12. Experimental UKA 387	0.08	0.17	0.25	0.27
Significance (33 df)	NS	NS	NS	NS
LSD	0.086	0.274	0.374	0.665

Effect of leaf wetness duration on downy mildew

Introduction

Leaf wetness duration was shown to be a critical factor influencing development of rose downy (Xu & Pettitt, 2003), a disease caused by the same fungus (*P. sparsa*) as that causing blackberry downy mildew. The incidence of rose downy mildew increased gradually from 16 to 96 h, and then increased sharply from 96 to 120 h. The effects of temperature in the range 9 - 18°C were generally small. An experiment was therefore devised to determine the influence of leaf wetness duration ranging from nil to 48 h, on infection of blackberry by *P. sparsa*. Knowledge of the effect of leaf wetness duration on blackberry downy mildew could be useful in assessing the risk of this disease in commercial crops.

Materials and methods

Crop and site details

The experiment was located in a polythene tunnel at ADAS Arthur Rickwood. Plants of the susceptible variety Loch Ness were potted into 1 L pots on 22 August 2007 and stood in plastic gravel trays. Plants were watered by hand into the base of trays. Plants were arranged in groups of five around a central plant with symptoms of downy mildew.

Treatments

Leaf wetness durations of 0, 8, 16, 24 and 48 h were applied weekly for 7 weeks by watering plants with a watering can and then covering them with polythene for the requisite period. Overhead irrigation lines were not used to water plants. The tunnel was ventilated by leaving the sides and ends open to encourage rapid drying of leaves after removal of the polythene.

Experimental design and statistical analysis

Treatments were arranged in four randomised blocks along the length of the tunnel. Results were examined by ANOVA.

Assessments

Plants were individually assessed for the percentage leaf area affected by downy mildew. At 2, 4, and 8 weeks after the start of the experiment the undersides of leaves were examined for sporulation of downy mildew. Sporulation intensity was assessed on a 0 – 3 scale (nil, slight, moderate, profuse) according to the density of sporangiophores.

Results and discussion

At the start of the experiment downy mildew was present at trace levels in all treatment (Table 2.1). At the final assessment, 1 week after the seventh application of wetness treatments, the severity of downy mildew remained low, affecting <2% leaf area. Although there were statically significant differences in disease severity between some treatments at some assessments, there was no evidence of a trend of increasing downy mildew with increasing leaf wetness duration (Table 2.1). Leaf wetness duration had no significant effect on the incidence of symptomatic leaves with sporulation of downy mildew, or the intensity of sporulation (Table 2.2).

No firm conclusions on the effect of leaf wetness duration can be drawn from this experiment due to the lack of significant levels of disease. The reasons for the lack of disease development are unclear. Possibly there was some residual fungicide on plants initially, but this would not be expected to prevent disease development for more than 8 weeks. Possibly temperatures in excess of those most favourable to downy mildew (9 - 18°C) reduced disease development. Leaves of plants were visibly wet at removal of the polythene covers, indicating that the planned leaf wetness durations had been achieved; leaves were generally visibly dry within 1 hour of removal of the polythene covers. Coverage of container-grown rose plants with polythene for 72 h on a weekly basis for 3 weeks resulted in their severe defoliation due to epidemic development of rose downy mildew (HNS 135). Possibly blackberry leaves in the autumn are more resistant to downy mildew than young leaves developing in the spring. It is suggested that this experiment is repeated in spring 2008 and that weekly wetness duration treatment are extended to 96 h.

Table 2.1: Effect of leaf wetness duration on downy mildew leaf symptoms – autumn 2007

Weekly wetness duration (h)		Mean % leaf area affected						
		Week 0	1	2	3	4	6	8
1.	Nil	0.05	0.35	0.50	1.21	1.61	1.68	1.94
2.	8	0.05	0.19	0.20	0.21	0.21	0.21	0.21
3.	16	0.05	0.70	1.04	1.52	1.81	1.86	1.86
4.	24	0.14	0.46	0.71	0.86	1.12	1.22	1.32
5.	48	0.06	0.29	0.31	0.46	0.58	0.75	0.86
Significance		NS	NS	0.049	0.012	0.016	0.022	0.032
LSD		0.109	0.479	0.568	0.721	0.958	0.462	1.142

Table 2.2: Effect of leaf wetness duration on downy mildew sporulation after 8 weeks – autumn 2007

Weekly wetness duration (h)		No. leaves (of 5) with sporulation	Sporulation intensity (0-3)
1.	Nil	2.3	0.49
2.	8	0.5	0.08
3.	16	3.0	0.94
4.	24	3.3	1.45
5.	48	2.8	0.86
Significance		NS	NS
LSD		3.327	1.127

Effect of fungicides on germination of *Septocyta ruborum spores*

Introduction

Six fungicides were evaluated for their effect on germination of *S. ruborum* spores in a laboratory test. The aim was to identify fungicides with potential for control of purple blotch in the field.

