

## TIMING OF FUNGICIDE APPLICATION FOR *BOTRYTIS CINEREA* CONTROL IN BLACKCURRANT (*RIBES NIGRUM*)

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### ABSTRACT

Flower blight in blackcurrants caused by the fungus *Botrytis cinerea* has received little attention in New Zealand. To determine the efficacy of a fungicide application in relation to the timing of infection, fungicides were applied 5, 3 and 1 day before as well as immediately after, 1, 2 and 5 days after an infection event. Cyprodinil and fludioxonil (Switch®) were chosen because of the translaminar systemic and protective mode of action. Studies were conducted during the 2006 flowering season in the laboratory and in the field on commercial properties in Canterbury and Nelson. Natural *Botrytis* flower inoculation and infection events were common during the 2006/07 production season. Results suggest that timing of fungicide application is critical for *B. cinerea* control in blackcurrants. Protection of flowers was achieved with cyprodinil and fludioxonil applications from 3 days before to 3 days after an inoculation and/or infection event. Reduction of flower infections resulted in increased fruit set.

**Keywords:** cyprodinil, fludioxonil, flower blight, flower drop, grey mould, berry.

### INTRODUCTION

The New Zealand blackcurrant industry has been a relatively small horticultural industry with approximately 50 growers producing 2260 t on approximately 1300 ha (Kerr et al. 2004). However, in recent years production has increased to around 10,000 t with a current export value of free on board (FOB) NZ\$15 million. This sudden, fast growth of the industry, particularly in non-traditional growing areas, has increased grower awareness of production related problems, such as pests and diseases, as well as fruit set and pollination issues. Anecdotal evidence suggests that flower blight and/or poor pollination are the two main factors affecting yields in New Zealand blackcurrant (*Ribes nigrum* L.) production. Flower blight in blackcurrant is caused by stilar infections of the flower with *Botrytis cinerea* (Perk.: Fries) (Pappas & Jordan 1997). In response to the infection, the flower produces ethylene, which causes flower abscission and hence die-back of the whole or part of the flower strig (McNicol et al. 1989). This naturally affects yield (McNicol et al. 1989). Research in Poland and the UK has shown that strobilurin fungicides (azoxystrobin, kresoxim-methyl and trifloxystrobin), fenhexamid, dichlofluanid and tolyfluanid applications during flowering can decrease the number of infection sites per shoot thereby increasing yield (Brennan et al. 2002; Bielenin 2002; Duben & Rosslenbroich 2002).

This work was commissioned by the blackcurrant industry in New Zealand. The aim was to evaluate the importance of timing of fungicide application on reducing blackcurrant

flower infections by *B. cinerea* using cyprodinil and fludioxonil (Switch®) as the model product. This product was chosen because the two active ingredients create a protectant and translaminar systemic fungicide with three different modes of action known to affect four sites on *Botrytis* development (Anon. 1997).

## MATERIALS AND METHODS

### Inoculum

*Botrytis cinerea* was isolated from blackcurrant in November 2005 during preliminary trials monitoring flower infections (incidence). The two isolates, BCJ1 and BCH, were taken from actively sporulating flowers sampled from two commercial fields in the Nelson region. Isolates were maintained on potato dextrose agar (PDA, Merck) at 4°C. Inoculum was produced by growing the *Botrytis* isolates on oatmeal agar plates (OA, 30 g Flemings oatmeal and 20 g agar (Merck) per litre distilled water) for 2-3 weeks at room temperature under a light bank (12 h photoperiod). Fungal spore suspensions were prepared by flooding plates with 10 ml aqueous Tween-80 solution (0.05%) and scraping gently with a sterile loop. The resulting crude suspension was filtered through a layer of sterile lens tissue to remove mycelial fragments. Inoculum concentration was estimated using a haemocytometer and adjusted to  $5 \times 10^4$  conidia/ml for a mixed *B. cinerea* spore suspension at a ratio of 1:1 of the two isolates.

### Detached shoot assays on timing of fungicide applications

Unsprayed cv. Ben Rua blackcurrant shoots were collected on 3 October 2006 (40% flowering) from a commercial property in Tai Tapu, Canterbury, and cut into approximately 50 cm long stems. All the leaves and buds were removed approximately a third of the way up from the shoots and 2-3 shoots were then placed into sterile 1 litre containers filled with tap water.

