# A Survey of Sensitivity of *Botrytis cinerea* to cyprodinil, fludioxonil, boscalid, pyrimethanil, carbendazim and iprodione from one Blueberry Orchard in Northland.

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## Introduction

Fungicides for the control of *Botrytis cinerea* are an important tool in helping to reduce the incidence and severity of blueberry fruit rots. An understanding of the current sensitivity of *B. cinerea* populations in the orchard to some of the more commonly used fungicides will help in providing a benchmark and will help in the development of robust resistance management strategies.

In this study the sensitivity profiles of *B. cinerea* isolates, approximately 20 from an orchard in Northland selected by BerryCo, were tested in an *in vitro* agar plate assay to the active ingredients cyprodinil, fludioxonil, boscalid, pyrimethanil, carbendazim and, iprodione.

## Methods

Blueberry flowers and twigs were sampled at random from one blueberry orchard during August 2023. Up to 30 samples of flowers and twigs were placed directly into individual zip-lock bags and couriered to Plant Diagnostics Limited for incubation under humid conditions at room temperature to allow sporulation of *Botrytis.* Single spore isolates were obtained, and agar plugs of each isolate stored in sterile distilled water in 2 mL microcentrifuge tubes at 4°C until required for testing.

*Botrytis cinerea* isolates were tested for their sensitivity to the active fungicide ingredients as individual components in an agar plate assay in a similar way to the methods described by Beresford et. al. (2017).

The individual components of the fungicide Switch® (cyprodinil and fludioxonil) were supplied by Syngenta New Zealand as Chorus® and Geoxe® respectively. Boscalid, one component of the fungicide Pristine® was tested as the formulated product Unistar® (Adria Crop Protection). Pyrimethanil, as Scala was supplied by Bayer. Carbendazim, as Goldazim 500 SC and iprodione, as Ippon 500 SC were supplied by Adria Crop Protection.

The *Botrytis cinerea* isolates were tested on agar plates supplemented with each fungicide at either one or two discriminatory doses (see Table 1). A non- fungicide amended agar control was also used for each isolate in order to calculate relative growth rates.

Cyprodinil and pyrimethanil were tested at 0, 1 and 10 mg/L on Modified GGA medium; fludioxinil was tested at 0, 1 and 10 mg/L on potato dextrose agar; boscalid was tested at 0, 2 and 20 mg/L on succinate medium; carbendazim was tested at 0 and 10 mg/L on potato dextrose agar and iprodione was tested at 0 and 5 mg/l on potato dextrose agar. Known standard sensitive and resistant isolates where available were included in the testing as controls.

*Botrytis cinerea* isolates were initially cultured on PDA at 20 °C in the dark for three days after which a 6 mm diameter core taken from just behind the colony edge was transferred to each test plate. Two isolates per plate were placed at opposite sides of the plate. Plates were incubated in the dark at 20°C for 3 days when the radial growth was measured. Each isolate was replicated randomly on a second true replicate plate.

For cyprodinil, fludioxonil boscalid and pyrimethanil each *Botrytis cinerea* isolate was categorised into one of three sensitivity groups by comparing the relative growth rate on fungicide amended agar as a percentage of the growth on non-fungicide amended agar at each discriminatory dose (Table 2). For carbendazim, sensitivity was categorised from a single discriminatory dose (10 mg/L) based on active growth at this concentration. For iprodione, isolates were classified as resistant when the level of growth reduction on 5 mg/L was less than or equal to 75% of the growth on non-amended potato dextrose agar. Sensitivity was categorised with reference to the findings of Beresford et al (2017), Myresiotis et al (2008) and Braithwaite et al (1991).


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Table 1: Percent Relative Growth Rate (RGR) and Percent Growth Reduction Sensitivity Categories

| Active ingredient | Sensitive | Moderately Resistant | Resistant |
|---|---|---|---|
| cyprodinil | ≤ 50% RGR at 1 mg/L | >50% RGR at 1 mg/L and ≤ 50% at 10 mg/L | > 50% RGR at 10 mg/L |
| fludioxonil | ≤ 50% RGR at 1 mg/L | >50% RGR at 1 mg/L and ≤ 50% at 10 mg/L | > 50% RGR at 10 mg/L |
| [1]boscalid | ≤ 50% RGR at 2 mg/L | >50% RGR at 2 mg/L and ≤ 50% at 20 mg/L | > 50% RGR at 20 mg/L |
| pyrimethanil | ≤ 50% RGR at 1 mg/L | >50% RGR at 1 mg/L and ≤ 50% at 10 mg/L | > 50% RGR at 10 mg/L |
| carbendazim | na | na | Active growth at 10 mg/L |
| iprodione | na | na | < 75% growth reduction at 5 mg/L |

[1]A new sample of boscalid was not available due to the product Unistar being currently unavailable. Discussions with the supplier suggested that the batch used in this testing could be at least 6 years from manufacture. BerryCo was advised of this prior to the start of testing and the decision was made to proceed. Results should be interpreted with this in mind but comparative differences in sensitivity between isolates should be relevant.

