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A Survey of Sensitivity of *Botrytis cinerea* to cyprodinil, fludioxonil and boscalid from Five Blueberry Orchards.

Report prepared for BerryCo NZ Ltd.
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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 each of 5 orchards selected by BerryCo, were tested in an *in vitro* agar plate assay to components of the fungicides Switch® and one component of the fungicide Pristine®.

Methods

Blueberry flowers and fruits were collected by BerryCo NZ Ltd from five separate blueberry orchards during the 2022-2023 season. Up to 30 samples of either flowers or fruit were randomly collected from each orchard and placed directly into individual zip-lock bags. Samples were couriered to Plant Diagnostics Limited where they were frozen at -18°C for 4 hours and then incubated under humid conditions at room temperature to allow sporulation. 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 each of three 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).

The *Botrytis cinerea* isolates were tested against each fungicide on agar plates supplemented with each fungicide at 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.

Cyrodinil was 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; and boscalid was tested at 0, 2 and 20 mg/L on succinate medium. Known standard sensitive and resistant isolates were included in the testing as controls.

Botrytis cinerea isolates were initially grown on PDA 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 18°C for 3 days when the radial growth was measured. Each isolate was replicated randomly on a second plate.

For each of the three fungicides each 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). Sensitivity was categorised with reference to the findings of Beresford et al (2017) and Myresiotis et al (2008).

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Table 1: Percent Relative Growth Rate (RGR) 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

[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 and in graphical form in Figure 1. Recorded growth rates for each orchard isolate and every fungicide component are presented in Appendix I.

Sensitivity of *Botrytis cinerea* isolates to cyprodinil when summed across the orchards showed more than half of isolates tested had growth rates on fungicide amended agar that would place them in the moderately resistant or resistant categories. On an individual orchard basis, isolate sensitivity varied with orchards 1 and 3 having half or less of isolates in the moderate or high resistance categories compared to the other 3 orchards.

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).

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. 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 similar to that of cyprodinil with more than half of isolates tested being classified as moderately resistant or resistant. Once again there are differences

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between orchards with orchards 1 and 3 having a much lower proportion of isolates in the moderately resistant or resistant categories. 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. At the discriminatory doses used in this study, there is quite a range in sensitivity of isolates which is a likely indicator of a loss of sensitivity in those isolates growing at the higher dose rate. 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. Its growth was completely inhibited by all rates tested in this agar plate assay.

Differences noted between orchards are interesting, but it may require testing of greater numbers of isolates to determine if this is a difference of significance. It could possibly be related to the fungicide programmes used on these orchards if there had been lower exposure to the fungicides in this study. However, many others have examined the relationship between the proportion of resistant isolates on an orchard and fungicide use and it often does not correlate well (Beresford 2017). This is thought to be due to the probable movement of *B. cinerea* spores within at least a 5 km radius.

