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DISCLAIMER

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

Defra project HH3233SSF – Biology, epidemiology and management of leaf spot and botrytis on black currant

Draft final report on Objective 2 – Biology and epidemiology of black currant leaf spot and Objective 4 – Effect of fruit age on susceptibility to Botrytis

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1 Executive summary

1.1 Scientific summary

1.1.1 Leaf spot

1. *Drepanopezizza ribis* causes the leaf spot disease of blackcurrant and may lead to severe premature leaf-fall, leading to reduction in yield and quality. This disease is currently managed by frequent fungicide applications.
2. Research was carried out to investigate infection of leaves in relation to environmental conditions and leaf age on cvs. Baldwin and Ben Hope in controlled inoculation studies.
3. All leaves on selected shoots were inoculated and then incubated under different initial conditions: 10, 17.5 and 25°C each with five wet periods (4, 8, 12, 24 and 30 h). Number of infected leaves was determined.
4. The two cultivars differed significantly in the susceptibility to infection by *D. ribis* – cv. Baldwin was much more susceptible than cv. Ben Hope.
5. Older leaves were more susceptible to infection with conidia (asexual spores) than younger leaves. Increasing length of wetness duration led to increasing incidence of leaves infected.
6. The effects of temperature were inconclusive and generally very small in comparison with other factors.
7. Monitoring of field epidemics three years (2005-07) confirmed the main findings from controlled inoculation studies: severe disease was associated with extreme wet conditions and older leaves.
8. Field data suggested that significant disease increase only occurred from the July onwards.
9. Previous studies indicated that ascospores released from apothecia (sexual fruiting bodies) on overwintering leaves could infect both young and old leaves and were responsible for initiating the spring epidemic. It seems likely that most of the observed leaf spot infection in the field plot resulted from conidia rather than ascospores and this probably accounts for the lack of early spring infection and the clear association with old leaves.

10. Overwintering of leaf spot as lesions on twigs and fruit strigs may be more important than previously thought.
11. From these findings, a control strategy was developed and this should be evaluated in the near future.

1.1.2 Botrytis

1. *Botrytis* is an important disease of blackcurrant that reduces yield and quality, and currently managed by frequent fungicide applications.
2. Research was carried out to investigate infection of flowers and fruit in relation to environmental conditions and fruit age on cvs. Baldwin and Ben Hope.
3. The two cultivars did not differ significantly in the susceptibility of flowers to *Botrytis* infection. Nearly 75% of flowers were infected or abscised one week after inoculation and nearly all remaining flowers failed to develop into mature fruit.
4. Fruits were inoculated at different growth stages and then incubated under different initial conditions: 10, 15, 20 and 25°C each with four wet periods (4, 8, 12 and 24 h). Number of infected flowers, aborted young fruits, and fruit with visual symptoms and latent infection was determined.
5. Infection of fruit was not significantly affected by the temperature and duration of wetness.
6. The two cultivars differed significantly in their responses to *Botrytis* infection depending on the fruiting stage at the time of inoculation. Inoculation of young fruitlets resulted in nearly 50% of fruits aborted on cv. Baldwin, compared to ca. 10% on cv. Ben Hope.
7. Inoculation of fruit near harvest resulted in significantly fewer fruit aborted.
8. The incidence of latent infection decreased with increasing fruit age at the time of inoculation.
9. Frequent sampling of blackcurrant fruit in an unsprayed planting of these two cultivars in open-field and under-protection conditions supported the main conclusions drawn from the controlled inoculation studies: flowers are most susceptible to infection and fruit became less susceptible as they age.
10. The results of the botrytis spray timing trial (not included in this report) largely agree with the inoculation studies in that the best control of botrytis was achieved in plots sprayed during flowering and green fruit.

11. Thus irrespective of weather conditions, strategies must be adopted to reduce inoculum and the extent of flower infections.
12. In addition, effective control of powdery mildew is also important since visible or microscopic mildew lesions on fruit may facilitate infection of fruit by *Botrytis*.

1.2 Growers' summary

1.1.1 Leaf spot

1. Baldwin was much more susceptible than Ben Hope.
2. Older leaves were more susceptible to infection than younger leaves.
3. Increasing length of wetness duration led to increasing incidence of leaves infected.
4. The effects of temperature were inconclusive and generally very small in comparison with other factors.
5. Significant disease increase in field conditions only occurred from the July onwards (2005-07).
6. From these findings, a control strategy was developed
    a. Strict control of infection on rosette leaves may not be essential because much of the epidemic and leaf fall associated with leaf spot was mainly observed on extension shoots. This is particularly true when crop husbandry measures had been applied during overwintering phase to reduce overwintering inoculum and hence minimise the ascospore risk.
    b. Rosette leaves (strig leaves) may be important in supplying nutrients to the fruit so as insurance, one spray against infection on rosette leaves may be used if the disease the previous season was very severe and extreme wet weather was forecast or experienced or if there is excessive leaf litter in the plantation and therefore a possibility of ascospore infection.
    c. From late June onwards, application of fungicides to control leaf spot must be timed to coincide with weather patterns. Here, the model may be used to indicate the magnitude of risks. Correct choice of fungicides may also depend on our knowledge on their physical mode of actions against *D. ribis*.
7. This strategy should be evaluated in experimental plots and in trial plots on commercial farms in the near future before being adopted widely.

1.1.2 Botrytis

1. Baldwin and Ben Hope did not differ significantly in the susceptibility of flowers to *Botrytis* infection: most inoculated flowers were infected or abscised one week after inoculation and nearly all remaining flowers failed to develop into mature fruit.
2. The two cultivars differed significantly in their responses to *Botrytis* infection depending on the fruiting stage at the time of inoculation. Inoculation of young fruitlets resulted in nearly 50% of fruits aborted on cv. Baldwin, compared to ca. 10% on cv. Ben Hope.
3. Infection of fruit was not significantly affected by the temperature and duration of wetness.
4. The incidence of latent infection decreased with increasing fruit age at the time of inoculation; inoculation of fruit near harvest resulted in significantly fewer fruit aborted.
5. Frequent sampling of blackcurrant fruit in an unsprayed planting of these two cultivars in open-field and under-protection conditions supported the main conclusions drawn from the controlled inoculation studies: flowers are most susceptible to infection and fruit became less susceptible as they age.
6. From these findings, a control strategy was formulated:
    a. Irrespective of weather conditions, strategies must be adopted to reduce inoculum (experiments looking at inoculum suppression will be conducted in late winter) and the extent of flower infections – reducing debris and applying fungicides one or twice during the period when most flowers were open.
    b. Stringent control was also needed during the four weeks post blossom (i.e. sprays applied during flowering and early green fruit); this is particularly true for susceptible cultivars like Baldwin
    c. Effective control of powdery mildew is important since visible or microscopic mildew lesions on fruit may facilitate infection of fruit by *Botrytis*.
    d. If fruit are processed within 7-10 days of harvest, then stringent botrytis control is only necessary during the flowering period and early fruiting

period (2 weeks after blossom). This is because infection on fruit normally results in visible rot systems only after harvest.

7. This should be evaluated in the near future before being adopted widely.

Objective 2 – Biology and epidemiology of black currant leaf spot

2 Infection of blackcurrant leaves by **Drepanopezizza ribis**

2.1 Introduction

There are about 2100 ha of blackcurrants in the UK; most of the blackcurrant crop is processed for juice. Blackcurrant is attacked by many pests and diseases, which are mainly controlled by scheduled application of pesticides. As with other fruit crops there is pressure from consumers to reduce pesticide input. Recent research on developing integrated pest management systems, e.g. on apple (Berrie and Xu, 2003) and strawberry (Berrie *et al.*, 2002), demonstrated that effective IPM is dependent on a good understanding of the biology and epidemiology of the diseases.