The 10 treatments consisted of cyprodinil and fludioxonil (Switch®, Syngenta Crop Protection Ltd, New Zealand) fungicide applications 5, 3, 1 and 0 days before and 1, 3, 5 days after challenge inoculation with *B. cinerea*. There was one nil control, applying only water instead of the *Botrytis* inoculum; and two *Botrytis* controls applying only the pathogen. For the day 0 treatment, fungicide was applied approximately 2 h before the *Botrytis* inoculation. Cyprodinil and fludioxonil were applied at 0.3 and 0.2 g active ingredient (a.i.) per litre, respectively. Fungicide and pathogen were applied in a fume hood using 1 litre hand-held misters (Yates) creating a fine mist. During application, shoots were turned to facilitate even spray coverage of all flower strigs resulting in approximately 10 ml water rate per shoot and application. Shoots were saturated, but run-off did not occur. Immediately after the *Botrytis* inoculation, shoots were incubated under high humidity for 24 h by bagging each container and shoots using clear polyethylene plastic bags. Throughout the trial, treatments and replicate containers were completely randomised on the bench in an air-conditioned laboratory (17-19°C). There were three replicates per treatment.

Ten days after *Botrytis* inoculation six or more buds, chosen at random from each replicate container (2-3 buds per shoot) were removed. Individual flowers were separated and incubated on sterile, moist paper towels, at high humidity for 6 days at room temperature (20-23°C). Incidence (%) of flowers showing *Botrytis* sporulation was assessed. The assay was repeated with shoots collected on 12 October 2006 (60% flowering).

### Field experiments on timing of fungicide applications

The detached shoot assay was repeated for all 10 treatments described above in the field at two locations. The first trial site was in Canterbury at the Tai Tapu field where shoots were collected for the laboratory trials. The second field site was in Upper Moutere, Nelson. For the field trials, whole plants (3-5 plants/replicate) were sprayed with cyprodinil and fludioxonil (0.3 and 0.2 g a.i./litre, respectively), using a Cropliner™ knapsack with 3-5 unsprayed buffer plants between treated plots. Plants were sprayed until just before run-off. For the *Botrytis* application, 3-5 shoots were selected from the centre of the plot, tagged, and inoculated with the mixed spore suspension

using a hand held mister, but shoots were not bagged. No other fungicides were applied in the field before or during the trials. Treatments were arranged in a randomised block design, with a block consisting of a row. There were four replicate blocks per trial site. *Botrytis* was applied on the 23 October 2006 in Canterbury and on the 26 October 2006 in Nelson.

In the field, disease assessments consisted of incidence of *B. cinerea* sporulation on flowers (%) and fruit set (%). To determine flower infections, five buds were randomly picked from the *Botrytis*-inoculated shoots 1 and 2 weeks after *Botrytis* inoculation. Flowers were incubated as described above and incidence (%) of flowers showing *Botrytis* sporulation determined. During the mid season, approximately 4 weeks after *Botrytis* inoculation, fruit set data were recorded by counting for each shoot: the number of strigs per bud, the number of flower pedicels per strig, and the actual number of green fruit. A strig was also scored for absence or presence of blight symptoms (i.e. dead flowers and browning of the strig). Fruit set was scored in Canterbury on 30 November 2006 and in Nelson on 29 November 2006. Weather data were obtained from weather stations 5 and 14 km from the actual research sites in Canterbury and Nelson, respectively. The Broome model (Broome et al. 1995) was used for *Botrytis* risk prediction to identify potential natural inoculation and infection events.

#### **Field experiment on determining number of fungicide applications**

An additional field experiment was conducted in parallel on the Nelson site to determine the number of fungicide applications required to optimise control of *Botrytis* on flowers in blackcurrant. There were up to eight fungicide applications during the 4-week flowering period. These consisted of two (18 and 21 October 2006), four (18, 21, 24 and 27 October 2006), six (18, 21, 24, 27 and 30 October and 2 November 2006) or eight (18, 21, 24, 27 and 30 October and 2, 6 and 9 November 2006) applications of cyprodinil and fludioxonil (Switch®). These were compared with no fungicide or the grower standard treatment of tolyfluanid (Euparen® Multi, Bayer New Zealand Ltd.) applied at 250 g a.i./100 litre at a water rate of 400 litres/ha once only on 12 October. Peak flowering period was on 27 October. Flower (19 and 26 October, 2 and 9 November 2006) and fruit set (29 November 2006) assessments were conducted as described above for the field experiments on timing of fungicide applications.