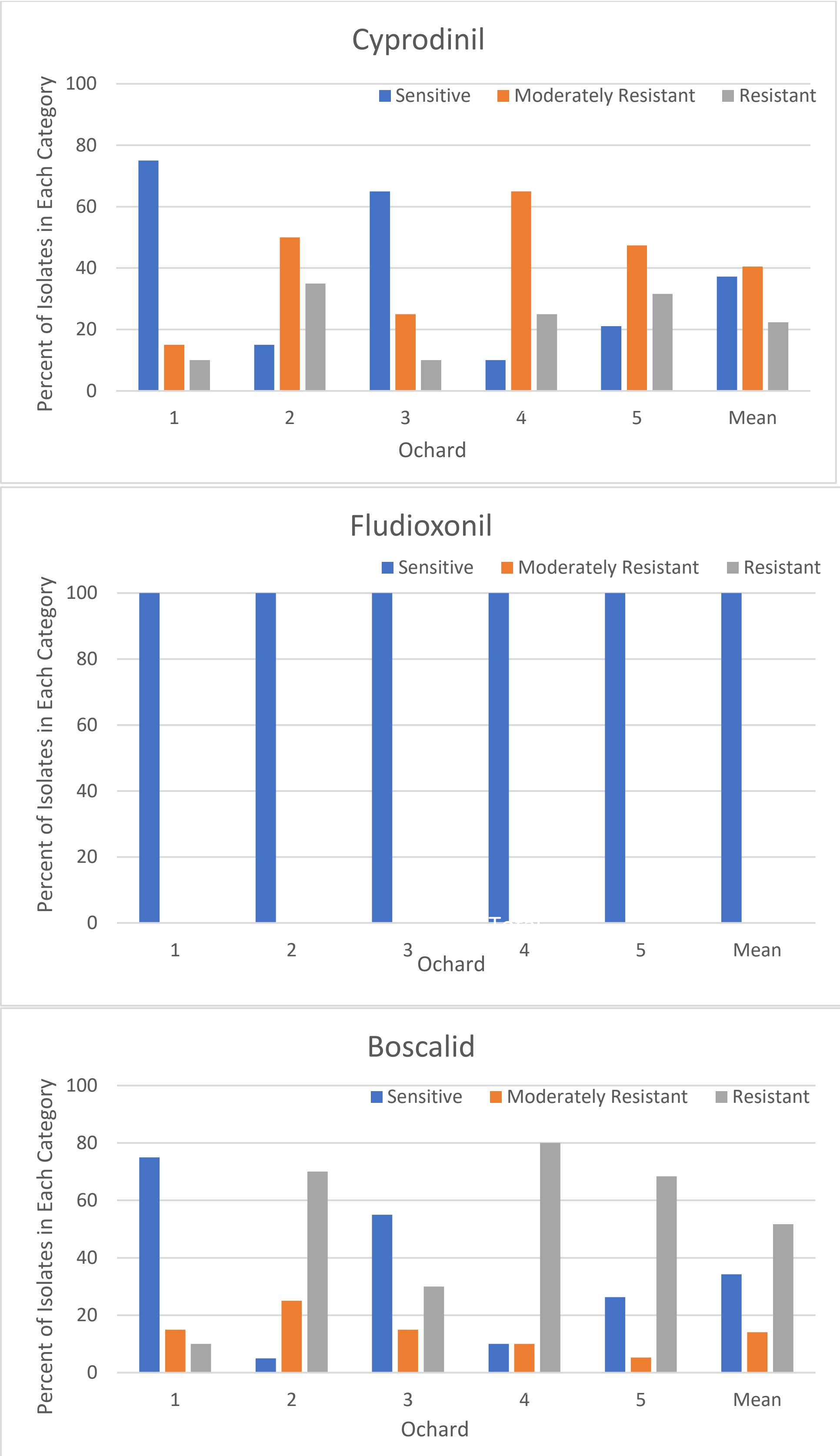
In order to determine if reduced sensitivity or resistance of isolates as characterised in this study is already resulting in the likelihood of reduced field control, detached fruit assays could be carried out. This would involve inoculating sprayed and non-sprayed fruit with a range of 'resistant' isolates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

Table 2: Fungicide sensitivity characterisation of *Botrytis cinerea* isolates to three fungicides collected from five blueberry orchards. Numbers of isolates in each category are presented.

Fungicide	**cyprodinil**			**Fludioxonil**			**boscalid**		
Orchard	**S[1]**	**MR**	**R**	**S**	**MR**	**R**	**S**	**MR**	**R**
1	15	3	2	20	0	0	15	3	2
2	3	10	7	20	0	0	1	5	14
3	13	5	2	20	0	0	11	3	6
4	2	13	5	20	0	0	2	2	16
5	4	9	6	19	0	0	5	1	13
Total	**37**	**40**	**22**	**99**	**0**	**0**	**34**	**14**	**51**

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

Figure 1: Fungicide sensitivity characterisation of *Botrytis cinerea* isolates to three fungicides collected from five blueberry orchards. Percent of isolates in each category are presented.



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®, showed across the orchards that more than half of isolates were moderately resistant or resistant. 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.

Boscalid appears to be at risk of the increasing development of *B. cinerea* isolates resistant to it. 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 practised. 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®.