The two main diseases in blackcurrant are leaf spot (*Drepanopezizza ribis*) and *Botrytis* (*Botrytis cinerea*). Leaf spot caused by the fungus *Drepanopezizza ribis* is an important disease of blackcurrants wherever they are grown (Locke et al., 2002; Muller and Gottwald, 1989; Zakharieva, 1978). Much research was done on the biology and epidemiology of the disease in the early part of the last century which is reviewed by Blodgett (Blodgett, 1936). In the UK the life cycle of *D ribis* and the overwintering as the sexual state was confirmed in work at Long Ashton in 1953 (Corke, 1953). Since then there appears to have been very little research on leaf spot in the UK other than evaluation of fungicides for control (Locke et al., 2002). Much of the research in Europe, mainly Eastern Europe has also focused on control and evaluation of fungicides (Burth and Ramson, 1985; Grongorg, 1984), but limited studies in Poland identified the timing of ascospore release during spring and the need to apply fungicides pre bloom to achieve control (Borecki, 1962).

There appears to be little recent data on timing of ascospore maturation in spring, the conditions for spore release and infection and the duration of ascospore release. Similarly there is little information available on conditions needed for infection of leaves by conidia. This project aims to generate this information which will enable better spray timing in spring and give an indication when the risk from ascospores has finished, allowing better management of the disease. Studies by Corke indicated that the leaf spot fungus only overwinters on dead leaves (Corke, 1953) similar to the apple scab fungus. The impact of this disease the following spring can be considerably reduced by elimination of leaf litter and Corke (Corke, 1953) also demonstrated this for leaf spot. Studies have also shown for scab that post harvest treatment with a DMI fungicide reduced the incidence of the sexual state overwintering on leaves (O'Leary and Sutton, 1986). Use of urea also has a similar effect on apple scab as well as encouraging leaf breakdown (Burchill, 1968; Burchill et al., 1965). Such treatments may also have similar effects of the overwintering stage of leaf spot and could be exploited to reduce overwintering inoculum. In apple orchards earthworms play an important part in removal of leaf litter. Limited evidence suggests that populations of earthworms are very low in blackcurrant plantations (Burchill, 1968).

Experiments were conducted to investigate infection of blackcurrant leaves by *D. ribis* in relation to other factors. Specific objectives include (1) susceptibility of leaves to *D. ribis* in relation to leaf age, and (2) effects of temperature and duration of wetness on infection of leaves by *D. ribis*.

2.2 Materials and Methods

2.2.1 Controlled inoculation studies

Potted plants were inoculated in Sanyo controlled environment (CE) growth cabinets (model SGC170.CFX.J), equipped with fluorescent/tungsten lights, humidity, temperature and misting controls in 2007. Inoculation was conducted in April and July.

Plants: Potted three-year-old bushes of two cultivars: Baldwin and Ben Hope were used in all controlled inoculations. Plants were kept in a polythene tunnel prior to inoculation; no overhead irrigation or watering was applied to these plants as they were irrigated directly into the pots. Only fungicides effective against powdery mildew (Nimrod (bupirimate)) were used on these plants. At the time of the first inoculation in April, no fungicides had been applied.

Inoculum: For the April inoculation, inoculum (conidia) was made from those leaves with leaf spot lesions collected from a sprayed blackcurrant plot at East Malling Research in 2006 and stored in a cold room (5°C). For the July inoculation, fresh leaves with leaf spot lesions were collected from the same plot and used. On the day of inoculation, these collected leaves were washed in distilled water and shaken vigorously to wash spores off. Conidial concentration was adjusted to 1×10^5 conidia.ml^{-1} using a haemocytometer.

Treatment and Inoculation: Two inoculation studies were conducted; each cultivar was inoculated on different days: cv Baldwin – 18/04/07 and 05/07/07, cv. Ben Hope – 20/04/07 and 07/07/07. For each inoculation, there were three temperatures (10, 17.5 and

25°C) and five wetness periods (4, 8, 12, 24 and 30 h), giving a total 15 treatments per inoculation. There were two plants for each temperature and wetness duration combination. For the first inoculation in April, about 8-12 shoots were inoculated on each plant, each shoot with 6-8 leaves. For the inoculation in July, all shoots were inoculated on each plant, each shoot with 15-25 leaves; shoots had almost stopped growing at the time of inoculation in July.

The day before inoculation, 30 plants were randomly selected on the condition that they had a sufficient number of shoots with healthy leaves to inoculate. These plants were then moved to the CE cabinet to acclimatise for at least 16 h under an appropriate experimental temperature. Three cabinets were randomly allocated to one of the three temperatures (10, 17.5 and 25°C). The position of each plant in a cabinet, irrespective of its wetness duration was randomly allocated. The wetting required was supplied and maintained by programmable sprinklers built into the cabinets. Each cabinet was programmed for a daily cycle of 16 h light/8 h dark (light intensity *c.* 300 $\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ at the plant height) and constant 95 relative humidity (rh).

Plants were sprayed with water using a knapsack sprayer before inoculation. All shoots on each plant were sprayed with the spore suspension using a fine hand-held aerosol sprayer. Immediately after inoculation, sprinklers were switched on to maintain surface wetness. Two plants were moved out of each cabinet after an appropriate number of wetting hours, gently shaken manually to remove excess water and moved to the polythene tunnel and well spaced out along an irrigation line. No overhead watering or irrigation was used. This tunnel was closed all the time apart from the side venting (about c. 80 cm high from the ground).

Disease assessment: For the April inoculation, only the bottom six leaves were assessed four weeks after inoculation. Because of the huge number of lesions, disease was scored on a scale of 0-5: 0 – no lesions, 1 - < 10 lesions, 2 < 50 lesions, 3 < 100 lesions, 4 < 200 lesions and 5 >= 200 lesions. For the July inoculation, because of the presence of other spots on the leaves exact estimates of lesion numbers was more problematic. Instead, each leaf was scored as infected or not infected 12 weeks after inoculation; all leaves on three randomly selected shoots were scored on inoculated shoots and leaves dropped before assessment were noted.

2.2.2 Monitoring field epidemic development of leaf spot

The temporal dynamics of leaf spot was regularly monitored in 2005, 2006 and 2007 on an unsprayed mixed field planting of cv. Baldwin, planted with 3 m between rows and 0.5 m between plants within a row. Number of lesions was estimated on all leaves of five shoots of each of the four plants randomly chosen. Monitoring started in May or June and stopped in September; assessment was made at intervals of 1-4 weeks depending on the year. Number of dropped leaves and their positions on the shoot were recorded. Weather conditions were recorded with an automatic data logger, sited about 200 meters from the plot.

2.2.3 Data analysis

Logistic regression analysis (Cox and Snell, 1989), which is based on the logit transformation of the proportion (p) of leaves infected ($\ln\left(p/(1-p) \right)$), was used to assess the effects of treatment factors on the incidence of leaves infected by *D. ribis*. In this analysis, the number of infected leaves per plant, hence pooling all inoculated shoots, was assumed to be binomially distributed. In addition to temperature, wetness and cultivar, leaf age or position was also included as a treatment factor. For the first inoculation, six individual leaf positions were treated as a factor with six levels; whilst for the July inoculation, leaves were divided into three age groups (i.e. three levels): young (top five leaves), medium (leaf 6 to 10) and old (all others). Separate logistic analysis was applied to the April and July inoculation.

To assess the effects of temperature and duration of wetness on infection, these two factors were first included in the logistic regression as categorical variables (i.e. as factors). Only when they were shown to have significant effects as individual factors, were they included in the logistic regression as continuous variables to determine whether their effects could be described by regression models. Genstat (Payne, 2006) was used for statistical analysis.

