A Survey of Sensitivity of *Botrytis cinerea* to pyrimethanil, carbendazim, and iprodione, from five Blueberry Orchards

Report prepared for BerryCo NZ Ltd.
Mark Braithwaite, Uykim Lim, Emily Hicks and Bronwyn Braithwaite.
PLANT DIAGNOSTICS Limited.
November 2023

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 five orchards were selected by BerryCo, and tested in an *in vitro* agar plate assay to the active ingredients, pyrimethanil, carbendazim and iprodione.

Methods

Blueberry flowers and fruits were sampled at random from five separate blueberry orchards during the 2022-2023 season. Up to 30 samples of flowers and fruit were placed directly into individual zip-lock bags and 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 the active fungicide ingredients in an agar plate assay in a similar way to the methods described by Beresford et. al. (2017). 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 (see Table 1). A non- fungicide amended agar control was also used for each isolate.

Pyrimethanil was tested at 0, 1 and 10 mg/L on Modified GGA medium. Carbendazim was tested at 10 mg/L on potato dextrose agar and iprodione was tested at 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 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. For carbendazim, sensitivity was categorised from a single discriminatory dose (10 mg/L) based on active growth at this concentration. Classification of resistant isolates for iprodione, was determined to be 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 (Table 1). Sensitivity was categorised with reference to the findings of Beresford et al (2017), Myresiotis et al (2008) and Braithwaite et 1991.

184 Kirk Road, Templeton, Christchurch 7678. Phone: 03 377 9026
email: enquiries@plantdiagnostics.co.nz

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

Active ingredient	Sensitive	Moderately Resistant	Resistant
pyrimethanil	≤ 50% RGR at 1 mg/L	>50% RGR at 1 mg/L and ≤ 50% at 10 mg/L	> 50% at 10 mg/L
carbendazim	No growth at 10 mg/L	NA	Growth at 10 mg/L
iprodione	> 75% growth reduction at 5 mg/L	NA	< 75% growth reduction at 5 mg/L

Results and Discussion

Fungicide sensitivity characterisation in summary form is presented in Table 2 and graphical form for pyrimethanil (Figure 1). Recorded growth rates for each isolate and every fungicide component are presented in Appendix I.

Sensitivity of *Botrytis cinerea* isolates to pyrimethanil across all 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.

Across all five orchards the sensitivity of isolates to carbendazim showed that 61 of 99 isolates (61%) had lost sensitivity to this fungicide. However, there was considerable variation between orchards. Orchard 1 had predominantly sensitive isolates whereas orchards 2 and 4 predominantly resistant isolates. The other orchards had a mixture of sensitive and resistant isolates.

Across all five orchards the sensitivity of isolates to iprodione showed that only 19 of 99 isolates (19%) had lost sensitivity to this fungicide. Hence in general, orchards had mostly sensitive isolates. No resistant isolates were detected on orchard one and orchards 2-4 had only low numbers. Orchard 5 had an even mixture of resistant and sensitive isolates.

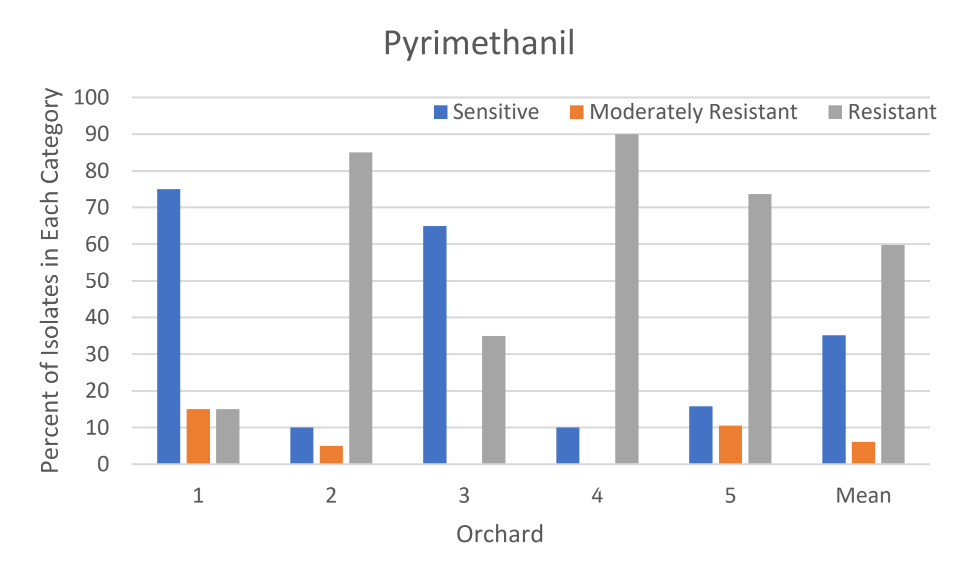
Table 2: Fungicide sensitivity characterisation of *Botrytis cinerea* isolates to three fungicides collected from 5 orchards.