Material and methods

Preparation of spore suspension

Pycnidia of *S. ruborum* were excised from blackberry canes and allowed to soak in sterile distilled water (SDW) for 24 h. The resultant suspension was filtered through muslin, centrifuged and re-suspended in 5 ml SDW.

Fungicide sensitivity test

Potato dextrose agar (PDA) plates amended with fungicides at 2 and 20 mg/L were prepared (Table 3.1). Twenty µL of spore suspension of *S. ruborum* was streaked onto the agar plates which were then incubated at 20°C. After 48 h, plates were examined microscopically, making three transects per plate until 50 spores of *S. ruborum* were located. Spores were deeded to have germinated when they had produced a germ tube at least as long as the spore.

Table 3.1: Fungicides evaluated for effect on germination of *S. ruborum*

Product	Active ingredient
1. Cleancrop Curve	50% Carbendazim
2. Bravo 500	50% Chlorothalonil
3. Cuprokylt Fl	27% cupric ammonium carbonate
4. Amistar	25% Azoxystrobin
5. Folicur	25% Tebuconazole
6. Signum	6.7% pyraclostrobin + 26.7% boscalid

Results and discussion

Germination of *S. ruborum* spores after incubation for 48 h was completely inhibited by chlorothalonil (Bravo 500) at both 2 and 20 mg/L, and was greatly inhibited by azoxystrobin (Amistar) and pyraclostrobin + bascalid at 20 mg/L (Signum) (Table 3.2). These three fungicides gave a greater inhibition of spore germination than the standard fungicides (carbendazim and copper) that have been used against purple blotch in recent years.

These results suggest that Bravo 500, Amistar and Signum warrant inclusion in a field experiment evaluating fungicides for control of purple blotch. In practice, Bravo 500 was not included (see section 4) due to the loss of approval for use of this product, or similar chlorothalonil-containing products, on blackberry.

Table 3.2: Effect of six fungicides on germination of *S. ruborum* spores – 2007

Treatment	% a.i.	Spore germination (%)	Inhibition (%)
1. Unamended PDA	-	88	-
2. Cleancrop Curve	2	70	20
	20	90	0
3. Bravo 500	2	0	100
	20	0	100
4. Cuprokylt FL	2	90	0
	20	88	0
5. Amistar	2	88	0
	20	8	91
6. Signum	2	44	50
	20	12	86

Evaluation of fungicides for control of purple blotch

Introduction

An experiment was devised to evaluate five fungicides (four approved for use on blackberry and one novel product), for control of blackberry purple blotch. A range of treatment timings was examined. Additionally, the effects of three crop management practices were investigated: i) primocane allowed to trail on the ground; ii) primocanes wound into support wires as they grew; iii) biennial cropping.

Infection of blackberry by *S. ruborum* occurs on primocanes, but symptoms do not develop until the following season when they are fruiting canes. In this experiment, the crop was treated in 2007 and the effects of treatments on purple blotch were determined in 2008. In the biennial cropping system, the crop is allowed to fruit only once every two years, so that primocanes and fruiting canes are never present together; theoretically this should significantly reduce the risk of purple blotch as the spores are considered to be largely spread by water splash and wind-driven rain, not in the air, so there should be limited opportunity for infection of the primocanes, when there are no floricanes close-by for most of the season.

Materials and methods

Crop and site details

The experiment was located in a naturally infected plantation of cv. Silvan at Terrington St Clement, Kings Lynn, Norfolk. The crop was around 6 years old. Except where stated otherwise, primocanes were allowed to trail on the ground and were tied-up in the winter, after removal of old fruiting canes, the standard training system used on the nursery. The crop was left uncovered. Fruiting canes were cut out at the end of each season and chopped up and allowed to decay in the grassed pathways between rows. A strip of ground around 1 m wide at the base of plants was un-grassed and maintained weed-free.

Experimental design and statistical analysis

Treatments were arranged in four randomised blocks, consisting or four adjacent rows (c. 50 m long). Each plot was 3 m long and contained at least 8 fruiting canes. The central 2.5 m of each plot was assessed for disease. Guard areas (3 m long) were left at the ends of rows and thin areas of crop were excluded from the experiment. Results were examined by analysis of variance.