## Results and Discussion

Fungicide sensitivity characterisation in summary form is presented in Table 2. Recorded growth rates for each isolate and every fungicide component are presented in Appendix I.

**Table 2: Fungicide sensitivity characterisation of *Botrytis cinerea* isolates to six fungicides collected from a Northland orchard. Numbers of isolates out of 20 in each category are presented.**

| **cyprodinil** | | | **fludioxinil** | | | **boscalid** | | | **pyrimethanil** | | | **carbendazim** | | **iprodione** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S** | **MR** | **R** | **S[1]** | **MR** | **R** | **S** | **MR** | **R** | **S** | **MR** | **R** | **S** | **R** | **S** | **R** |
| 0 | 11 | 9 | 20 | 0 | 0 | 0 | 0 | 20 | 1 | 1 | 18 | 9 | 11 | 7 | 13 |

**[1] Sensitivity categories are as follows: S = sensitive, MR = moderately resistant, R = resistant**

Sensitivity of *Botrytis cinerea* isolates to cyprodinil from this orchard showed that all isolates tested had growth rates on fungicide amended agar that would place them in the moderately resistant or resistant categories.

All isolates tested were sensitive to fludioxinil, the other component of the fungicide Switch® at the rates tested (1 mg/L and 10 mg/L).

Sensitivity of *Botrytis cinerea* isolates to pyrimethanil from this orchard showed that nearly all isolates tested had growth rates on fungicide amended agar that would place them in the moderately resistant or resistant categories.

Sensitivity of isolates to carbendazim from this orchard showed that 11 of 20 isolates (55%) were resistant to this fungicide.

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Sensitivity of isolates to iprodione from this orchard showed that 13 of 20 isolates (65%) were resistant to this fungicide.

It is difficult to predict whether the presence of resistant isolates would translate in the field situation to a loss of sensitivity. The field application rate of cyprodinil, for example, equates to 300 mg/L which is much higher than the 10 mg/L threshold used as the cut-off for categorising an isolate as resistant. However, the actual amount of fungicide that the fungus is exposed to in the field would normally be less than the application rate due to incomplete exposure or breakdown by environmental elements. Even an isolate categorised as resistant in this testing may be controlled if exposed to the field rate but there is an indication of a reducing sensitivity in some isolates of *B. cinerea* when exposed to the 10 mg/L rate and a risk that this could translate into a loss of sensitivity in the field to cyprodinil, if not currently then at some point in the future, without careful management. This situation appears similar with pyrimethanil with the possibility of cross resistance between the two fungicides. At present, the fludioxinil component of Switch® is allowing the fungicide to continue to perform and the cyprodinil component is continuing to show evidence of some continuing efficacy but with the proviso that some shift of sensitivity appears to be occurring.

The situation with boscalid is that all isolates from this orchard were classified in the moderately resistant or resistant category. This is of concern in relation to use of the fungicide Pristine® because the other component of this fungicide (pyraclostrobin) which was not tested in this study has been found in numerous studies to have reduced efficacy against isolates of *B. cinerea* due to decreasing fungicide sensitivity (Fernandez-Ortuno et al 2014).

It should be noted that with boscalid, it is unknown as to what degree, if any, the age of the product tested may have influenced its activity, but fresh product was not available. It is possible that if activity had been lost, the loss of sensitivity may not be quite as much as indicated here. In addition, some other researchers have classified isolates based on a 5 mg/L and a 50 mg/L discriminatory dose as compared to the 2 mg/l and 20 mg/L doses used here. However, Myresiotis et al (2008) has determined that growth of fungi at a dose of < 2-3 mg/L denotes sensitivity to this fungicide. As further evidence of the activity of the boscalid sample used in this study, the 'wild' strain isolate used as a control has had no known fungicide exposure and unlike the orchard isolates, was classified as sensitive to the discriminatory doses used in this study.

The situation with carbendazim and iprodione is that approximately half the isolates tested were categorised as resistant. This would potentially place these two fungicides at risk of failing to control disease if they were to be in frequent use.

In order to determine if reduced sensitivity or resistance of isolates as characterised in this study is already resulting in the likelihood of reduced field control, detached fruit assays could be carried out. This would involve inoculating sprayed and non-sprayed fruit with a range of 'resistant' isolates and then assessing the amount of disease development.

## Conclusions and Recommendations

Results of this sensitivity testing showed that all orchard *B. cinerea* isolates were sensitive to fludioxinil at the lowest concentration tested. Sensitivity of *B. cinerea* to cyprodinil, the other component of the fungicide Switch®, and pyrimethanil indicated that all or most isolates were moderately resistant or resistant to these two fungicides. It is therefore likely that effective control of *B. cinerea* is becoming increasingly dependent on the fludioxinil component of this fungicide. Although reduced sensitivity of fungi to fludioxinil is not commonly reported, it has been detected (Fernandez-Ortuno et al 2014). Resistance management strategies should be employed where Switch® is used. Care should be taken with pyrimethanil as this fungicide is at risk similar to cyprodinil.