2.3 Results

2.3.1 First inoculation in April 2007

Disease symptoms first appeared about 3 weeks after inoculation. Nearly a quarter (23%) of all inoculated leaves was infected. Overall, most variability in disease incidence was attributable to cultivar, leaf age and their interactions, accounting for 85% of the total variation. There were a greater (*P* < 0.001) proportion of leaves infected on cv. Baldwin (46%) than on cv. Ben Hope (5%) (Fig. 1a). The proportion of infection decreased significantly (*P* < 0.001) with decreasing leaf age (Fig. 1a). On cv. Baldwin, nearly all leaves at the bottom two positions of the shoot (two oldest leaves) were infected but only just 1% infected on the leaf at the sixth position (nearly the shoot tip at the time of inoculation). On cv. Ben Hope, about 18% and 9% of two oldest leaves infected; none of the leaves at the fifth and six positions were infected (Fig. 1a). Average disease severity nearly followed the identical pattern as the incidence (Fig. 1b).

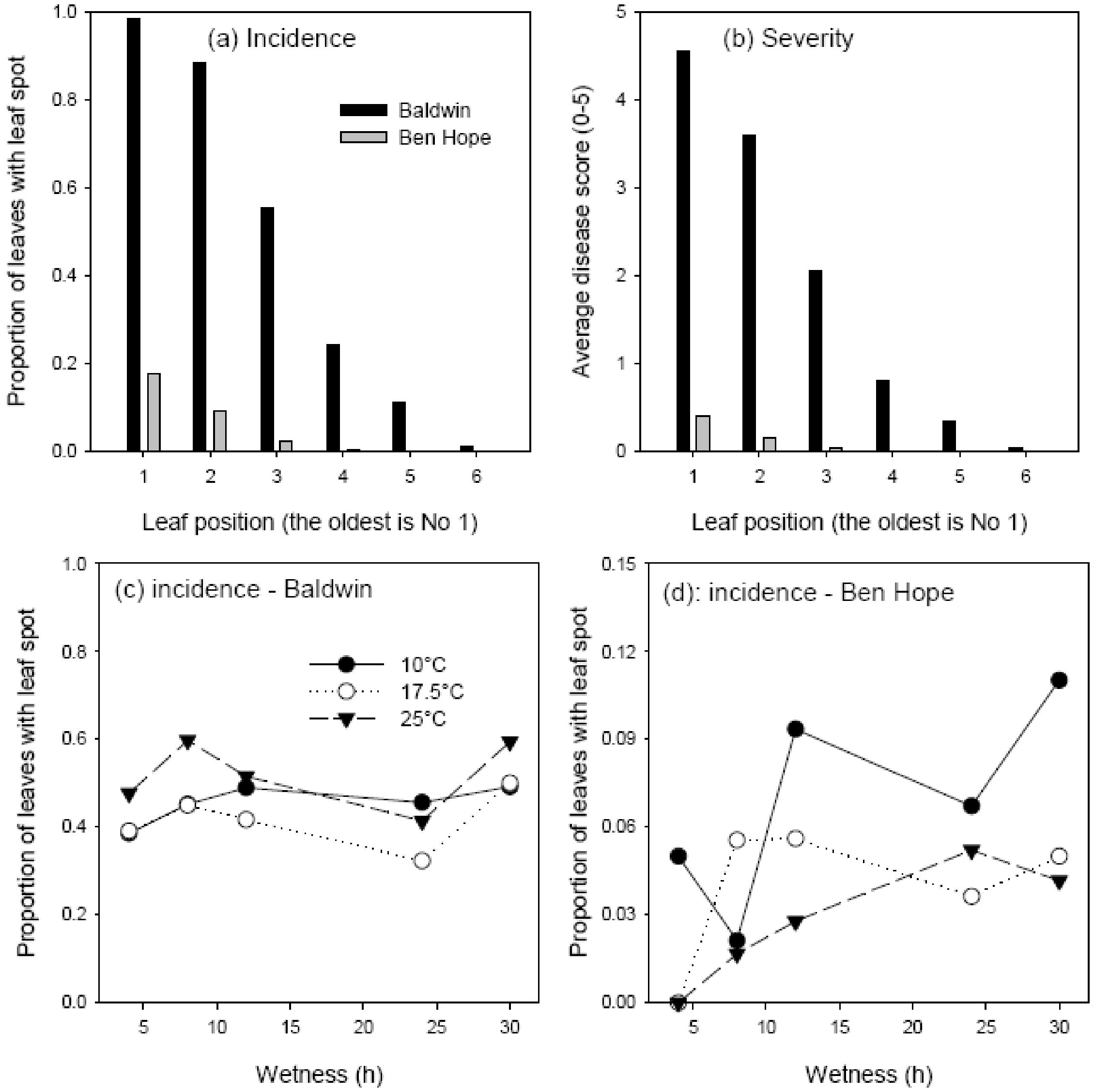

Figure 1. Proportion (and severity) of inoculated leaves with leaf spot lesions in relation to cultivar (Baldwin and Ben Hope), leaf age, temperature and wetness duration. Plants were inoculated in late April 2007 when there were about 6-8 leaves per shoot.

The effects of temperature and duration wetness were statistically significant ($P < 0.01$) but were very small in comparison with cultivar and leaf age. On cv. Baldwin, the effects of temperature and wetness duration was close to the statistical significance at 5% (Fig. 1c). In contrast, the effect of temperature and wetness were greater on cv. Ben Hope: increasing length of wetness led to greater incidence and the incidence was greater at 10°C than 25°C (Fig. 1d).

2.3.2 Second inoculation in July 2007

Nearly quarter of inoculated leaves had dropped by the time of the assessment but the number of leaves dropped did not show any clear relationships with temperature, wetness and cultivars. About 42% of all inoculated leaves were infected. As in the inoculation in April, cultivar and leaf age accounted for a considerable proportion of the variability in the observed incidence data, accounting for 14% and 17% of the total variation. There were a greater ($P < 0.001$) proportion of leaves infected on cv. Baldwin (53%) than on cv. Ben Hope (27%) (Fig. 2a). The proportion of infection decreased significantly ($P < 0.001$) with decreasing leaf age (Fig. 2a). On cv. Baldwin, there were 36%, 64% and 75% leaves infected for the leaves of young, medium, and old age group, respectively; the corresponding values on cv. Ben Hope were 19%, 40% and 38% (Fig. 2a).

In contrast, there were greater effects of wetness duration on disease development, accounting for nearly 23% of the total variability. For both cultivars, increasing length of wetness led to increases in incidence of infection (Fig. 2bc). Overall incidence was 17%, 35%, 48%, 53% and 57% for the wetness duration of 4, 8, 12, 24 and 30 h, respectively. The main effects of temperature were not statistically significant ($P > 0.05$), but its interactions with wetness duration were significant ($P < 0.001$), and accounted for 4% of the total variation. This significant interaction resulted mainly from the differential effects of wetness duration at 25°C from 10°C and 17.5°C (Fig. 2bc). The effect of wetness duration on the incidence of leaf infection can be well described by the following model:

$$\ln\left(\tfrac{p}{1-p}\right) = -1.204 + 0.0554Wet$$

where Wet is the duration of wetness in hours. The standard errors of the two parameter estimates were 0.353 and 0.019, respectively.

2.3.3 Field monitoring

Figure 3 shows the summary of disease development together with daily temperature, relative humidity and rainfall in the three years. Of the three years, the highest disease was observed in 2007 and least in 2005; average number of lesions per leaf was 19, 104 and 268 for 2005, 2006 and 2007, respectively. During the period from 01/05 to 05/09, there were 53, 60 and 71 days with more than 0.2 mm rainfall recorded in 2005, 2006 and 2007, respectively; the corresponding total rainfall during this period was 132, 222 and 333 mm.