Treatments

Details of fungicide products (Table 4.1) and treatments (Table 4.2) are given below. Fungicides were applied using a knapsack sprayer and lance with 02F110 nozzles operating at 2 bar pressure. Sprays were applied to both sides of the row and to primocanes trailing on the ground at 1,000 L/ha.

Table 4.1: Fungicides evaluated for control of blackberry purple blotch - 2007

Fungicide	Active ingredient	Rate of use	Approval status on blackberry
Amistar	Azoxystrobin	1.0 L/ha	SOLA 0365/03 Expires 31-12-11. Max 2 sprays 7d hi (outdoor).
Bravo 500	Chlorothalonil	5.0 L/ha	No longer approved. Label revoked in 2007
Cuprokylt Fl	Cupric ammonium carbonate	5.0 L/ha	SOLA 3139/06. Expires 31-12-13. Max 4 sprays.
Folicur	Tebuconazole	0.8 L/ha	SOLA 0897/05. Expires 6-5-09. Max 3 sprays, 14 d hi
Signum	Pyraclostrobin + boscalid	1.8 Kg/ha*	Not approved

*Rate used on strawberry.

Table 4.2: Treatments evaluated for control of purple blotch - 2007

Treatment	Crop management	Fungicide	Spray timings
1.	Primocanes on ground	-	-
2.	Primocanes on ground	Bravo 500	15-30cm, 60-90cm, Sep
3.	Primocanes on ground	Cuprokylt Fl	15-30cm, 60-90cm, Sep
4.	Primocanes on ground	Amistar	15-30cm, 60-90cm, Sep
5.	Primocanes on ground	Signum	15-30cm, 60-90cm, Sep
6.	Primocanes on ground	Folicur	15-30cm, 60-90cm, Sep
7.	Primocanes wound-in	-	-
8.	Primocanes wound-in	Folicur	15-30cm, 60-90cm, Sep
9.	Primocanes wound-in	Folicur	May, June, July (monthly)
10.	Primocanes wound-in	Folicur	According to rainfall
11.	Biennial cropping, primocanes wound in	Folicur	15-30cm, 60-90cm, Sep

For treatment 10, Folicur was applied when the weekly rainfall total measured at ADAS Terrington (around 0.5 mile distant) exceeded 5 mm, providing no spray had been applied in the preceding 2 weeks, up to a maximum of three sprays.

The actual dates of treatment application are shown in the results.

Assessment of purple blotch

The occurrence of purple blotch cane lesions in each plot was assessed on a 0 – 3 scale (nil, slight, moderate, severe) in April 2007, before any fungicides were applied. At the same time, the proportion of dead or wilting canes was determined. Cane death and wilting of laterals in the spring is considered to be a consequence of purple blotch cane infection and is more common after a cold winter.

On 22 January 2008, the incidence of the new season floricanes affected by purple blotch lesions, and the severity of the disease were determined. Disease severity was measured by estimating the % cane length affected by purple blotch symptoms on 10 canes/plot (5 on each side of the row).

Crop growth

At each spray application date, the length of 20 primocanes in an untreated area of the crop was measured. When the final spray application was applied on 7 September, floricanes had not been removed and primocanes had not been wound into the support wires.

Crop management

Primocanes were wound-in as required at each site visit (see Appendix 2). T11 fruiting canes were removed in May 2007.

Results and discussion

Control of purple blotch

In May 2007, purple blotch was widespread in the experimental area with a mean disease severity of 1.5 – 2.3 (0 – 3 index). There were no significant differences between treatments in the severity of purple blotch of the incidence of dead and wilting canes (Table 4.3). Around 4 – 11% of fruiting canes in each plot were wilting or dead at this time, most probably due to purple blotch. No symptoms of purple blotch were observed on primocanes during the period of fungicide treatment, the final inspection being on 7 September 2007.

By 14 January 2008, purple blotch lesions were visible on many floricanes (these having been primocanes in 2007). Spores characteristic of *S. ruborum* were found when pycnidia within lesions were crushed and examined microscopically, confirming that this fungus was the probable cause of the purple lesions. There were generally numerous lesions per cane, which often merged together making it impractical to count them. Disease severity was therefore assessed as the percentage cane length area covered by purple lesions.