Fungicide		pyrimethanil			carbendazim		iprodione	
Orchard	**Number isolates**	**S**	**MR**	**R**	**S**	**R**	**S**	**R**
1	20	14	3	3	18	2	20	0
2	20	2	1	17	1	19	16	4
3	20	13	0	7	10	10	18	2
4	20	2	0	18	3	17	17	3
5	19	3	2	14	6	13	9	10
Total	**99**	**34**	**6**	**59**	**38**	**61**	**80**	**19**

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

184 Kirk Road, Templeton, Christchurch 7678. Phone: 03 377 9026
email: enquiries@plantdiagnostics.co.nz

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



Conclusions and Recommendations

Resistance to pyrimethanil varied between orchards. There appears to be a fairly high correlation between the results of individual isolate sensitivity to cyprodinil in the testing earlier this year with the testing of pyrimethanil in this current study. This suggests that there may by cross resistance between these two AP fungicides (FRAC Group 9). Therefore, the degree of resistance to pyrimethanil may not be entirely related to usage but may also be related to the usage of cyprodinil. A study by Beresford et al 2017 on wine grapes demonstrated cross-resistance between these AP fungicides.

Sensitivity testing to carbendazim showed variation between orchards from low to high numbers of isolates classified as resistant. Generally, there was a good degree of sensitivity to iprodione except for orchard 5 where there was an approximately even mix of sensitivities. Presumably the degree of sensitivity to these fungicides follows the usage pattern across the orchards.

These results show that the management of fungicide usage will be essential in maintaining sensitivity thereby extending the useful life of these products. At this stage the indications are that iprodione may prove useful if used sparingly in orchards where there is currently good sensitivity.

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.

References

Beresford RM, Wright PJ, Middleditch CL, Vergara M, Hasna L, Wood PN and Agnew RH. Sensitivity of *Botrytis cinerea* to fungicides used in New Zealand wine grapes spray programmes. New Zealand Plant Protection 70: 285-294.

Braithwaite M, Elmer PAG and Ganev S 1991. Survey of fungicide resistance in *Monilinia fructicola* in New Zealand. New Zealand Plant Protection 44: 220-224.

Myresiotis CK, Bardas GA, and Karaoglanidis GS 2008. Baseline sensitivity of *Botrytis cinerea* to pyraclostrobin and boscalid and control of anilinopyrimidine- and benzimidazole-resistant strains by these fungicides. Plant Disease 92: 1427-1431.

DISCLAIMER: All reasonable efforts have been made to ensure the information contained in this report is correct, and is provided on the basis that PLANT DIAGNOSTICS Limited accepts no liability for any loss or damage resulting from actions or non-action taken on the information provided.

184 Kirk Road, Templeton, Christchurch 7678. Phone: 03 377 9026
email: enquiries@plantdiagnostics.co.nz

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

		Pyrimethanil Relative growth rate			Carbendazim Relative growth rate		Iprodione Percent growth reduction	
	Isolate	1	10	Category	10		5	
Orchard 1	1	10	0	S	0	S	100	S
	2	96	8	MR	1	S	100	S
	3	4	4	S	0	S	100	S
	4	58	32	MR	0	S	100	S
	6	45	5	S	0	S	100	S
	7	67	5	MR	0	S	100	S
	8	106	109	R	0	S	100	S
	10	41	20	S	0	S	100	S
	11	8	0	S	0	S	100	S
	12	2	5	S	0	S	100	S
	14	29	24	S	0	S	100	S
	15	34	5	S	0	S	100	S
	16	96	74	R	100	R	100	S
	17	4	4	S	0	S	100	S
	18	28	0	S	0	S	100	S
	19	38	22	S	0	S	100	S
	20	40	4	S	0	S	100	S
	22	19	15	S	0	S	100	S
	24	40	4	S	0	S	100	S
	26	91	79	R	103	R	100	S
Orchard 2	41	41	35	S	102	R	78	S
	42	97	89	R	77	R	94	S
	43	22	17	S	0	S	100	S
	45	81	73	R	103	R	100	S
	46	112	88	R	107	R	90	S
	47	90	83	R	103	R	100	S
	48	91	81	R	87	R	92	S
	49	86	79	R	104	R	74	R
	50	118	46	R	93	R	88	S
	52	95	67	R	81	R	73	R
	53	103	94	R	98	R	98	S
	54	100	21	MR	114	R	100	S
	55	88	78	R	99	R	78	S
	56	104	92	R	96	R	83	S
	57	93	81	R	105	R	100	S
	58	90	81	R	96	R	75	R
	59	96	72	R	89	R	75	R
	61	106	84	R	100	R	80	S
	62	107	53	R	111	R	84	S
	63	89	76	R	98	R	100	S