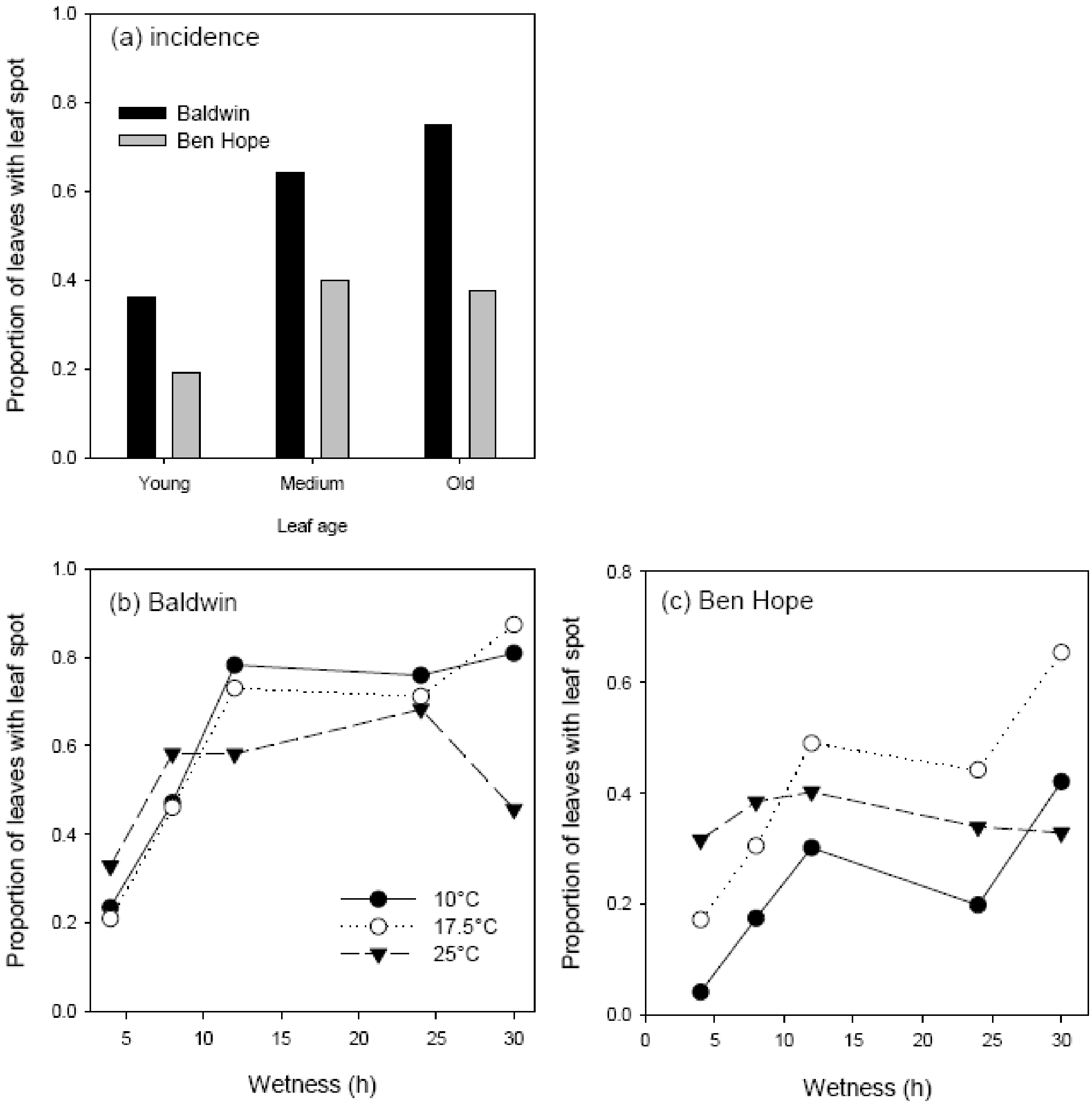

Figure 2. Proportion of inoculated leaves with leaf spot lesions in relation to cultivar (Baldwin and Ben Hope), leaf age, temperature and wetness duration. Plants were inoculated in early July 2007 when there were about 6-8 leaves per shoot.

In 2005, first lesions were seen on 10 June on rosette leaves and only four assessments were made on extension shoots. Number of lesions increased considerably from 0.6 per leaf on 28/07 to 8 on 26/08, and to 18 on 21/09 (Fig. 3a). Given the length of incubation period of ca. 2-3 weeks, these two increases in lesion number is likely to have resulted from infections associated with the two rainy periods in late July and mid-late August (Fig. 3a). The weather was very dry from early June to late July (Fig.3a).

In 2006, first lesions seen on 11 May on rosette leaves and a total of fourteen assessments were made on extension shoots. Number of lesions increased slowly until the last three assessments (Fig. 3b); lesions increased from 10 on 09/06 (1st assessment) to 25 on 24/08, to 56 on 31/08, to 80 on 06/09 and, to 104 on 18/09 (last assessment). The initial relative slow increase in the number of lesions is likely due to the drier conditions in the period from early June to early August (Fig. 3b). The rapid increase in disease from the late August onwards was due to the long rainy periods in mid- to late August (Fig. 3b).

In 2007, first lesions seen on 31 May on rosette leaves and only five assessments were made on extension shoots – assessment was terminated on 24/08, much earlier than in 2005 and 2006 because of impracticality of counting/estimating such a huge number of lesions (Fig. 3c). Number of lesions increased rapidly from 6 on 06/07 (1st assessment) to 268 on 24/08 (last assessment) (Fig. 3c). This rapid increase in the number of lesions was due to the virtually continuous rainy period from mid-July onwards (Fig. 3c).

2.4 Discussion

Controlled inoculation with conidia showed that infection of blackcurrant leaves by *D. ribis* is critically influenced by leaf age, cultivar and wetness duration. However, the effects of temperature (10-25°C) in general did not much affect incidence of leaves infected. Of the two cultivars tested, cv. Baldwin was much more susceptible to infection than cv. Ben Hope. Susceptibility also increased with increasing leaf age irrespective of cultivar. Increasing wetness led to higher incidence of infection.