On 22 January 2008, the incidence of fruiting canes affected by purple blotch lesions ranged from 90 – 100%. There was no significant difference between treatments. The severity of purple blotch (% cane length affected) ranged from 17% to 62% with significant differences between treatments (Table 4.4). Compared with the untreated (T1), disease severity was significantly reduced by Signum (T5), Folicur applied to primocanes that were trained onto wires as they grew (T8, T9, T10), and Folicur applied to the biennial cropping system (T11). Bravo 500, Cuprokylt FL and Amistar all appeared to reduce purple blotch severity, compared with untreated plants, though differences were not quite significant at the 5% probability level. Folicur (T6) appeared slightly less effective than these fungicides when applied at the same timings.

Recent studies in the USA, in a year with little purple blotch development, reported significant reductions in the disease following use of boscalid + pyraclostrobin (Pristine, at 1.4 kg/ha) applied one or twice to new flushes of primocane that appeared after the final application of herbicide to burn-off early primocanes (Kaufman *et. al.,* 2006). The use of a herbicide to burn-off early primocanes also reduced the disease.

The severity of purple blotch on untreated primocanes that were trained as they grew onto the support wires (53.9%), was slightly less than that on untreated primocanes allowed to trail on the ground until autumn (61.8%). Canes that were trained as they grew onto the support wires and treated with Folicur (T8, T9, T10), had significantly less purple blotch than canes allowed to trail on the ground and treated with the same fungicides (T6). Application of Folicur at monthly intervals (T9), appeared to give slightly better control of purple blotch than application according to growth stage (T8) or accumulated rainfall (T10), though these differences were not significant. Possibly application of Folicur on a monthly basis reflects protection of the developing primocane for a greater period of time, as application according to growth stage resulted in the first two sprays being applied just 14 days apart. However, a Folicur application on 4 July as used in T9 would not be practical in a commercial crop without some fruit destruction as this fungicide has a 14 day harvest interval and fruit picking commenced on 30 June.

Although it did not eliminate purple blotch, management of plants as a biennial crop and treatment with Folicur at the conventional spray timings (T11), resulted in the lowest disease severity (17.1%). Possibly the level of control may have been greater if the floricanes had been removed before May 2007. Results elsewhere in this project indicate there is potential for spore release from floricanes from as early as January. Obviously the loss of yield in non-cropping years needs to be taken into account if biennial cropping is to be recommended as a means of reducing purple blotch.

For treatments 2 – 6, 8 and 11, the target growth stage for application of the first and second sprays were primocanes at 15 – 30 cm and 60 – 90 cm length respectively (Table 4.5). The actual mean lengths at these times were 38.5 cm and 80.1 cm respectively. Possibly a greater level of control may have been achieved if the first spray had been applied earlier. Our measurements indicate that between 2 May and 4 July, primocanes grew in length at a rate of around 2 cm/day. It would be difficult to maintain complete protection of the developing primocane with fungicides during this period of rapid growth, especially with fungicides that are not systemic in blackberry, or are not re-distributed on the cane surface by rainfall. When considering which fungicides to use in a programme of sprays for control of purple blotch, it would seem appropriate to use Amistar as the final pre-fruiting spray, as this has a shorter harvest interval (7 days) than Folicur, and appears to give slightly better control.

Table 4.3: Levels of purple blotch prior to start of spray program (May 2007)

Treatment	Mean purple blotch severity (0-3 index)	% dead or wilting canes
1. Untreated	1.5	4.7

2. Bravo 500	2.0	8.2
3. Cuprokylt FL	1.8	4.0
4. Amistar	2.3	9.4
5. Signum	2.0	9.6
6. Folicur	1.8	1.8
7. Untreated	2.0	11.0
8. Folicur	2.0	7.1
9. Folicur	1.8	5.8
10. Folicur	1.8	9.1
11. Folicur (biennial crop)	-	-
Significance (30 df)	NS	NS

Table 4.4: Effect of fungicide programmes and crop management practices on purple blotch of blackberry, cv. Silvan – 22 January 2008

Treatment	Spray date (2007)			Mean % cane length affected
Primocanes left on the ground				
1. Untreated	-	-	-	61.8
2. Bravo 500	2 May	16 May	07 Sept	45.2
3. Cuprokylt FL	2 May	16 May	07 Sept	45.7
4. Amistar	2 May	16 May	07 Sept	40.6
5. Signum	2 May	16 May	07 Sept	**27.8**
6. Folicur	2 May	16 May	07 Sept	53.8
Primocanes trained onto wires as they grow				
7. Untreated	-	-	-	53.9
8. Folicur	02 May	16 May	07 Sept	**32.8**
9. Folicur (3x, monthly)	02 May	01 June	04 July	**17.1**
10. Folicur (rain triggered)	11 May	01 June	20 Jun	**26.5**
Biennial crop				
11. Folicur	02 May	16 May	07 Sept	**17.1**
Significance (30 d.f.)				0.013
LSD				26.05

Results in bold are significantly lower than the untreated (T1).