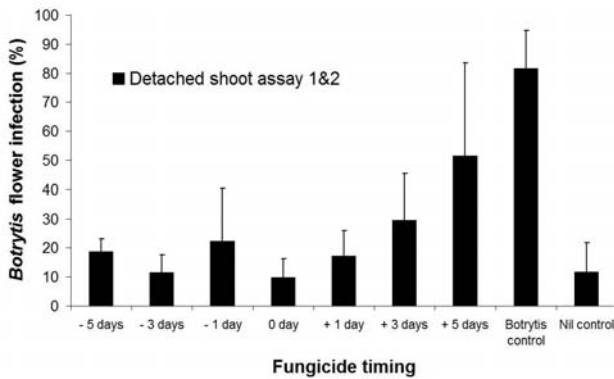
#### **Statistical analyses**

Laboratory and field trials data were subjected to analysis of variance (ANOVA) to evaluate the effect of treatment, number, time of application and their interactions using Genstat 9.1 (Lawes Agricultural Trust, Rothamsted Experimental Station). Mean comparisons for laboratory and field data were conducted with Fisher's unprotected least significant difference (LSD) test at the 5% probability level. Residual plots showed that data were normally distributed, therefore no transformation was required. Regression was used to identify optimum timing of fungicide application in response to the artificial inoculation event.

## **RESULTS AND DISCUSSION**

### **Detached shoot assays**

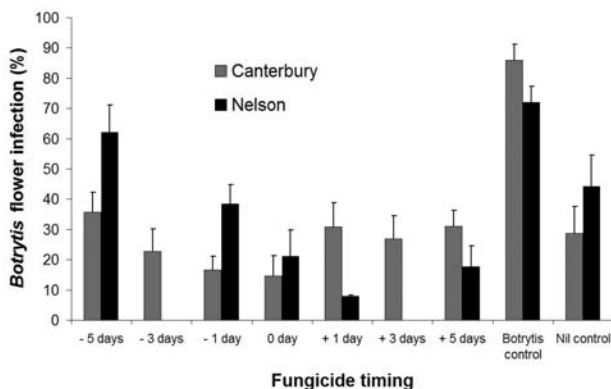
There was no trial effect, nor interactions between replicate trials and treatments ( $P > 0.05$ ). Therefore results from the pooled data are presented. The nil control treatment showed that there was some background *Botrytis* on flowers (12%) with *Botrytis* inoculations resulting in approximately 82% flowers infected. There was a significant effect ( $P < 0.001$ ) of fungicide timing on reducing *Botrytis* sporulation on flowers (Fig. 1). Cyprodinil and fludioxonil applications 5 days before the inoculation event still provided a 77% reduction in sporulation. However, fungicide applications after the inoculation had to be within 3 days of *Botrytis* applications to reduce flower infections significantly (Fig. 1). Inoculation and/or infection events occurring before the trial, or background *Botrytis* levels as monitored with the nil control treatment, were not reduced ( $P > 0.05$ ) with the fungicide treatments (Fig. 1).



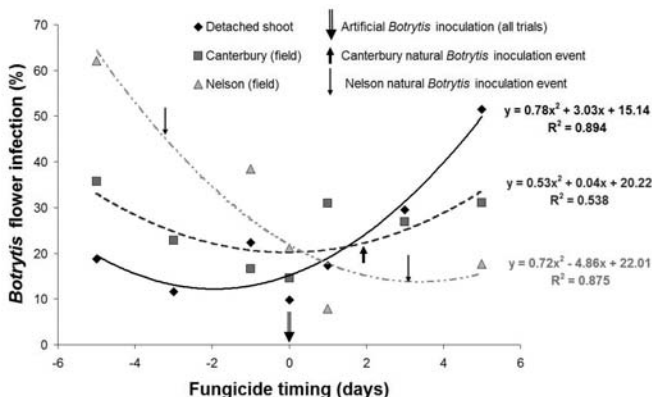
**FIGURE 1:** *Botrytis cinerea* flower infections (%) on detached blackcurrant shoots for fungicide (cyprodinil and fludioxonil) applications ranging from 5 days prior (-5 days) to 5 days after (+5 days) artificial inoculation with the pathogen at day 0. Data were pooled from two trials, n = 6.