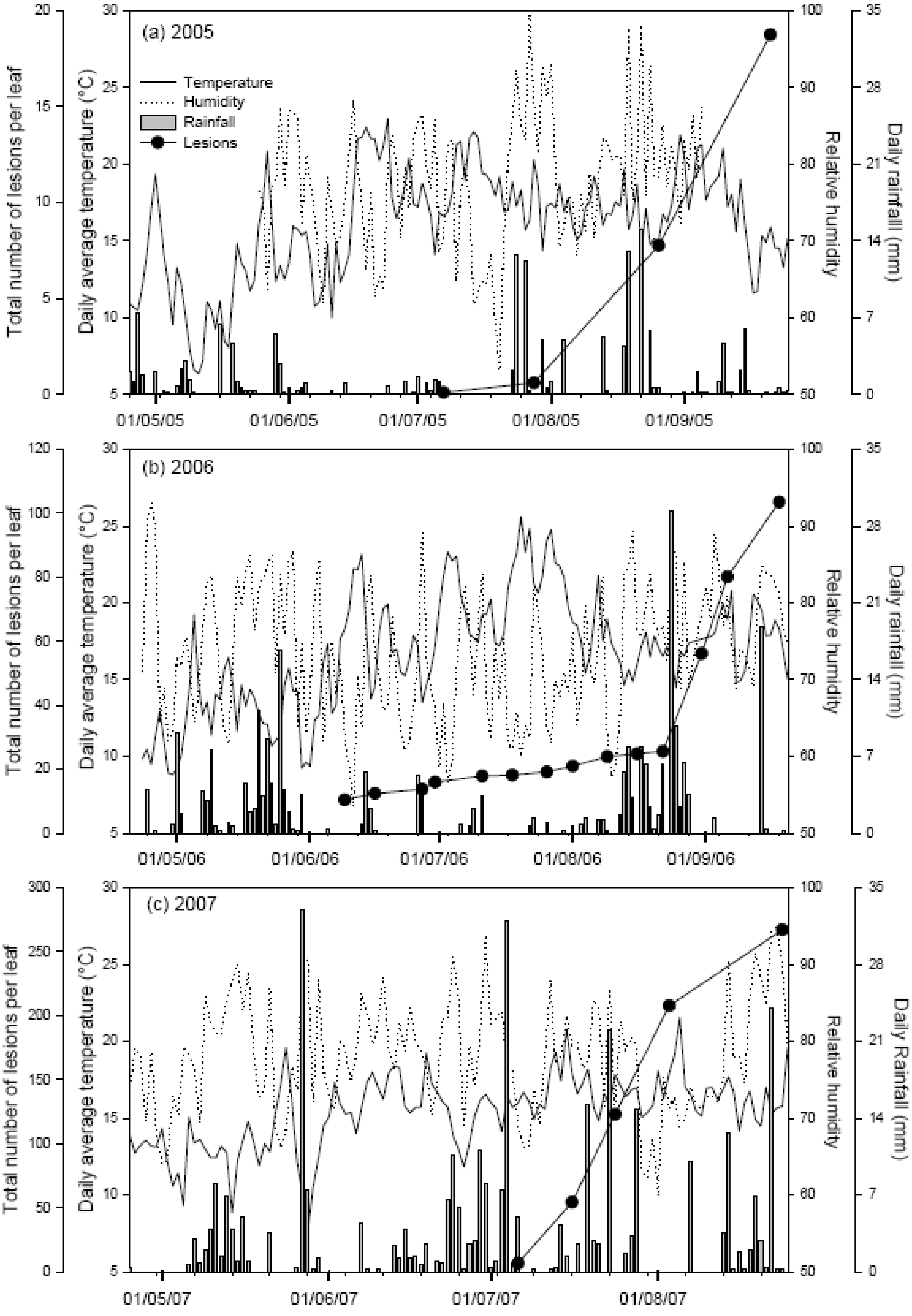

Figure 3. Observed (circles) number of leaf spot lesions per shoot in 2005, 2006 and 2007 on an unsprayed plot of cv. Baldwin at EMR, together with several weather variables: temperature (solid line), relative humidity (dotted line) and rainfall (bar).

Overall disease development on cv. Baldwin differs considerably between the two inoculations (April and July). For the April inoculation the incidence of leaves infected did not vary much with duration of wetness but increased with increasing wetness in the July inoculation. Exact reasons for such differences were not clear. The effects of temperature were only significant on cv. Ben Hope but its exact effect differed between the two inoculations: the overall incidence at 10°C was the highest in the April inoculation but the lowest in the July inoculation. However, it should be noted that the incidence level was very low for cv. Ben Hope in the April inoculation; thus any small variation in disease development may lead to an 'apparent' large difference. Conidia can germinate over range temperature between 2 to 28°C with an optimum 16-24^{o}C (Booth and Waller, 1979). The wetness requirement is much shorter than previously suggested 12-24 h (Booth and Waller, 1979). These differences may be due to the fact that different cultivars and inoculum concentration were used.

In the controlled inoculation, disease is expected to be much more severe than in field conditions, mainly because of higher inoculum dose received by leaves. Thus, any predictive model developed from the controlled inoculation studies is likely to lead to over-forecasting (or conservative predictions) predictions for the worst scenarios. Thus, for this purpose, a useful compromise would be to use the model developed for Ben Hope in practice. This is because that over-forecasting by higher inoculum doses might be negated to a larger extent by its lower susceptibility to the disease.

As expected, disease development in field conditions was closely related to wet conditions. Thus, average number of lesions per leaf increased over the three years as the total amount of rainfall (mm) in the growing season was 132, 222 and 333 mm in 2005, 2006 and 2007, respectively. Any probable reason for this increase in disease severity over the three years is the increase of primary inoculum in the spring because the plot was unsprayed in the three years but sprayed in 2004. This pathogen is believed to overwinter in a sexual state and primary infection on rosette leaves in the spring result hence from ascospores (Blodgett, 1936); thereafter, conidia from these primary infections on rosette are the main source of inoculum. Indeed, in all three years, lesions were first observed on rosette leaves in the period from early May – early June, suggesting infection may have taken place from mid April onwards. However, during the three years, we have failed to observe fungal sexual bodies on leaves over the winter and in spring despite of regular sampling and assessment in the laboratory. Thus, further studies are needed to clarify fungal overwintering mechanisms for this pathogen. Survival of leaf spot as lesions on twigs and fruiting strigs on the bush may be more important as an inoculum source in spring than previously thought.

Within each year, disease development followed closely with rainy periods. However, it does appear that significant amount of disease become visible only from late July onwards. For example, in 2007 average number of lesions per leaf is only about 6 in spite of extreme wet weather in late May and mid June (shoots have already started growth during this period). This may be explained by several factors, including lack or low level of inoculum during the early season as discussed above. Another important factor concerns leaf age. Controlled inoculation with conidia clearly showed that young leaves are resistant or less susceptible to the fungus. Thus successful infection of young leaves with conidia is not likely during the early stage of extension growth even under conducive weather conditions. However, ascospores are reported to be able to infect both young and old leaves (Blodgett, 1936; Borecki, 1962) and where these are likely to be present early infection of rosette leaves may result.

In summary, current results suggest that, in the absence of ascospores, infection is only likely to take place on relatively old leaves and wet conditions are essential for the disease to develop. A simple model was developed to describe the effect of wetness duration on disease development. Temperature was not included in this model because its effect is inconclusive and relatively small compared to wetness duration. Field monitoring over three years confirmed these findings and showed that severe disease development was only observed from late July onwards, suggesting infection from late June onwards may be responsible for this. Combining these findings we may suggest the following control strategies:

1) Strict control of infection on rosette leaves is may not be essential because much of the epidemic and leaf fall associated with leaf spot was mainly observed on extension shoots. This is particularly true when crop husbandry measures had been applied during overwintering phase to reduce overwintering inoculum and hence minimise the ascospore risk.

2) Rosette leaves (strig leaves) may be important in supplying nutrients to the fruit so as insurance, one spray against infection on rosette leaves may be used if the disease the previous season was very severe and extreme wet weather was

forecast or experienced or if there is excessive leaf litter in the plantation and therefore a possibility of ascospore infection.

3) From late June onwards, application of fungicides to control leaf spot must be timed to coincide with weather patterns. Here, the model may be used to indicate the magnitude of risks. Correct choice of fungicides may also depend on our knowledge on their physical mode of actions against *D. ribis*.

This strategy should be evaluated in experimental plots and in trial plots on commercial farms in the near future before being adopted widely.

3. Infection of blackcurrant flowers and fruits by *Botrytis cinerea*

3.1 Introduction

The two main diseases in blackcurrant are leaf spot (*Drepanopezizza ribis*) and *Botrytis* (*Botrytis cinerea*). *Botrytis* is often difficult to control in blackcurrants and results in considerable losses in yield and fruit quality. Currently there are several gaps in knowledge such as spray timing and effect of fruit age on susceptibility to *Botrytis*. Flowers of blackcurrant are sprayed routinely with fungicides to control *Botrytis* on the maturing fruit. Post harvest rots are usually not serious mainly because fruits are cold stored in very high carbon dioxide atmospheres for relatively short periods before processing (Dennis, 1983). Fungicides are also applied post flowering to harvest for *Botrytis* although it is not clear whether green fruit are susceptible to infection. Several studies (McNicol and Williamson, 1989; McNicol et al., 1989) showed that premature fruit drop or run-off in some varieties of blackcurrant could be attributed to symptomless infection of flowers by *Botrytis* which triggered ethylene production resulting in fruit abscission. Premature fruit drop can result in considerable yield loss in some years and fungicides applied at flowering can result in yield increases (Jorg *et al.*, 2003) and also delay *Botrytis* rot at harvest (Heltbech *et al.*, 2000). The latter indicates that infection at flowering may be later expressed as rot in the mature fruit, although another study (Pappas and Jordan, 1997) indicated that symptomless fruit colonisation initiated from flower infection did not occur because infected flowers aborted.