184 Kirk Road, Templeton, Christchurch 7678. Phone: 03 377 9026
email: enquiries@plantdiagnostics.co.nz

A Survey of Sensitivity of *Botrytis cinerea* to pyrimethanil, carbendazim and iprodione from five Blueberry Orchards.

		Pyrimethanil Relative growth rate			Carbendazim Relative growth rate		Iprodione Percent growth reduction	
	Isolate	1	10	Category	10		5	
Orchard 3	64	29	23	S	0	S	100	S
	65	44	15	S	0	S	100	S
	66	30	19	S	0	S	100	S
	68	40	21	S	101	R	87	S
	69	27	8	S	0	S	100	S
	70	13	18	S	103	R	82	S
	71	89	70	R	94	R	71	R
	72	111	86	R	102	R	100	S
	73	8	8	S	0	S	100	S
	74	20	5	S	0	S	100	S
	75	0	0	S	0	S	100	S
	76	96	73	R	108	R	100	S
	77	79	74	R	100	R	100	S
	78	31	12	S	0	S	100	S
	79	35	15	S	0	S	100	S
	80	93	72	R	107	R	100	S
	81	106	94	R	97	R	100	S
	82	32	13	S	0	S	100	S
	83	93	73	R	110	R	100	S
	84	24	19	S	110	R	71	R
Orchard 4	85	87	87	R	94	R	73	R
	86	98	54	R	90	R	81	S
	87	0	0	S	0	S	100	S
	88	0	0	S	0	S	100	S
	89	94	89	R	111	R	83	S
	90	91	72	R	105	R	100	S
	91	108	87	R	100	R	88	S
	92	104	82	R	102	R	74	R
	93	102	88	R	112	R	81	S
	94	90	79	R	142	R	70	R
	95	80	57	R	95	R	84	S
	96	84	64	R	96	R	69	S
	97	78	78	R	102	R	100	S
	98	118	82	R	99	R	84	S
	99	87	87	R	112	R	100	S
	100	105	74	R	97	R	100	S
	101	108	87	R	108	R	100	S
	102	87	66	R	100	R	85	S
	103	100	98	R	5	S	79	S
	104	88	77	R	105	R	88	S

A Survey of Sensitivity of *Botrytis cinerea* to pyrimethanil, carbendazim and iprodione from five Blueberry Orchards.

		Pyrimethanil Relative growth rate			Carbendazim Relative growth rate		Iprodione Percent growth reduction	
	Isolate	1	10	Category	10		5	
Orchard 5	105	98	75	R	96	R	71	R
	106	10	4	S	0	S	100	S
	107	72	77	R	100	R	75	R
	108	103	100	R	105	R	66	R
	109	102	78	R	107	R	63	R
	110	95	62	R	105	R	100	S
	111	97	85	R	100	R	100	S
	112	94	84	R	0	S	59	R
	113	100	87	R	107	R	100	S
	114	93	70	R	93	R	70	R
	115	102	42	MR	110	R	71	R
	116	80	66	R	113	R	75	R
	117	0	0	S	0	S	100	S
	118	4	0	S	0	S	100	S
	119	96	89	R	0	S	62	R
	121	90	8	MR	102	R	86	S
	122	84	105	R	105	R	69	S
	123	86	89	R	108	R	100	S
	124	111	89	R	0	S	62	R
Stds[1]	BC 34	8	0	S	2	S	100	S
	BC 153	-	-		103	R	66	R
	Isolate 22	105	91	S	-		-	

[1] Standard strains: BC 34, wild isolate with no known exposure to any of these fungicides; BC153, carbendazim and iprodione resistant isolate; Isolate 22, cyprodinil resistant isolate. Green shading represents resistant isolates.

184 Kirk Road, Templeton, Christchurch 7678. Phone: 03 377 9026
email: enquiries@plantdiagnostics.co.nz