Table 4.5: Growth of primocanes at different spray dates in 2007 (mean length of 20 canes)

Date spray applied	Mean primocane length (cm)	Crop growth stage
2 May	38.5	Pre-flowering
11 May	70.4	-
16 May	80.1	Fruiting buds removed T5
1 June	95.5	-
20 June	122.3	Developing fruit visible
4 July	169.9	-
7 September	213.8	Post-harvest

Fruit was picked in this crop from 30 June to 19 July.

Occurrence of *S. ruborum* spore production

Introduction

Information on when spores of *S. ruborum* are produced in UK blackberry crops could help to define the period of time when newly developing primocanes are likely to become infected or contaminated by the fungus. Current information on both the period of spore production and recommended fungicide timing is conflicting. A crop of blackberry cv. Silvan naturally infected by purple blotch was examined at intervals in 2007 to determine the presence of pycnidia of *S. ruborum* and their ability to produce spores. The effect of three temperature and two moisture levels on spore exudation from pycnidia was also examined.

Materials and methods

Five stem pieces bearing purple lesions indicative of purple blotch were collected on five occasions between 1 May and 3 August 2007. Stems were examined microscopically within a few hours of collection and again after incubation for 3 – 5 days in a damp chamber at ambient temperature in the laboratory. Lesions were examined for the presence of pycnidia and the occurrence of cirrhi (spore masses) exuding from them; a sample of spores was examined using a high power microscope to confirm that the spore shape and size was typical of *S. ruborum*.

On 18 May, five stem pieces affected by purple lesions were placed on damp paper towel or dry paper towel in closed plastic boxes and incubated at 5, 10 and 15°C. Additionally, stem pieces were incubated on damp power towel and dry paper towel on the laboratory bench and outside. Stems were examined microscopically after 3, 6 and 18 days for the occurrence of pycnidial spore exudates.

Results and discussion

All of the stem pieces collected between 1 May and 3 August bore pycnidial fungi. None of the pycnidia were found to be exuding spores on immediate examination. After damp incubation for 3 – 5 days, spores of *S. ruborum* had exuded from pycnidia on most stems and were visible as white tendrils (Table 5.1). The proportion of purple lesions producing spores of *S. ruborum* was fewer on samples collected on 4 July and 3 August, than on earlier samples.

High humidity was found to be necessary for spore exudation. None of the lesions incubated under dry conditions had produced spores after 18 days, whereas all pycnidia incubated in humid conditions had produced spores after 3 days. Temperatures in the range 5 - 20°C had no obvious influence on spore exudation (Table 5.2).

A sample of canes with purple blotch lesion collected on 14 January 2008 bore pycnidia. There was no exudation of spores on immediate examination. Pycnidia examined after crushing were found to contain spores typical of *S. ruborum*. After damp incubation of canes at ambient room temperature for 18 days, spore exudates from pycnidia were confirmed as *S. ruborum.* Ten cane slivers with purple blotch lesions were collected on 7 March 2008. Although none bore spores on immediate examination, all were exuding spores of *S. ruborum* after damp incubation for 3 days.

These results indicate that pycnidia of *S. ruborum* are likely to exude spores in wet weather from May to August, and probably for a longer period. The potential for pycnidia of *S. ruborum* to produce spores in a crop during this period suggest that developing primocanes should be protected against infection during this period.

Table 5.1: Production of spores from pycnidia of *S. ruborum* on blackberry canes: May – August 2007

Sample date (2007)	Pycnidia visible on purple blotch lesion	Spore exudation visible (out of 5)	
		Immediately	After humid incubation
1 May	Yes	0	5
11 May	Yes	0	5
16 May	Yes	0	5
20 June	Yes	0	5
4 July	Yes	0	2
3 August	Yes	0	3 (out of 7 pieces

Table 5.2: Effect of temperature and humidity on exudation of spores by pycnidia of *S. ruborum*

Incubation:			N° stems with spore exudation (out of 5) after:			Mean number pycnidia (or 20) exuding spores after 6 days
Moisture		Temperature (°C)	3 days	6 days	18 days	
1.	Moist	5	5	5	-	20
2.	Moist	10	5	5	-	20
3.	Moist	15	5	5	-	20
4.	Moist	20	5	5	-	20
5.	Dry	5	0	0	0	0
6.	Dry	10	0	0	0	0
7.	Dry	15	0	0	0	0
8.	Dry	20	0	0	0	0
9.	Dry	Lab bench	0	0	0	0
10.	Moist	Lab bench	5	5	-	20
11.	Dry	Outside	0	0	0	0
12.	Moist	Outside	5	5	-	20