Experiments were carried out to investigate infection of blackcurrant flowers and fruit by *Botrytis* in relation to other factors. Specific objectives include (1) susceptibility of flowers to *B. cinerea*, (2) susceptibility of fruit to *Botrytis* at different fruit ages, and (3) effects of temperature and duration of wetness on infection of fruit by *B. cinerea*. Results from these studies may determine whether post blossom sprays for *Botrytis* are necessary.

3.2. Materials and Methods

3.2.1 Controlled inoculation blackcurrant flowers and fruits

Potted plants were inoculated in Sanyo controlled environment (CE) growth cabinets (model SGC170.CFX.J), equipped with fluorescent/tungsten lights, humidity, temperature and misting controls, during 2006 and 2007 (Table 1). In 2006, the susceptibility of both flowers and fruit was investigated in addition to the effects of temperature and duration of wetness on infection of fruit at several development stages. In 2007, experiments focused on the latent infection in relation to the fruit age at the time of inoculation.

Plants: Potted two- or three-year-old bushes of Baldwin (highly susceptible to *Botrytis*) and Ben Hope (moderately susceptible to *Botrytis*) were used in all controlled inoculations. Plants were kept in a polythene tunnel prior to inoculation; no overhead irrigation or watering was applied to these plants as they were irrigated directly into the pots. No fungicides that were effective against Botrytis were applied; only fungicides effective against powdery mildew were used.

Inoculum: One single Botrytis isolate originating from an infected blackcurrant fruit was used for all inoculation experiments. The isolate was multiplied and maintained on potato dextrose agar (PDA) plates. Plates were incubated at 20°C for one week and then placed under UV lights to encourage sporulation for one week before inoculation. On the day of inoculation, plates were washed with distilled water to make a spore suspension, which was homogenised to break the chains of conidia and filtered through muslin. A haemocytometer was used to estimate and adjust, if necessary, the concentration of the conidial suspension. Because of variable spore productions over time, conidial concentration varied with different experiments, from 4 to 8 x 10^5 conidia.ml^{-1}. Inoculum was tested for germination on PDA and in all cases more than 60% conidia germinated after 4-6 hours of incubation at 20°C.

Treatment and Inoculation: The day before inoculation, appropriate number of plants of each cultivar (Table 1) were randomly selected on the condition that they had a sufficient number of flowers or healthy fruit to inoculate. Any unopened flowers (in the tight-bud stage) were carefully removed before inoculation. These plants were then moved to the CE cabinet to acclimatise for at least 16 h under an appropriate experimental temperature (Table 1). For fruit inoculation in 2006, four cabinets were randomly allocated to one of the four temperatures (Table 1). The position of each plant in a cabinet, irrespective of its origin (cultivar) and wetness duration, where appropriate, was randomly allocated. The wetting required was supplied and maintained by programmable sprinklers built into the cabinets. Each cabinet was programmed for a daily cycle of 16 h light/8 h dark (light intensity *c.* 300 $\mu\,\mathrm{mol\,m^{-2}\,s^{-1}}$ at the plant height) and constant 95 relative humidity (rh).

Plants were sprayed with water using a knapsack sprayer before inoculation. All flower trusses and fruit on each plant were sprayed with the spore suspension using a fine hand-held aerosol sprayer. Each flower truss (or fruit) received about 0.4 ml spore suspension. Immediately after inoculation, sprinklers were switched on to maintain surface wetness. Flower inoculation, misting was switched off after 24 h and plants were gently shaken manually to remove excess water. These plants were then left inside the cabinet for assessment one week later. After assessment, all plants were moved to another polythene tunnel and well spaced out along an irrigation line; as before, no overhead watering or irrigation was used. This tunnel was closed all the time apart from the side venting (about c. 80 cm high from the ground) in order to reduce aerial botrytis spore concentration inside the tunnel. For fruit inoculation in 2006, plants were moved outside of the cabinet after an appropriate number of wetting hours, gently shaken manually to remove excess water and kept inside the building until plants of the longest wet period were moved out. Then all plants were moved to the polythene tunnel. Plants inoculated in 2007 were similarly handled.

Table 1 Summary of experiments conducted to investigate the infection of blackcurrant flowers and fruit by *Botrytis cinerea*

Factors	2006	Fruit inoculation	
	Flower inoculation	2006	2007
Cultivars	Ben Hope, Baldwin	Ben Hope, Baldwin	Ben Hope, Baldwin
Date	BH: 03/05, 08/05 BW: 27/04, 03/05	BH: 16/05, 01/06, 27/06 BW: 11/05, 23/05, 22/06	09/05, 20/06, 30/05, 05/07
Temperature	15°C	10, 15, 20, 25[a]	17.5°C
Wetness (h)	24	4, 8, 12, 24[b]	24
No of plants per treatment	4	2 (3 for the last inoculation: 27/06 & 22/06)	4
Harvest date	19/07	19/07	11/07

[a]: for the last inoculation (Ben Hope - 27/06 and Baldwin – 22/06), only 12 and 20°C were used.

[b]: for the last inoculation of Baldwin, the four wetness periods were 4, 8, 24 and 30 h.

Disease assessment: For inoculation of flowers, an assessment was made one week after inoculation by counting number of aborted flowers and flowers with necrotic petals. At harvest, number of fruit with visible botrytis symptoms and number of aborted flowers or fruitlets but still attached to the plant were recorded for each inoculated plant; all remaining apparently healthy fruit were harvested and incubated for assessment of latent infection.

In 2006, harvested fruit from each plant were placed directly into a Petri dish lined with a piece of damp filter paper and incubated at 20°C. In 2007 harvested fruit from all plants of a single cultivar inoculated at the same time were surface sterilised together. Surface sterilisation was done by immersing fruit in a 0.5% Domestos® solution (the a.i. is sodium hypochloride so that 0.025% wt/vol chlorine is available) for 15 min and then immersed in sterile distilled water for 15 min. Surface-sterilised fruit were then placed into a Petri dish lined with a piece of damp filter paper and incubated as in 2006. Fruit were then assessed for botrytis 10-14 days later.

3.2.2 Incidence of latent infection in field conditions

The temporal dynamics of latent infection of botrytis on blackcurrant fruit were investigated in 2007 on an unsprayed mixed field planting of cvs. Ben Hope and Baldwin, planted with 3 m between rows and 0.5 m between plants within a row. Fruit were sampled from plants in two blocks in the planting: protected and open-field. The open-field block was about 40 m from the protected block. For the protected area (12 m x 18 m), an open-sided Spanish tunnel was erected two weeks before flowering (mid April 2007) to cover the plants. Each block consists of four rows of plants, each with 24 plants divided into four sections of six plants with 1.5 m between consecutive sections. At all sampling times, only fruit on the plants of the centre two sections (one for each cultivar) on the 3rd row were sampled.

Fruit were sampled at an approximately interval of two weeks, starting on 02/05/2007, one week after full bloom. In total, fruit were sampled on five occasions: 02/05, 17/05, 31/05, 13/06 and 26/06. On each occasion, 60 fruit were sampled for each cultivar: 10 fruit randomly from each of the six plants in the section. Fruit were surface sterilised first as described previously and then placed on paraquat chloramphenicol agar (PCA) media to induce sporulation of *B. cinerea* (Peng and Sutton, 1991). The Petri dishes were incubated for two weeks under UV light at 20°C; plates were about 35 cm below the UV light. An assessment was done 10-14 days later.