Boscalid, carbendazim and iprodione appear to be at risk of the increasing development of *B. cinerea* isolates resistant to them. The knowledge that fungal resistance to pyraclostrobin, the other component of Pristine®, has been frequently detected in other studies suggests that this fungicide should be used only in a programme where resistance management strategies are practiced, and its effectiveness should be carefully monitored. It may be advisable to test in a detached fruit assay whether some of the isolates characterised as resistant in this study are able to be controlled by field rates of Pristine®. Resistance to carbendazim can build up very fast making it advisable to limit its use or find alternative chemistry.

## References

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## Appendix I: Percent relative growth rate of B. cinerea isolates.

| | cyprodinil | | | fludioxonil | | | boscalid | | | pyrimethanil | | | carbendazim | | iprodione | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | Percent Relative growth rate | | Category | Percent Relative growth rate | | Category | Percent Relative growth rate | | Category | Percent Relative growth rate | | category | Percent Relative growth rate | Category | Percent growth reduction | Category |
| **Rate (mg/L)** | **1** | **10** | | **1** | **10** | | **2** | **20** | | **1** | **10** | | **10** | | **5** | |
| 1 | 104 | 24 | MR | 0 | 0 | S | 92 | 53 | R | 93 | 100 | R | 96 | R | 100 | S |
| 2 | 97 | 10 | MR | 0 | 0 | S | 90 | 55 | R | 88 | 75 | R | 104 | R | 100 | S |
| 3 | 89 | 61 | R | 4 | 0 | S | 79 | 67 | R | 100 | 59 | R | 0 | S | 53 | R |
| 4 | 71 | 32 | MR | 0 | 0 | S | 100 | 69 | R | 83 | 64 | R | 93 | R | 100 | S |
| 5 | 121 | 66 | R | 4 | 2 | S | 76 | 65 | R | 100 | 83 | R | 163 | R | 65 | R |
| 6 | 79 | 50 | MR | 5 | 0 | S | 111 | 81 | R | 110 | 39 | MR | 116 | R | 78 | S |
| 7 | 95 | 74 | R | 5 | 0 | S | 90 | 59 | R | 100 | 90 | R | 0 | S | 70 | R |
| 8 | 90 | 58 | R | 0 | 0 | S | 83 | 69 | R | 84 | 76 | R | 90 | R | 94 | S |
| 9 | 106 | 75 | R | 2 | 15 | S | 84 | 68 | R | 102 | 95 | R | 0 | S | 64 | R |
| 10 | 98 | 84 | R | 16 | 6 | S | 91 | 61 | R | 108 | 100 | R | 0 | S | 55 | R |
| 11 | 67 | 21 | MR | 4 | 0 | S | 67 | 64 | R | 103 | 97 | R | 62 | R | 72 | R |
| 12 | 77 | 62 | R | 0 | 0 | S | 90 | 60 | R | 93 | 84 | R | 90 | R | 66 | R |
| 13 | 88 | 44 | MR | 0 | 0 | S | 83 | 61 | R | 110 | 93 | R | 95 | R | 67 | R |
| 14 | 80 | 48 | MR | 0 | 0 | S | 78 | 64 | R | 0 | 0 | S | 0 | S | 65 | R |
| 15 | 97 | 23 | MR | 6 | 0 | S | 87 | 77 | R | 103 | 78 | R | 98 | R | 100 | S |
| 21 | 94 | 51 | MR | 14 | 0 | S | 112 | 88 | R | 117 | 80 | R | 0 | S | 55 | R |
| 22 | 91 | 57 | R | 10 | 0 | S | 100 | 76 | R | 89 | 71 | R | 0 | S | 64 | R |
| 23 | 20 | 20 | MR | 17 | 0 | S | 78 | 67 | R | 108 | 82 | R | 0 | S | 14 | R |
| 24 | 68 | 23 | MR | 6 | 0 | S | 89 | 66 | R | 107 | 90 | R | 117 | R | 100 | S |
| 25 | 90 | 86 | R | 15 | 27 | S | 77 | 72 | R | 117 | 120 | R | 0 | S | 53 | R |
| **Standards** | | | | | | | | | | | | | | | | |
| BC 34 | 3 | 0 | S | 5 | 0 | S | 39 | 37 | S | 8 | 0 | S | 0 | S | 100 | S |
| BC 153 | - | - | - | - | - | - | - | - | - | - | | - | 92 | R | 67 | R |
| COBC 350 | - | - | - | - | - | - | 82 | 61 | R | - | - | - | 93 | R | - | - |
| COBC 348 | - | - | - | - | - | - | 80 | 53 | R | - | - | - | 94 | R | - | - |
| Isolate 22 | - | - | - | - | - | - | - | - | - | 105 | 91 | R | - | - | - | - |

[1] Standard strains: BC 34 wild isolate with no known exposure to any of these fungicides; BC 153 has known resistance to carbendazim and iprodione. COBC 348 is moderately resistant; isolate 22 is resistant to cyprodinil and COBC350 a resistant standard to boscalid. Green shading represents moderately resistant or resistant isolate.


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