Occurrence of stem purpling and purple blotch on different varieties

Introduction

Although many blackberry varieties show stem purpling, in many instances there are no distinct lesions, or they are not typical of purple blotch caused by *S. ruborum*. This suggests that the cause of purpling in some cases may be due to a factor other than *S. ruborum*, and possibly the symptom is not always of pathogenic origin. A range of varieties of blackberry on a farm in Norfolk were examined for symptoms of purple blotch, occurrence of pycnidia and the presence of *S. ruborum*.

Materials and methods

Seven blocks of blackberry comprising six varieties were assessed on 26 June 2007. The plantation ranged in age from two to 15 years, with most at least 10 years old. Every tenth floricane in a row was examined until a total of 100 canes/variety was reached. Canes were assessed for the presence of wilting shoots, stem purpling with pycnidia visible (suspect purple blotch) and stem purpling without pycnidia. A sample of sections of purpled canes of each variety was collected. These were incubated in a damp chamber prior to microscope examination to check for *S. ruborum* spores.

Results and discussion

Symptoms suggestive of purple blotch were readily found on cv. Silvan and less commonly on cvs. Loch Ness (one block), Kotata and Koraka Black (Table 6.1). Purple lesions bearing pycnidia were absent on cv. Loch Tay and present at trace levels on cvs. Chester and one block of cv. Loch Ness. Stem purpling without pycnidia was common on cv. Kotata and relatively common on cvs. Koraka Black and Chester. Shoot wilting on canes affected by purple blotch was observed on cv. Silvan at a low level (3% of canes). No symptoms suggestive of purple blotch were observed on primocanes of any of the varieties.

Spores typical of *S. ruborum* conidia were found in pycnidia on cv. Silvan and not on the other varieties (Table 6.1). Spore cases exuding a spore different from *S. ruborum* conidia were found on both blocks of cv. Loch Ness. These results suggest that purple lesions on cvs. Loch Ness, Kotata and Koraka Black may not be due to *S. ruborum*. Possible explanations for the cause of purple areas on canes where *S. ruborum* was not confirmed are: i) symptoms were caused by infection from *Botrytosphaeria dothidiea* (Botrytosphaeria cane canker), *Elsinoe veneta* (cane spot), downy mildew (*Peronospora sparsa*) or another pathogen; ii) infection by *S. ruborum* on varieties other than Silvan does not result in the production of any spores within pycnidia by early summer. Further investigation of lesions on these varieties is required to determine their probable cause.

Table 6.1: Occurrence of stem purpling with and without fungal spore cases, shoot wilting and confirmation of *S. ruborum* on blackberry varieties on a farm in Norfolk – 2007

Blackberry variety and age of planting (years)	N° of canes (of 100) with:			Fungal identification where spores produced
	Stem purpling with fungal spore cases	Stem purpling without fungal bodies	Wilted shoots and purple blotch present	
1. Silvan (15)	99	1	3	*S. ruborum*
2. Chester (10)	1*	22	0	Unidentified
3. Loch Ness (15)	3*	9	0	Unidentified
4. Loch Tay (2)	0	0	0	No pycnidial fungi
5. Loch Ness (10)	15*	7	0	Unidentified
6. Kotata (10)	31	69	0	No spore exudate
7. Koraka Black (3)	32	49	0	No spore exudate

* Pycnidia in white patch around purple lesions, especially in lower 50 cm of cane; possibly cane spot.

Development of methods for production of purple blotch

Introduction

Evaluation of treatments for control of purple blotch is a long procedure (around 10 months) due to the extended latent period between cane infection and symptom development. Symptoms are reported to develop after cold damage in winter. An experiment was devised seeking to speed the development of purple blotch symptoms by applying cold stress and herbicide damage, and thereby allow more rapid treatment evaluation.

Materials and methods

Young blackberry plants cv. Silvan were potted in March 2007 and grown in 3 L pots outside in a sheltered area at ADAS Arthur Rickwood. The following procedures for inoculation with *S. ruborum* were tested in May 2007:

1. Uninoculated control.
2. Spray-inoculation of primocanes with a spore suspension of *S. ruborum.*
3. Blackberry canes sections (c. 10 cm long) naturally infected by *S. ruborum* were suspended above and close to the primocane tips.
4. Inoculation of primocanes with a 8 mm diameter mycelial plug of *S. ruborum*. Three plugs were applied at 5, 15 and 25 cm from shoot tips.
5. As in T4, with mycelial plugs applied to wounded primocanes. Wounds were created by removing the epidermis with a sterile scalpel blade.