3.2.3 Data analysis

Logistic regression analysis (Cox and Snell, 1989), which is based on the logit transformation of the proportion (p) of flowers or fruit infected ($\ln\left(p/(1-p)\right)$), was used to assess the effects of treatment factors on the incidence of flowers or fruit infected by *B. cinerea*. In this analysis, the number of infected flowers or fruit per plant or per treatment was assumed to be binomially distributed. Thus for assessing flower infection in 2006, the following logistic model was fitted to the data

$$\ln\left({p}/{(1-p)} \right) = Time + Variety + Variety \times Time$$

where *Time* and *Variety* represent the inoculation time (full or late bloom) and cultivars, respectively. A similar model was used to investigate the effects of temperature, duration of wetness and fruit age, and protection/open field conditions in the case of 2007 on the infection of fruit by *B. cinerea*. To assess the effects of temperature and duration of wetness on infection, these two factors were first included in the logistic regression as categorical variables (i.e. as factors). Only when they were shown to have significant effects as individual factors, were they included in the logistic regression as continuous variables to determine whether their effects could be described by regression models. For fruit inoculation in 2006, disease development was expressed in terms of the following variables: percentage of aborted flowers/fruitlets (but still attached to the plant), percentage of total infection (including aborted flowers/fruitlets and fruit with latent infection), and fruit with latent infection as the percentage of apparently 'sound' fruit at harvest.

For the data obtained from the 2007 controlled inoculation, a standard Normal approximation is used for two-sample tests of binomial data to compare the incidence of latent infection between cvs. Baldwin and Ben Hope at each inoculation, and between pairs of inoculation time for each cultivar (Arimitage and Berry, 1994). Genstat (Payne, 2006) was used for statistical analysis.

3.3 Results

2.3.1 Inoculation in 2006

One week after inoculation, nearly 75% of flowers were infected or had dropped following inoculation at the full bloom and late flowering stages. The two cultivars did not differ significantly in their susceptibility of flowers to *Botrytis*. At full bloom, 76% and 72% of flowers/fruitlets were infected or aborted on cvs. Baldwin and Ben Hope, respectively. The corresponding figures were 75% and 71% at the late flowering stage. There were also no significant effects of flowering time or its interaction with cultivar on the incidence of flower infection. Nearly all those remaining flowers (i.e. not infected) failed to develop into healthy mature fruit and thus post-harvest assessment was not done on these plants.

Table 2 summarises the results of inoculation studies where blackcurrant flowers and fruit of cvs. Baldwin and Ben Hope were inoculated on three occasions and subjected to various combinations of temperature and duration of wetness. At harvest, none of the fruit showed visual *Botrytis* symptoms. Logistic regression showed that neither temperature (10-25°C) nor duration of wetness (4-30 h) had significantly affected the incidence of flowers and fruit infected. For example, about 89%, 95%, 83% and 91% of flowers and fruit of cv. Ben Hope were infected following the inoculation two weeks after full bloom for wetness duration of 4, 8, 12 and 24 h respectively. In contrast, the incidence of fruit infection was significantly ($P < 0.01$) affected by fruit age and cultivar as well as their interactions. On cv. Baldwin, the incidence of total infection (i.e. including aborted flowers/fruit and latent infection) did not differ between the 1st and 2nd inoculation but significantly more ($P < 0.01$) than the 3rd inoculation (Table 2). On cv. Ben Hope, the incidence of total infection progressively decreased significantly ($P < 0.01$) as the fruit age at the time of inoculation increased (Table 2). For the first inoculation, the two cultivars did not differ significantly in their susceptibility; for the 2nd and 3rd inoculations, cv. Ben Hope had significantly ($P < 0.01$) lower incidence of aborted flowers/fruitlets and latent infection (Table 2). Over the three inoculations, the incidence of total infection was 91% and 80% for cvs. Baldwin and Ben Hope, respectively.

Table 2 Summary of inoculation studies where blackcurrant flowers and fruit of cvs. Baldwin and Ben Hope were inoculated on three occasions under various conditions (temperature and duration of wetness) in controlled environment growth cabinets. Temperature and wetness duration did not significantly affect the incidence of botrytis infection.

		Inoculation date			Weighted average
	Cultivar	Early	Mid	Late	
% aborted flowers/fruit					
	Baldwin	42.74	48.5	0	41.4
	Ben Hope	7.8	2.4	2.3	5.6
Weighted average		26.5	34.4	1.0	26.9
% total infection (aborted flowers/fruit and latent infection)					
	Baldwin	91.6	93.2	78.8	91.1
	Ben Hope	89.4	74.5	45.7	80.0
Weighted average		90.6	87.5	63.5	86.6
% fruit with latent infection (excluding aborted flowers/fruit)					
	Baldwin	85.2	86.7	78.8	84.8
	Ben Hope	88.5	73.9	44.4	78.8
Weighted average		87.2	80.9	63.1	81.7

There were many more ($P < 0.01$) aborted flowers/fruitlets on cv. Baldwin following the 1st (43%) and 2nd (49%) inoculation than the 3rd inoculation (0%). In contrast, there was a very low level of aborted flowers/fruitlet on cv. Ben Hope (Table 2). There were no significant differences in the incidence of latent *Botrytis* on cv. Baldwin between the three inoculations; on average about 85% of harvested fruit on cv. Baldwin had developed *Botrytis* following 1-2 week incubation. However, on cv. Ben Hope the incidence of latent infection progressively decreased significantly ($P < 0.01$) as fruit age at the time of inoculation increased (Table 2). The incidence of latent infection was similar following the first inoculation between cvs Baldwin (85.2%) and Ben Hope (88.5%); but the incidence following the late inoculations was significantly higher on cv. Baldwin (87%, 79%) than on cv. Ben Hope (74%, 44%).

3.3.2 Inoculation in 2007

At harvest, none of fruit showed visual *Botrytis* symptoms. On cv. Baldwin, about 88%, 9%, 2% and 15% of latent infection was observed following four inoculations, respectively; these were significantly ($P < 0.01$) different among themselves. About 27% latent infection was observed on cv. Ben Hope following the 1st inoculation, significantly ($P < 0.01$) greater than that achieved at the 2nd and 3rd inoculation (15% and 14%), which in turn was greater ($P < 0.01$) than that achieved from the last inoculation (9%). At the same inoculation stage, the two cultivars differed significantly ($P < 0.01$) in their responses to *Botrytis*: the incidence of latent infection was greater on cv. Baldwin on the 1st and last inoculation than on cv. Ben Hope, and *vice versa* on the other two inoculations.

3.3.3 Field sampling

Average incidence of latent infection increased with fruit age, from *c.* 30% on 02/05/07 to *c.* 60% on 13/06/07, which was similar to the incidence observed on the last sampling date (26/06/07) (Fig. 4). Logistic modelling suggested that there were no significant differences in the incidence of latent infection between the two cultivars or between the open-field and under protection (Fig. 4). The effect of fruit age on the incidence of latent infection can be well described by the following model: $\ln\left(\frac{p}{1-p}\right) = 0.839 + 0.022\mathit{Days}$, where p is the incidence of latent infection and *Days* is the number of days from 30/04/07. The standard errors of the two estimated parameters are 0.247 and 0.007, respectively. This model is also shown in Fig. 4. There were many rainy days with heavy rainfall throughout the sampling period (Fig. 4).