### Field experiments

Similar to the detached shoot assays, the field trials also showed significant ( $P < 0.001$ ) fungicide timing effects (Fig. 2). In Canterbury, all fungicide timing applications reduced blackcurrant flower infections compared with the *Botrytis* control ( $P < 0.001$ ). However, in Nelson, the protective spray application at 5 days before the inoculation event did not reduce flower infections ( $P > 0.05$ ). This might be attributed to a rain and a natural inoculation event 2 days after the application as well as high levels of background infections (Fig. 3, Table 1). While the timing of fungicide applications was important, different patterns and regressions could be observed for the different experiments (Fig. 3). In the detached shoot assays a protective application 1-3 days before *Botrytis* inoculation was most desirable (Fig. 3), which is in contrast with the field results. For example, in Canterbury, fungicide applications on the day of the inoculation and 1 day before or after resulted in the least amount of *B. cinerea* flower infections. For the Nelson site, the results seem to suggest that fungicide applications 3 or 5 days post inoculation was most successful. This difference in inoculation and potential infection events may explain the differences in optimum timing for fungicide applications in the detached shoot assay and field trials. In the field, additional natural inoculation events occurred before and after the artificial inoculation at day 0 (Fig. 3).



**FIGURE 2:** *Botrytis cinerea* flower infections (%) in blackcurrants in the field at the Canterbury and Nelson trial sites. Fungicide (cyprodinil and fludioxonil) applications ranged from 5 days before (-5 days) to 5 days after (+5 days) artificial inoculation with the pathogen at day 0. Note: for the Nelson site, the -3 days and +3 days fungicide applications are missing because of rain events; n = 4.



**FIGURE 3:** Regression equations for timing of fungicide applications in relation to control of *Botrytis cinerea* flower infections (%) in blackcurrants as observed from the detached shoot assay or the field trials in Canterbury and Nelson. Fungicide applications ranged from 5 days before (-5 days) to 5 days after (+5 days) the artificial infection event at day 0, with predicted natural infection events also indicated for the field trials. Note: for the Nelson site, the -3 days and +3 days fungicide applications are missing because of rain events; n = 6 and 4 for the detached shoot assay and field trials, respectively.

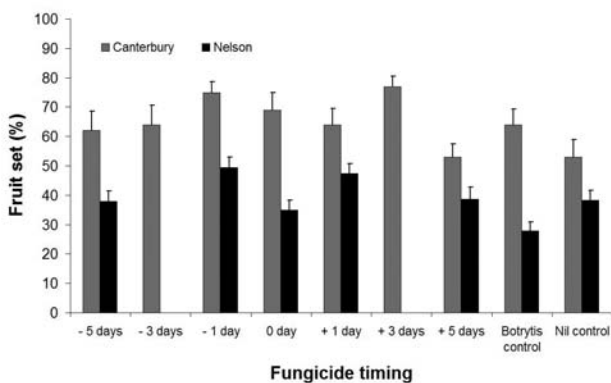
High flower infections translated into a high proportion of dead or blighted flowers at the fruit set assessment. This relationship was linear and could be described by  $y=0.326x + 20.3$ , where  $y$  is the proportion of dead or blighted flowers at fruit set and  $x$  the proportion of flower infection at full bloom ( $R^2=0.574$ ;  $P<0.05$ ). Incubation of dead flowers or brown strigs yielded *Botrytis* only >90% of the time (data not shown). Increased control of flower infections also resulted in increased fruit set (Fig. 4). This improvement in fruit set was particularly apparent in the separate Nelson field trial studying the effect of increasing number of fungicide applications on fruit set (Fig. 5) and flower infections (Fig. 6). Multiple fungicide applications improved fruit set from 31% (nil control) to 62% (eight cyprodinil and fludioxonil applications) with a steady increase in fruit set with increasing number of fungicide applications (Fig. 5). Flower infections similarly were highly controlled by multiple cyprodinil and fludioxonil fungicide applications (Fig. 6). The flower infection and fruit set data clearly indicate that uncontrolled *Botrytis* flower infections are a major contributor to fruit loss by causing flower damage. This was particularly true for Nelson growers during the 2006/07 production season, with seven high risk *Botrytis* infection periods during bloom (Table 1). However, in blackcurrant, fruit set is not only affected by the level of *Botrytis* control. Fertilisation, inadequate pollination and abortion of young seeds can also cause 30-50% of fruit drop (Wellington et al. 1921; Neumann 1955).