References

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

		cyprodinil (Chorus) GGA Percent Relative growth rate			fludioxonil (Geoxe) PDA Percent Relative growth rate			boscalid (Unistar) Succinate Percent Relative growth rate		
	Isolate	1	10	Category	1	10	Category	2	20	Category
Orchard 1	1	0	0	S	0	0	S	45	17	S
	2	98	56	R	0	0	S	45	41	S
	3	10	4	S	0	0	S	37	31	S
	4	7	2	S	0	0	S	45	23	S
	6	0	0	S	0	0	S	45	18	S
	7	17	5	S	0	0	S	37	29	S
	8	92	63	R	0	0	S	56	38	MR
	10	2	0	S	0	0	S	40	19	S
	11	3	3	S	0	0	S	39	13	S
	12	0	0	S	0	0	S	51	23	MR
	14	8	2	S	0	0	S	36	24	S
	15	16	16	S	0	0	S	41	25	S
	16	90	48	MR	0	0	S	74	53	R
	17	2	2	S	0	0	S	39	24	S
	18	2	2	S	0	0	S	47	26	S
	19	33	4	S	0	0	S	41	27	S
	20	67	5	MR	0	0	S	48	27	S
	22	0	0	S	0	0	S	63	37	MR
	24	0	0	S	0	0	S	37	13	S
	26	95	15	MR	0	0	S	81	51	R
Orchard 2	41	13	3	S	0	0	S	76	50	R
	42	82	35	MR	0	0	S	67	46	MR
	43	3	3	S	0	0	S	39	26	S
	45	102	57	R	0	0	S	83	61	R
	46	98	52	R	0	0	S	75	59	R
	47	95	56	R	0	0	S	86	58	R
	48	98	53	R	0	0	S	68	49	MR
	49	98	39	MR	0	0	S	83	63	R
	50	78	36	MR	0	0	S	73	62	R
	52	69	24	MR	0	0	S	112	100	R
	53	103	58	R	0	0	S	57	45	MR
	54	39	0	S	0	0	S	105	82	R
	55	83	55	R	0	0	S	71	46	MR
	56	80	35	MR	0	0	S	75	57	R
	57	100	56	R	0	0	S	94	72	R
	58	103	46	MR	0	0	S	91	59	R
	59	70	25	MR	0	0	S	62	56	R
	61	86	44	MR	0	0	S	75	58	R
	62	77	37	MR	0	0	S	68	49	MR
	63	81	37	MR	0	0	S	94	61	R
Orchard 3	64	26	24	S	0	0	S	40	23	S
	65	13	10	S	0	0	S	46	22	S
	66	35	51	S	3	2	S	49	34	S
	68	0	0	S	0	0	S	67	55	R
	69	9	5	S	0	0	S	47	36	S
	70	3	3	S	0	0	S	55	45	MR
	71	102	61	R	0	0	S	83	61	R
	72	89	42	MR	0	0	S	92	100	R
	73	19	13	S	0	0	S	41	28	S
	74	17	11	S	0	0	S	65	29	MR
	75	0	0	S	0	0	S	44	26	S
	76	100	47	MR	0	0	S	63	53	R
	77	75	36	MR	0	0	S	75	53	R
	78	2	0	S	0	0	S	35	18	S
	79	0	0	S	0	0	S	33	17	S
	80	91	55	R	0	0	S	74	59	R
	81	97	43	MR	0	0	S	39	12	S
	82	43	17	S	0	0	S	44	24	S
	83	83	40	MR	8	0	S	46	27	S
	84	0	0	S	0	0	S	60	42	MR

		cyprodinil (Chorus) GGA Percent Relative growth rate			**fludioxonil (Geoxe) PDA Percent Relative growth rate**			**boscalid (Unistar) Succinate Percent Relative growth rate**		
	Isolate	1	10	Category	1	10	Category	2	20	Category
Orchard 4	85	98	45	MR	0	0	S	79	60	R
	86	90	38	MR	0	0	S	74	46	MR
	87	34	34	S	0	0	S	41	21	S
	88	0	0	S	0	0	S	45	26	S
	89	86	41	MR	0	0	S	80	59	R
	90	70	39	MR	0	0	S	86	74	R
	91	53	23	MR	0	0	S	81	70	R
	92	90	42	MR	3	0	S	83	51	R
	93	103	46	MR	0	0	S	63	44	MR
	94	89	41	MR	7	0	S	73	53	R
	95	79	43	MR	0	0	S	77	56	R
	96	88	47	MR	0	0	S	80	62	R
	97	84	50	R	0	0	S	60	52	R
	98	98	57	R	10	0	S	81	58	R
	99	81	49	MR	0	0	S	80	50	R
	100	95	55	R	0	0	S	90	65	R
	101	76	47	MR	0	0	S	88	82	R
	102	85	46	MR	0	0	S	80	61	R
	103	94	61	R	0	0	S	74	61	R
	104	95	58	R	0	0	S	81	67	R
Orchard 5	105	86	39	MR	9	1	S	82	58	R
	106	2	2	S	0	0	S	37	35	S
	107	74	42	MR	0	0	S	49	29	S
	108	72	33	MR	0	0	S	66	49	MR
	109	93	50	R	0	0	S	74	52	R
	110	81	5	MR	0	0	S	154	85	R
	111	82	36	MR	0	0	S	49	31	S
	112	96	83	R	0	0	S	76	54	R
	113	83	34	MR	0	0	S	72	53	R
	114	100	57	R	5	0	S	79	65	R
	115	100	69	R	0	0	S	74	65	R
	116	30	24	S	0	0	S	56	50	R
	117	0	0	S	0	0	S	36	27	S
	118	11	18	S	0	0	S	46	34	S
	119	83	60	R	0	0	S	80	57	R
	121	82	11	MR	0	0	S	84	59	R
	122	80	49	MR	0	0	S	82	61	R
	123	97	37	MR	0	0	S	80	63	R
	124	123	109	R	0	0	S	91	65	R
Stds[1]	BC 34	0	0	S	0	0	S	41	14	S
	COBC 348	81	51	R	0	0	S	67	48	MR
	COBC 350	6	6	S	3	0	S	79	62	R

[1] Standard strains: BC 34 wild isolate with no known exposure to any of these fungicides; COBC 348 cyropdinil resistant isolate; COBC350 boscalid resistant isolate.

Green shading represents moderately resistant or resistant isolates.

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