3.4 Discussion

This study showed that infection of blackcurrant flowers and fruit may lead to premature flower abscission, aborted fruit, and latent infection on fruit, and that disease development was not significantly affected by climatic conditions. Conidia of *B. cinerea* can germinate on the stigmatic surface of blackcurrant flowers (McNicol and Williamson, 1989) as flowers of raspberry (McNicol *et al.*, 1985; Williamson *et al.*, 1987) and strawberry (Bristow *et al.*, 1986). Thus even under dry laboratory conditions, conidia could germinate in the stigmatic fluid. This may explain why *Botrytis cinerea* on strawberry and raspberry grown under protection is still a major problem as infection of flowers may lead to rot development when fruit ripens. This may also explain why the incidence of flower infection on open-field strawberry plants was best predicted by temperature and relative humidity (Xu *et al.*, 2000) instead of the usual wet related variables (e.g. rainfall duration or wetness duration) required for many other wet pathogens, such as apple scab (Xu *et al.*, 1995) and pear scab (Li *et al.*, 2006). Field sampling has also shown that rainfall during the flowering and fruiting period did not significantly affect the incidence of latent infection on blackcurrant fruit. Controlled inoculation of fruit at several growth stages showed that temperature and the duration in the test range did not significantly affect the incidence of latent infection. Thus, it suggests that a wet period as short as 4 h should be sufficiently long enough to result in maximum possible infection of fruit. Similar findings on the lack of effect of duration of wetness on infection of fruit were also obtained for the infection of cherry by *M. laxa* (Xu *et al.*, 2007). The incidence of latent infection in both the open and under-protection plots increased steadily during the sampling period, indicating some of the fruit

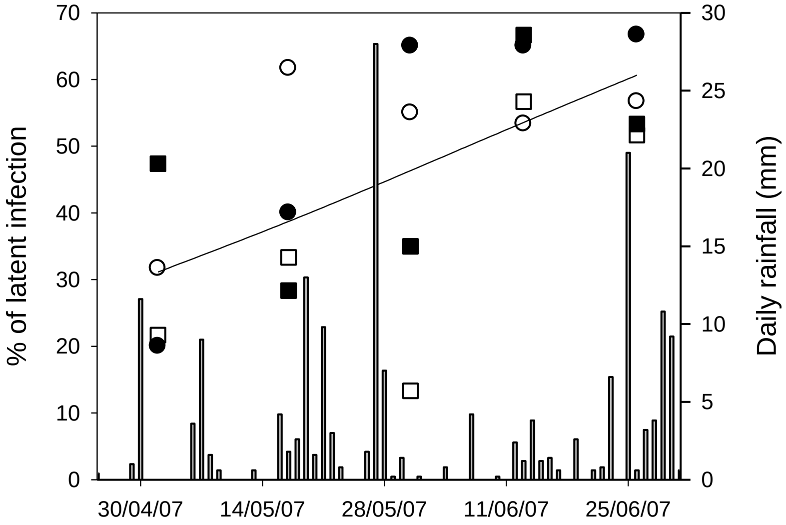

Figure 4. Observed (symbols) and fitted (line) percentage of latent infection of blackcurrant fruit plotted against sampling time in two plots (under protection – filled symbols; open field – unfilled symbols) of an unsprayed mixed planting of cvs. Baldwin (circle) and Ben Hope (square) in 2007.

with latent *Botrytis* must have resulted from post-blossom infection of fruit. This further supports the conclusion that infection of blackcurrant flowers and fruit by *B. cinerea* may not need free water.

There was a strong association between the incidence of flower infection and failure of the fruits to reach maturity. Inoculation with dry conidia increased the incidence of premature fruit drop compared to that where flowers were not inoculated and delayed inoculation in relation to pollination also resulted in fewer premature fruit drop (McNicol and Williamson, 1989). The premature flower abscission is related to the increased ethylene production following the infection by *B. cinerea* (McNicol *et al.*, 1989). Present studies showed high percentages of premature flower abscission or aborted flowers irrespective of inoculation time (full or late blossom) in both cultivars. However, the two cultivars differed significantly in their responses to infection of fruit. On cv. Baldwin, inoculation of young fruitlets even 2 and 4 weeks after full bloom resulted in nearly half of young fruits aborted though most were still attached to the plants. In contrast, inoculation of fruit at the similar times on cv. Ben Hope resulted in very little aborted fruit. However, most harvest fruit (apparently healthy) developed *Botrytis* rot after 1-2 weeks post-harvest incubation. The overall incidence of latent infection decreased as fruit age increased at the time of inoculation, particularly for cv. Ben Hope. The decreased fruit susceptibility to infection by *Botrytis* was further confirmed by the field data. At the first sampling, about one week after full bloom, there were already about 30% of fruitlets infected; the incidence of latent infection increased gradually over the next six weeks to about 60% (about 3-4 weeks before harvest) and then remained at this level for the final sampling. This is different from strawberry where fruits become more susceptible to *Botrytis* as they are maturing (Hennebert and Gilles, 1958; Kovacs, 1968).

The extent of latent infection following inoculation of fruit at different stages differed greatly between the two years. Except for the first inoculation, the incidence was much lower in 2007 than in 2006, especially for cv. Baldwin. The difference between the two cultivars was also much smaller (except for the first inoculation) in 2007 than in 2006. These differences between the two years could be due to the consequence of mildew epidemics. In 2006, powdery mildew was not controlled in the tunnel prior to full bloom. By the time the first spray against mildew was applied, powdery mildew was already well established, particularly on cv. Baldwin, resulting in many fruit infected with mildew. Although these fruit with visible mildew symptoms were all removed before inoculation, it was probable that some inoculated fruit may already been infected by mildew but without visible symptoms, which may facilitate the infection by *Botrytis*. In 2007, fungicides were applied to the plants much earlier to control mildew such that mildew did not cause noticeable fruit or leaf infection. Thus, the significant differences in the incidence of latent infections between the two cultivars may be due to their differences in susceptibility to mildew. Similarly this may explain the higher incidence of aborted fruit on cv. Baldwin in 2006. The field data in 2007 appeared to support this. Occurrence of mildew on the field planting was sporadic in 2007 and overall there were no significant differences in the incidence of latent infection between the two cultivars over all sampling dates. Another possible cause for the differences between the two years may be due to the fact that surface sterilisation was only applied to 2007. However, this is not likely to be the main cause because all treatments would have similar incidence of latent infection if this is the main cause.

In both strawberry and raspberry, *Botrytis* may infect flower parts and remain latent until fruit ripening (Bristow et al., 1986; Dashwood and Fox, 1988). However, symptomless fruit colonisation initiated from flower infection on blackcurrant did not occur as infected flowers aborted (Pappas and Jordan, 1997). But fungicides applied at flowering delay *Botrytis* rot at harvest (Heltbech *et al.*, 2000), indicating that latent infection at flowering may later express as post-harvest rot in the mature fruit. Present data obtained from field sampling suggest that the phenomenon of latent infection resulting from flower infection is most likely also occurring on blackcurrant. One week after full bloom, there was already 30% of young fruitlets with latent infection of *Botrytis*. Whether infection of flowers would lead to premature abscission or latent infection on fruit is critically dependent on the time of inoculation in relation to pollination (McNicol and Williamson, 1989): latent infection is most likely to occur where infection took place several days after pollination. Furthermore, present data also suggest that the infection pressure can also influence whether infection of flowers would lead to premature abscission or latent infection. Heavy inoculation pressure, e.g. high inoculum dose and wet conditions (which is likely to hamper pollination), is more likely to cause premature flower abscission. Thus, nearly all flowers artificially inoculated in growth cabinets were prematurely abscised as in a previous study (Pappas and Jordan, 1997). In contrast, low infection pressure such as in field conditions is likely to result in latent infection on fruit. The loss due to latent infection in addition to premature abscission of flowers indicates the importance of preventing flowers from being infected, as showed in fungicide spray trials (Heltbech *et al.*, 2000; Jorg *et al.*, 2003). In addition to latent infection resulting from flower infections, infection of developing fruit may also contribute to the latent infection at harvest, albeit to a lesser extent.

In summary, the present study confirmed previous results that infection of blackcurrant flowers and fruit may lead to premature flower abscission, aborted fruit development, and latent infection on fruit. Furthermore, it also showed that infection of flowers and developing fruit was not affected much by climatic conditions (rainfall, temperature and duration of wetness). Thus irrespective of weather conditions, strategies must be adopted to reduce inoculum and the extent of infections of flowers and, in case of cultivars like cv. Baldwin, very young fruitlets as well. Furthermore, effective control of powdery mildew is also important since visible or microscopic mildew lesions on fruit may facilitate infection of fruit by *Botrytis*.

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