Each treatment was applied to 4 plants.

Spores of *S. ruborum* for treatment 2 were collected from naturally-infected cane sections. Plants in treatments 2 and 3 were maintained humid for 48 h after inoculation by covering

inoculated canes with a polythene bag, loosely tied around the canes. In September 2007, one plant from each treatment was sprayed with half-rate paraquat, one was placed in a cold store (4°C) for 48 h, and the remaining two plants were left outside. The inoculated canes were examined at intervals from August 2007 to March 2008 for occurrence of purple blotch lesions. Results were examined by ANOVA.

Results and discussion

No symptoms of purple blotch were observed on any of the plants until February 2008. Occurrence of purple blotch in March 2008 is shown in Table 7.1. The mean number of purple blotch lesions per cane was significantly affected by inoculation treatment (p-0.004), and not by the cold stress treatment (p=0.913). The development of purple blotch lesions was greater following inoculation with mycelium of *S. ruborum* and where cane pieces affected by purple blotch were suspended above developing primocanes than inoculation by a single spray with a suspension of *S. ruborum* spores or no inoculation. *S. ruborum* was confirmed in a sample of purple blotch lesions on green canes examined microscopically. Canes treated with paraquat were dead and no distinct purple blotch lesions or *S. ruborum* pycnidia were identified; another pycnidial fungus (*Phoma* sp.) was found on dead canes

Further work is required to devise an inoculation system that reliably results in infection by *S. ruborum* and more rapid development of purple blotch symptoms. Possible reasons for the lack of early symptom development in this study are inoculum level, incubation conditions after inoculation and the level of plant stress caused by cold damage and paraquat treatment.

Table 7.1: Effect of inoculation with *S. ruborum* and post-inoculation stress treatments on development of purple blotch in pot-grown blackberry, cv. Silvan, March 2008

Treatment of primocanes	Mean number of purple lesions/cane (March 2008)		
	No stress treatment	Cold-shock for 48h	Mean
1. Uninoculated	0.5	4.0	1.7
2. Spore inoculation	0.3	0.3	0.3
3. Adjacent infected canes	4.8	3.8	4.5
4. Mycelium inoculation	8.8	4.7	7.4
5. Mycelium on damaged area	2.5	3.7	2.9
Significance (9 df)			0.004
LSD			2.02

Conclusions

1. Amistar, Bravo 500, Folicur and Signum all have activity against *S. ruborum*, inhibiting spore germination.
2. Severity of purple blotch was reduced by three-spray programmes of Folicur and Signum; Amistar, Bravo 500 and Cuprokylt FL appeared to reduce disease severity but differences were not quite significant at the 5% probability level.
3. Tieing-in primocanes during the season and a biennial cropping system reduced purple blotch compared with allowing primocanes to trail on the ground.
4. Purple blotch pycnidia on floricanes have the potential to release spores during wet weather from at least May to August and probably over a longer period.
5. *S. ruborum* pycnidia containing spores were only found associated with purple lesions on canes of cv. Silvan; purpling on canes of other blackberry varieties may not be due to *S. ruborum.*

Technology transfer

Project review meeting, 13 March 2008, Norfolk.

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Appendix 1: Efficacy of fungicides against downy mildew (ADAS Arthur Rickwood 2007)

Trial diary.

Date	Action carried out
22/08/2007	400 blackberry cv. Loch Ness potted into 1 L pots containing C2 compost. 10 plants per pot.
05/09/2007	First spray applied. Paraat applied as drench
06/09/2007	% DM per plant recorded.
13/09/2007	2nd spray applied.
19/09/2007	% DM per plant recorded.
21/09/2007	3rd spray applied
28/09/2007	4th spray applied
01/10/2007	Assessed all plants for % DM. Covered all plants with polythene after wetting leaves.
03/10/2007	All plants covered with polythene after rewetting leaves.
04/10/2007	All polythene covers removed from plants.
11/10/2007	5th spray applied.
16/10/2007	Plants irrigated and then covered with polythene.
19/10/2007	Polythene covers removed.
25/10/2007	All plants assessed for % DM. Tiny tag logger downloaded. Spray 6 applied to trial.
07/11/2007	Spray 7 applied.
16/11/2007	Trial completed. No further disease assessment carried out as 1. disease has not increased, 2. leaves turning purple.