**TABLE 1: *Botrytis cinerea* risk prediction for Canterbury and Nelson blackcurrant field research sites based on the Broome model developed for grapes (Broome et al. 1995) from 1 September 2006 to 31 January 2007. Weather data were obtained from weather stations 5 and 14 km from the actual research sites in Canterbury and Nelson, respectively.**

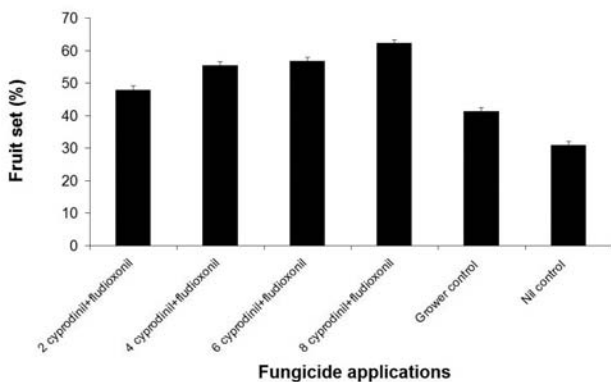
Canterbury <sup>1</sup>	Risk	Nelson <sup>2</sup>	Risk
–	–	03 September 2006	Severe
–	–	05 September 2006	Severe
–	–	28 September 2006	Severe
04 October 2006	Severe	03 October 2006	Severe
25 October 2006	Severe	24 October 2006	Severe
–	–	29 October 2006	Severe
07 November 2006	Severe	08 November 2006	Severe
18 November 2006	Severe	18 November 2006	Severe
–	–	19 November 2006	Low
–	–	24 November 2006	Moderate
–	–	02 December 2006	Severe
21 December 2006	Severe	–	–
–	–	10 January 2007	Severe
11 January 2007	Severe	11 January 2007	Severe
14 January 2007	Severe	14 January 2007	Severe
–	–	15 January 2007	Low
20 January 2007	Low	–	–
27 January 2007	Severe	27 January 2007	Severe
–	–	29 January 2007	Severe

<sup>1</sup>Date of artificial *B. cinerea* application: 23 October 2006.

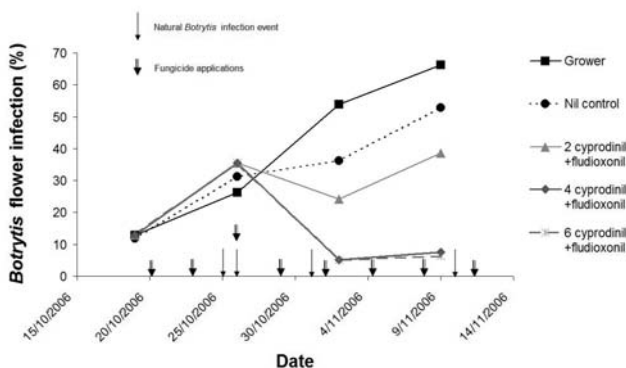
<sup>2</sup>Date of artificial *B. cinerea* application: 26 October 2006.



**FIGURE 4:** Blackcurrant fruit set (%) at the Canterbury and Nelson field trial sites after fungicide applications ranging from 5 days before (-5 days) to 5 days after (+5 days) artificial inoculation with the pathogen at day 0. Note: for the Nelson site, the -3 days and +3 days fungicide applications are missing because of rain events; n = 4.



**FIGURE 5:** Blackcurrant fruit set (%) at the Nelson field trial site after nil, two, four, six or eight applications of cyprodinil and fludioxonil (Switch®), or a single application of tolyfluaniid (grower control).



**FIGURE 6:** Blackcurrant flower infections (%) at the Nelson field trial site after nil, two, four or six applications of cyprodinil and fludioxonil (Switch®, indicated with wide arrows), or a single application of tolyfluand (Grower). Predicted natural *Botrytis* infection events based on the Broome model (Table 2) are indicated with a narrow arrow, n = 4. Note: data for the treatment '8 cyprodinil+fludioxonil applications' were not available for flower infections as the last fungicide application was after the disease assessment on 9 November 2006.

### CONCLUSION

These results suggest that *B. cinerea* can be a major problem in blackcurrant production in New Zealand, causing flower infections, flower blight and thereby reducing fruit set and yield, if left uncontrolled. Prediction of inoculation and infection events, tailored to blackcurrant, may aid timing of fungicide application to protect flowers from *B. cinerea* infections. Cyprodinil and fludioxonil applications had to be within 3 days prior or after the *Botrytis* inoculation and/or infection event to improve fruit set. While intensive fungicide applications have reduced flower infections, such a programme with two applications per week is neither realistic nor advisable for growers to use because of resistance management as well as the potential for high levels of fungicide residues in blackcurrant juice. The New Zealand blackcurrant industry currently runs a 'zero' pesticide residue programme, with a general move towards pesticide free production. Other solutions to *B. cinerea* control in blackcurrant are required, such as choice of growing sites, selection of less susceptible cultivars, plant architecture, inoculum management and disease prediction for optimum chemical or biological control.

### ACKNOWLEDGEMENTS

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