Appendix 2: Efficacy of fungicides against purple blotch (Terrington, Norfolk) - 2007

Date	Action carried out
01/05/2007	Trial marked out. Number fruiting canes / plot assessed
02/05/2007	First spray applied
11/05/2007	T10 Folicur applied. 20 random primocanes measured.
	Five purple blotch slivers were removed and checked for spores
16/05/2007	Trial area second spray applied to T2, T3, T4, T5, T6, T8, T11
	20 random primocanes measured in untreated plots
	Five purple blotch slivers were removed and checked for spores
01/06/2007	T9 and T10 sprayed. 20 random primocanes measured
20/06/2007	T10 Folicur applied. 20 random primocanes measured.
04/07/2007	T9 spray applied. 20 random primocanes measured.
	5 slivers of purple blotch removed and assessed in lab
07/09/2007	Final spray applied T2, T3, T4, T5, T6, T8, T11.
	20 random primocanes measured in untreated plots
	5 slivers of purple blotch removed and assessed in lab
22/01/2008	Assessment of purple blotch cover - 10 random canes
	from each plot assessed for % purple blotch cover

Appendix 3: Effect of leaf wetness duration on downy mildew (ADAS Arthur Rickwood Autumn 2007)

Trial Diary

Date	Action carried out
22/08/2007	100 blackberry plants c.v. Loch Ness potted out
18/09/2007	Leaf wetness trial week 1 started. T2, T4, T5 leaves wetted and covered with polythene (9 am) T3 plants leaves wetted and covered at 5 pm. Polythene removed from T2 at 5 pm. All treatment leaves rewetted
19/09/2007	Polythene removed from T3 and T4 (9 am). T5 leaves rewetted
20/09/2007	T5 polythene removed. All plants assessed for % DM Leaf wetness trial week 2. T2, T4 and T5 covered in polythene (9 am)
25/09/2007	T3 wetted and covered in polythene (5 pm). T2 uncovered (5 pm).
26/09/2007	T3 and T4 plots uncovered. 20 leaves (10 green, 10 with DM sporulation) assessed for presence of DM. Scored as per protocol.
27/09/2007	T5 uncovered. 20 leaves (10 green, 10 with DM sporulation) assessed On T5. All plots assessed for % DM sporulation.
02/10/2007	Leaf wetness trial week 3. T2, T4 and T5 wetted and covered (9 am) T3 plants wetted and covered in polythene, T2 plants uncovered (5 pm)
03/10/2007	T3 and T4 plots uncovered (9 am)
04/10/2007	T5 plants uncovered (9 am) All plants assessed for % DM including central infector plants.
09/10/2007	Leaf wetness trial week 4. T2, T4 and T5 wetted and covered with polythene at 9 am. T3 plots wetted and covered at 5 pm. T2 uncovered at 5 pm.
10/10/2007	T3 and T4 uncovered at 9 am.
11/10/2007	T5 plots uncovered (9 am). All plants assessed for % DM. 10 green leaves and 10 DM leaves assessed according to protocol for DM sporulation.
16/10/2007	Leaf wetness trial week 5. T2, T4 and T5 wetted and covered in polythene at 9 am. T3 plants wetted and covered with polythene at 5 pm. T2

	plots uncovered at 5 pm.
17/10/2007	T3 and T4 plots uncovered at 9 am.
18/10/2007	T5 plots uncovered (9 am). All plants assessed for % DM. 10 green leaves and 10 DM leaves assessed according to protocol for DM Sporulation
23/10/2007	Leaf wetness trial week 6. Experiment as per protocol. Loggers downloaded.
24/10/2007	Leaf wetness plots uncovered as per protocol.
25/10/2007	T5 plot uncovered (9 am). All plants assessed for DM.
30/10/2007	Leaf wetness trial week 7. Experiment started as per protocol.
31/10/2007	Plots T3 and T4 uncovered as per protocol
01/10/2007	Plot T5 uncovered as per protocol.
06/11/2007	Leaf wetness trial week 8 started as per protocol. T2 plot covers removed at 5 pm.
07/11/2007	T3 and T4 plot covers removed at 9 am
08/11/2007	T5 plot uncovered (9 am).
09/11/2007	% DM per plant assessed. DM sporulation assessed on 10 green and up to 10 DM leaves per plant. 3 leaves collected per plot with DM. Each examined for % area with sporulation.

Appendix 4: Weekly temperature and humidity records for polytunnel 4: