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BerryCo blueberry experiments 2023

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March 2024

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14630 Post-harvest Fruit Collapse in Blueberry, 14507 Botrytis Inoculum Control Trial, 14408 Spray Trial

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Executive summary

BerryCo blueberry experiments 2023

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March 2024

During July to December 2023 The New Zealand Institute for Plant and Food Research Limited (PFR) conducted three experiments with and for BerryCo. All experiments were conducted in covered cropping systems (plastic tunnel houses with bird netting). Plants were grown in pots with fertigation and irrigation. The experiments were:

- Nanric Inoculum Management
- Lanvale Spray Trial
- Postharvest Berry Collapse.

Three experiments were undertaken to illustrate the importance of cultivar susceptibility, inoculum management, spray timing and agrichemical choice, as well as postharvest fruit handling, on blueberry shelf-life as affected by *Botrytis cinerea* (B.c.) and berry collapse. The experiments were small and would be considered preliminary, owing to the short timeframe and urgency. However, the results provide good key insights.

With regard to cultivar susceptibility, ‘Masena’ consistently was the least susceptible compared with the other BerryCo varieties explored. Data from Experiments 1 and 2 also indicate that inoculum management affects berry infection, and that lowering the inoculum reduces infection risks. Corolla infections may be predictive of berry infections. The number and frequency of spray interventions should correspond to B.c. risk and cultivar susceptibility. Berry collapse is more likely to be driven by physiological factors and postharvest management than by biological causes. However, soft and collapsed berries express more disease than clean, healthy fruit.

Recommendations for the blueberry industry

Inoculum control

- Frequently sweep/vacuum dead flowers and leaves on the ground (weekly or more often).
- Gently shake or leaf blow plants to shake out dead flowers and aborted fruitlets (to be further tested).
- Avoid water pooling on the ground from fertigation/irrigation (as this aids *Botrytis cinerea* sporulation from infected tissues).
- Control humidity via fans or ventilation (to be further tested). *Botrytis cinerea* readily grows and infects at relative humidity (RH) greater than 93%.

Agrichemical use

- In a low-risk year (low tunnel house humidity), few sprays may be required. In a high-risk year combined with a susceptible cultivar more sprays will probably be required (to be further tested).
- Apply fungicides preventatively and according to *B. cinerea* sporulation and infection risk (before wet weather or extended periods of high humidity).
- Ensure good spray coverage with calibrated spray equipment.

Postharvest

- Over-ripe collapsed berries featured the most *B. cinerea* sporulation, followed by soft berries, compared with those firm to the touch.
- We believe *B. cinerea* fruit infection is secondary, due to spore contamination of berries before and during harvest. Harvest and postharvest handling then redistributes spores onto picking scars or other physical wounds/microcracks.
- The time from picking to on-orchard cooling should be 30 min or less.
- Avoid all unnecessary handling of fruit after picking.
- Avoid postharvest temperature fluctuations.

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1 Nanric inoculum management

This work has been funded by BerryCo to control *Botrytis cinerea* in BerryCo blueberry tunnels. The pathogen has caused severe postharvest fruit losses (up to 30%; Ewan Potgieter pers. comm) in BerryCo blueberry varieties produced in the northern, warm and humid, regions of New Zealand. *Botrytis cinerea* fruit losses are caused by flowering tissue infections and fruit contamination (Li et al. 2018). Inoculum management and protection of susceptible tissues is important in disease control. Here we investigate the use removing inoculum sources for control of *B. cinerea.*

1.1 Aim

For the Nanric Inoculum Management experiment, the aim was to remove *Botrytis cinerea* (B.c.) inoculum sources, to reduce flower, green and ripe berry infections. Inoculum control is important in disease management to reduce pathogen pressure, minimise exposure risk of susceptible tissues and optimise fungicide efficacy.

1.2 Methods

The trial was conducted at one grower site, using two tunnel houses. In Tunnel A, necrotic tissues were removed weekly to fortnightly from the plant canopy, but only from 50% of the designated plots (by manually picking out symptomatic tissues from one side only of the plant row) and from the ground (by sweeping both sides of the plant rows). This removal was done for approximately a 50-m section of Tunnel A for the whole tunnel width (Figure 1.1). Tunnel B was left as is and served as a control. Standard grower management was applied in both tunnels. Necrotic B.c. corolla infection *in planta*, green berry and ripe berry infections were monitored. The amount of debris was determined by dry weight, and the ratio of aborted flowers to corollas in the debris was determined. The number of symptomatic tissues in the plants was counted. The first samples were collected on 26 July 2023. To determine B.c. infection, tissues collected were incubated at room temperature with natural light in humidity chambers for 7–14 days (Figure 1.2). Green berries were surface sterilised and frozen overnight to allow latent infections to express. Surface sterilisation consisted of 1 min of 70% ethanol, followed by 1 min in 1% sodium hypochlorite, 1 min of 70% ethanol and two rinses of reverse osmosis water. Ripe berries were picked into individual cells (12-cell titre plates), collecting 24 berries per plot. These were not surface sterilised (NSS), but hands were

Figure 1.1. Swept trial section of Tunnel A in foreground.

sanitised between plots. For each sampling plot, both green berry and ripe berries were incubated for 7–14 days in humidity chambers (Figure 1.3).

Cultivars in the tunnels were two rows of ‘Eureka’ and one row of ‘First Blush’, with the centre plant row being ‘First Blush’. There were four monitoring plots (13 plants/plot) for ‘Eureka’ and two plots for ‘First Blush’ in each tunnel.

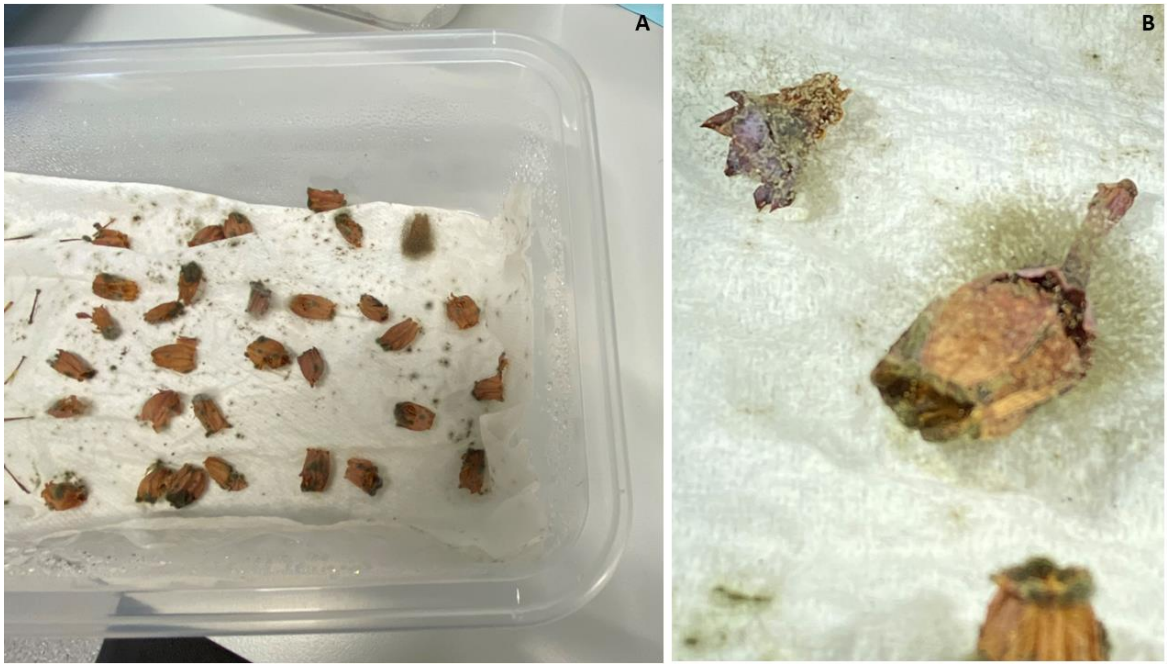

Figure 1.2**.** Humid incubation tray (A) and close up view of *Botrytis cinerea* sporulation on necrotic blueberry tissue (B).

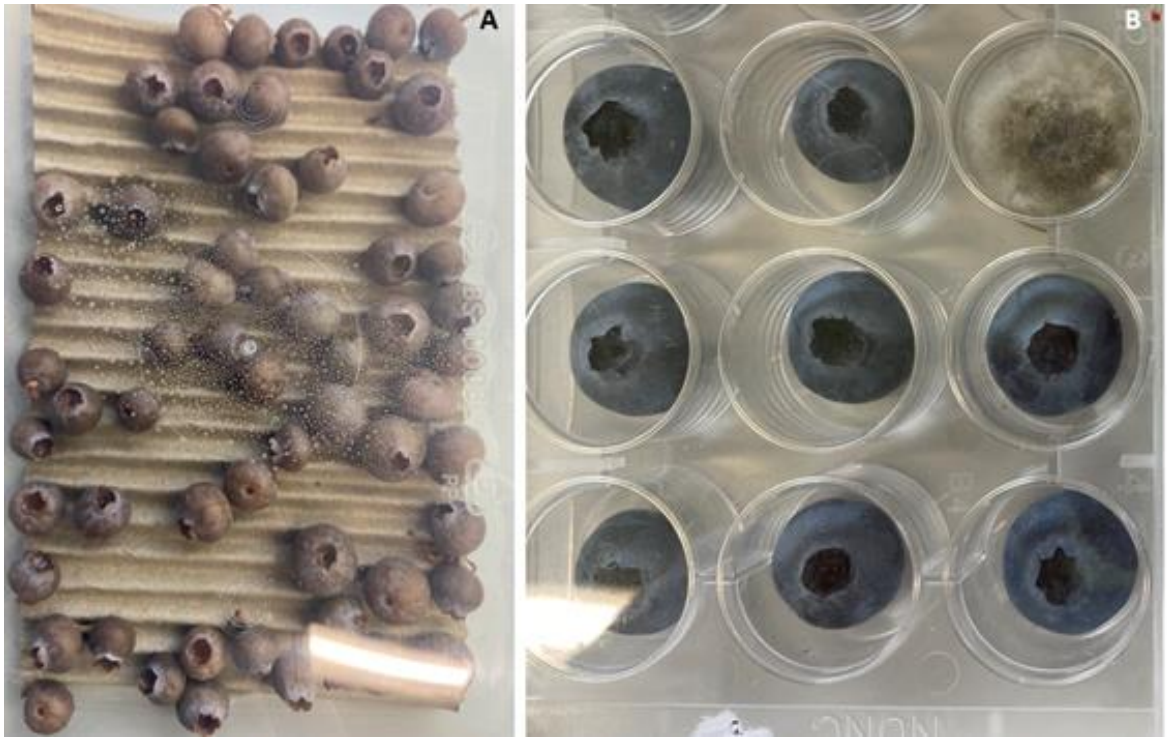

Figure 1.3. Incubation of green (A) and ripe blueberries (B) in their humid chambers, showing *Botrytis cinerea* sporulation.

1.3 Results

Results for this observational experiment are presented descriptively.

Over 70% of aborted flowers (in the canopy) yielded B.c. upon incubation in both tunnels, with all aborted flowers from ‘First Blush’ being colonised. Necrotic corollas collected in the canopy showed B.c. colonisation of 49% and 67% in Tunnels A (inoculum removed) and B (control), respectively (Figure 1.4). For styles, 36% and 51% respectively were colonised by B.c. The latent green berry infection was similar in both tunnels, with 9% and 11%, in Tunnel A and B respectively. This translated into 8.7% and 9.7% ripe berry infections respectively,. Considering all tissues incubated, 35% and 43% yielded B.c. in Tunnels A and B, respectively. Figure 1.5 shows the upward trend of infection in necrotic corollas and ripe berries during the season and through harvest, respectively. Corolla infection corelated more frequently and strongly with ripe berry infection in Tunnel A, where inoculum was removed (Figure 1.6).

Figure 1.4. Close up view of a representative ‘Eureka’ blueberry plant tissues in Tunnels A (left) and B (right) on the first week of November 2023. Tunnel A (left) is showing healthy berries, whereas Tunnel B (right) shows unhealthy/aborted berries.

The dry weight of the sweepings on the ground ranged from 535 g to 2825 g (Appendix 1, first graph). The initial debris for the first seven weeks consisted largely of leaves (from ‘First Blush’). However, with bloom starting (16 August 2023) and corollas dropping, the dry weight increased to up to 1850 g, then peaked again with fruit drop during harvest. The percentage of aborted flowers (vs corolla counts in the debris) ranged from 6% to 65%. The proportion of aborted flowers was high at early flowering (39% and 27% on 23 and 31 August 2023, respectively), dropping to 6–8% for the following six weeks and then increasing steadily from 21% to 65% during the last 6 weeks monitored (Appendix 1, second graph).

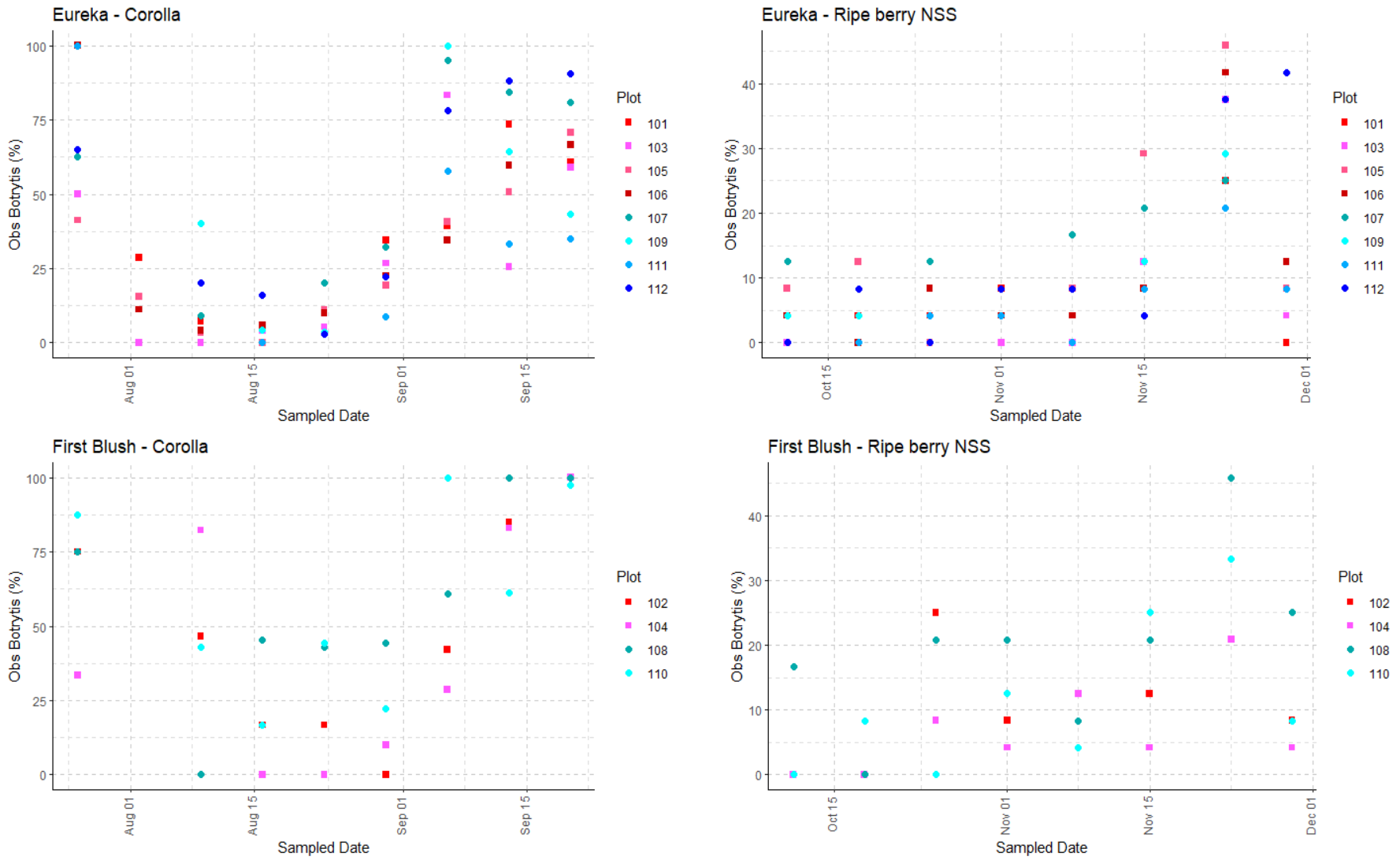

Figure 1.5. *Botrytis cinerea* infection (%) of necrotic blueberry corollas collected in the canopy and ripe berry infections (not surface sterilised, NSS) for 'Eureka' and 'First Blush' in Tunnel A (inoculum removed, red-pink coloured plots) and Tunnel B (control tunnel, blue coloured plots). A plot consisted of 13 blueberry plants. Note the different y-axis scales for corolla and ripe berries.

Throughout the monitoring period from 12 July to 23 November 2023, B.c. sporulation in the canopy and on the ground could be observed. The most sporulation was noticed in late August/early September during full bloom. In both tunnels, approximately 15% of all corollas on the ground sitting in wet patches from irrigation/fertigation run-off showed abundant B.c. sporulation (Figure 1.7).

The numbers of symptomatic tissues/plant were similar in both tunnels at the start of the *in planta* counts (the first count done on 13 September 2023, at full bloom). There were c. 3–5 symptomatic tissues per plant, which tripled to 13–14 by 27 September 2023 and continued to increase, peaking in October, particularly in Tunnel B (control). In Tunnel A, the maximum number of symptomatic tissues observed was 47, but in Tunnel B the maximum was 525 (25 October 2023 counts), and this number then decreased again with harvest. Figure 1.5 shows the data for B.c. corolla infections and ripe berries for both cultivars and all plots observed.

The correlation between B.c. infection in corolla and ripe berry in 'Eureka' (Figure 1.6) showed strong positive correlations. Correlations typically greater than 0.7 indicate strong predictability of ripe berry infection by corolla infection earlier in the season. As expected, the ripe berry infection could be predicted from the corolla infection with higher accuracy in the control tunnel, where the inoculum was not removed. However, the degree of variation was still high, showing considerable numbers of low or negative correlations in both graphs (Figure 1.6). Thus, there is evidence of impact of inoculum removal on breaking the link between corolla and ripe berry infection, although the results are highly variable. The limited number of data points did not allow for the same analysis for "First Blush".

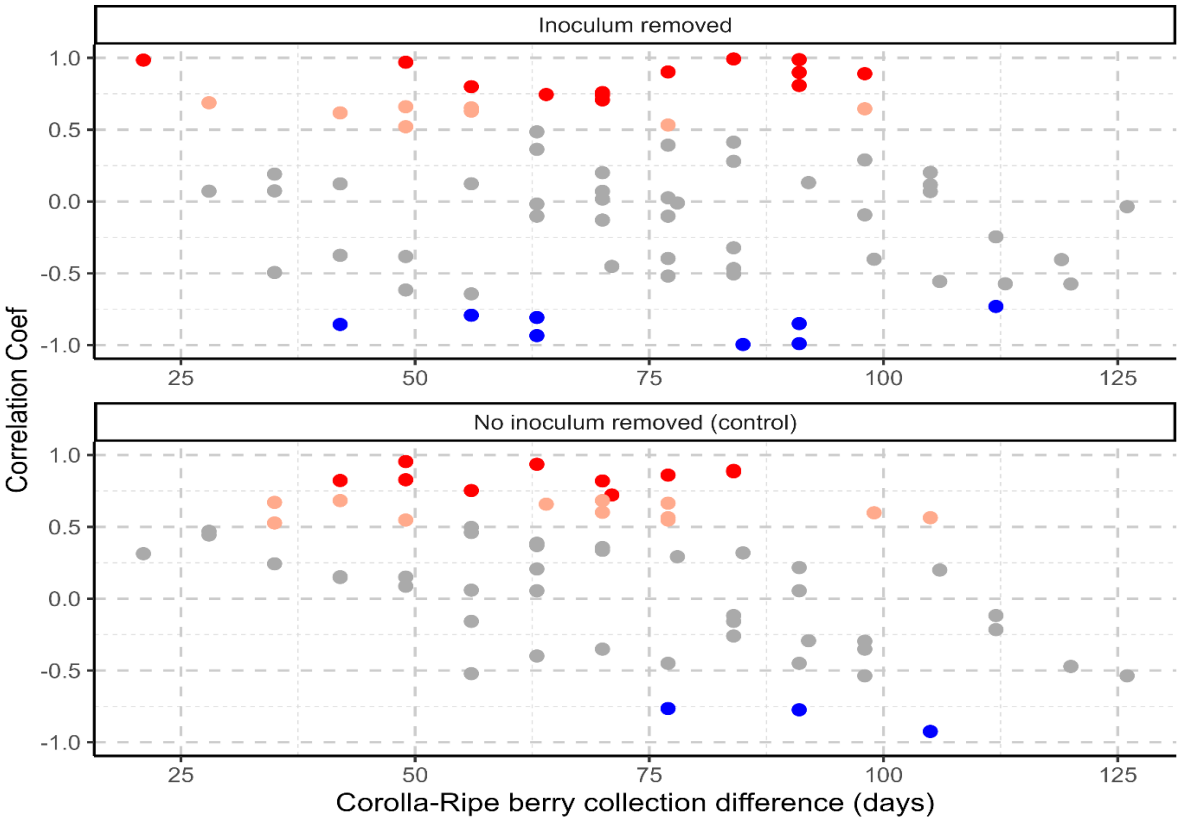

Figure 1.6. Correlation coefficients between *Botrytis cinerea* infection (%) of necrotic 'Eureka' blueberry corollas collected in the canopy and ripe berry infections (not surface sterilised, NSS) in both inoculum-removed and control tunnels versus the time difference in days between the dates when the corolla and ripe berry samples were taken for the same plot. The correlation coefficients are shown by dark red dots when >0.7 (strong positive correlation), light red dots when between 0.5 and 0.7 (moderate positive correlation), grey when between 0.5 and −0.5 (low correlation), light blue when between −0.5 and −0.7 (moderate negative correlation) and dark blue when <−0.7 (strong negative correlation).

Figure 1.7**.** Blueberry corollas sitting in wet patches between two plant pots (A). *Botrytis cinerea* sporulating corollas are seen in white circles (B) from an area of rectangle in photograph A.

1.4 Discussion

The inoculum removal was marginally beneficial in overall B.c. inoculum removal. The correlation analyses have indicated that inoculum reduction affects berry infections. The area treated was small, surrounded by inoculum in-drift from the adjacent rows. Also, inoculum was physically removed in Tunnel A from only half the canopy in the designated plots, which equates to 39 plants from 273 plants (14%) in the experimental area. The sweeping would have released spore clouds from sporulating aborted flowers and corollas on the ground. Which negates the sweeping. The fairly abundant sporulation observed throughout the monitoring period on necrotic tissues on the ground and in the canopy suggests that weekly inoculum removal at a larger scale will be required and possibly at a higher frequency. Vacuuming into a dust-bag might be a more appropriate approach.

Corolla infections increased over time as B.c. spores accumulated on the necrotic tissues (potentially with daily spore-release events). Green berry and ripe berry infections also increased over time, but at a lower rate (approximately 5-fold less) than corolla infections. Green berry infections were similar to ripe berry infections and therefore might be predictive of ripe berry infections. Similarly, the rate of corolla infections might be used as a risk predictor of B.c. green and ripe berry infections.

The overall B.c. disease expression in blueberries was low, or lower than those experienced in the previous two seasons (David Holmes, pers. comm). This is largely attributed to climatic drivers, temperature and humidity in the tunnels, which of course is driven by the macroclimate (rainfall, cloudiness, windrun, temperature) (Li et al. 2018). Wetness under the plants should be avoided at all times. Infected but dry debris will not produce B.c. spores. Infected and wet/imbibed necrotic tissues will support spore production. At 20°C, B.c. infection, colonisation and sporulation on necrotic tissues can happen in as little as 3–5 days. Indeed, aborted flowers, when incubated at 20°C and high humidity, produced spores within one day, indicating that aborted flowers are readily colonised by B.c. (Li et al. 2018). The highest count of aborted flowers/plant (in the canopy) was 975 (25 October 2023) in Tunnel B (control). Was this due to B.c. only? Was this poor pollination and/or associated with other plant health and physiological problems? We observed flower abortion during 6 September to 11 October (the main flowering period), but proportionally to the flowers available, aborted flower counts increased steeply in the latter part of flowering. We postulate that this is a combination of poor pollination at the later flowering stages and possibly higher B.c. pressure. Additional research will be required to understand the causes of flower abortion.

1.5 Conclusion

Sanitation and inoculum management (manual and/or agrichemical) are key control tools for managing flower infection with B.c.. Flower infections in berries may lead to flower abortion as well as causing latent green and ripe berry infections. These then manifest as grey mould postharvest. Latent berry infections are caused via stylar infections and occur at the time of pollination. This 2023 season, surface contamination and infections arising from berry handling during harvest (particularly picking wound infections) were rarely observed. This agrees with the ripe berry infections being similar to the latent green berry infections. In this study, berries were picked into individual cells, therefore cross-contamination of the picking wounds was very unlikely.

1.6 Recommendations

- There is evidence that early corolla infection predicts late ripe berry infection. Therefore, we should further explore the temporal corolla-ripe berry infection relationships.
- There is also evidence that by removing inoculum, the correlation is weakened, indicating the 'chain' between corolla and ripe berry infection can be broken. This should be further validated.
- Wetness of necrotic tissues on the ground should be avoided, to prevent B.c. spore production.
- Understanding of the drivers of flower abortion is needed. Is flower abortion primarily caused by lack of pollination (e.g., bee activity, pollen loading and/or pollen source), and/or B.c. (also vectored via the pollenising insects), and/or cool temperatures at pollination affecting pollen tube growth and fertilization, and/or other plant health/nutritional/physiological drivers?
- Removal of aborted flowers and corollas via a commercial vacuum should be trialled at a larger scale. As in grapes, (gentle) shaking of the plants may also aid abscission of necrotic tissues in the canopy, thereby decreasing inoculum in the canopy, but potentially also positively affecting the metabolic resistance to B.c. infections (Schwendel et al. 2021).
- Ventilation and humidity management for inoculum control should be explored.

1.7 References

Li H, Chen Y, Zhan Z, Li B, Qin G, Tian S 2018. Pathogenic mechanisms and control strategies of *Botrytis cinerea* causing post-harvest decay in fruits and vegetables. Food Quality and Safety 2(3), 111-119. https://doi.org/10.1093/fqsafe/fyy016.

Schwendel BH, Anekal PV, Zarate E, Bang KW, Guo G, Grey AC, Pinu FR 2021. Mass spectrometry-based metabolomics to investigate the effect of mechanical shaking on Sauvignon Blanc berry metabolism. J Agric Food Chem 69 (16), 4918-4933. https://doi.org/10.1021/acs.jafc.1c00413.

2 Lanvale spray trial

This work has been funded by BerryCo to control *Botrytis cinerea* in BerryCo blueberry tunnels. The pathogen has caused severe postharvest fruit losses (up to 30%; Ewan Potgieter pers. comm) in BerryCo blueberry varieties produced in the northern, warm and humid, regions of New Zealand. *Botrytis cinerea* fruit losses are caused by flowering tissue infections and fruit contamination (Li et al. 2018). Inoculum management and protection of susceptible tissues is important in disease control. Here we investigate the use of agrichemicals for control of *B. cinerea.*

2.1 Aim

For the Lanvale Spray Trial the aims were:

- to explore the fungicide efficacy of KENJA® 400 SC and ARMOUR-Zen® with that of the standard fungicide programme used by BerryCo growers
- to investigate the frequency of applications to control *Botrytis cinerea* (B.c.) corolla, green and ripe berry infections.

2.2 Methods

The Lanvale blueberry orchard in Pukekohe was used. Plants were young (1-year-old) and the 'Masena' (Mountain Blue) cultivar was used because of the availability of flowers. Tunnel houses 6 and 7 were used, with 10 plants in a treatment plot and four buffer plants between treatment plots. There were four rows of potted 'Masena' plants (one in Tunnel 6, three in Tunnel 7) which were used to randomly assign treatment plots (n=40) for the eight treatments and five replicates thereof. Eight treatments in total (Table 2.1) included two unsprayed controls (paired, i.e. adjacent to each other; Treatments 1 and 2) and two grower standard controls (also paired, i.e. adjacent to each other; Treatments 4 and 5; with 7-day interval spraying). The other treatments consisted of product evaluation of a single Kenja® 400 SC (UPL) application (Treatment 6), replacing one Switch® (Syngenta New Zealand) application in the grower standard treatment and weekly applications of the biological control agent Amour-Zen® (Botry-Zen (2010) Ltd). The grower standard treatments contained spray applications of Pristine® (BASF), Switch®, Esteem® (Arxada New Zealand) and Captan 600 Flo® (Nufarm). Lokit® (Grochem) was always applied with Captan 600 Flo® to lower the spray solution pH. The spray intervals for grower standard treatments were 14 days (Treatment 3), 7 days (Treatments 4 and 5) and 3-4 days (Treatment 8).

All products were applied according to label rates (Table 2.1). Each treatment was assigned a colour. The knapsack sprayer (Figure 2.1) used for fungicide applications was calibrated by BerryCo and triple rinsed between products. Plants were sprayed to the point of runoff at an approximate water rate of 350 L/ha or 80 mL/plant.

Application dates were based normally on Tuesdays, and for Treatment 8 (blue), Tuesdays and Fridays: for example week 1 Blue 'P, C' = Pristine on Tuesday 25 July, and Captan on Friday 28 July. Sprays for weeks 5, 8, and 11 were actually applied on the Wednesday. For Treatment 8, weeks 5 and 10, the Friday captan was not applied (Table 2.1).

All sprays were applied at approx. 80 mL/plant (approx. equivalent to 350 L/ha).

Table 2.1. Fungicide spray treatments, products and spray timing intervals for control of *Botrytis cinerea* infections of blueberry corollas, green and ripe berries.

		Sampling timing	Corollas (25 Jul – 11 Sep)							Green berries (11 Sep – 23 Oct)						Ripe (blue) berries (6 Nov – 11 Dec)			
		Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
		Application date	25-Jul	1-Aug	8-Aug	15-Aug	23-Aug	29-Aug	5-Sep	12-Sep	19-Sep	26-Sep	3-Oct	10-Oct	17-Oct	24-Oct	31-Oct	7-Nov	14-Nov
TRT#	**Colour**	**Treatment**																	
1	White	Unsprayed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	White +Black	Unsprayed (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Yellow	14 day, grower standard	P	-	S	-	P	-	S	-	E	-	E	-	E	-	C	-	C
4	Orange	7 day, grower standard (2x Switch®)	P	C	S	C	P	C	S	C	E	C	E	C	E	C	C	C	C
5	Orange +Black	7 day, grower standard (1x Switch®)	P	C	-	C	P	C	S	C	E	C	E	C	E	C	C	C	C
6	Pink	7 day, Kenja	P	C	K	C	P	C	S	C	E	C	E	C	E	C	C	C	C
7	Green	7 day, ARMOUR-Zen	P	C	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ
8	Blue	3–4 day, grower standard	P, C	C, C	S, C	C, C	P,-	C, C	S, C	C, C	E, C	C,-	E, C	C, C	E, C	C, C	C, C	C, C	C, C

P=Pristine® (BASF), C=Captan 600 Flo® (Nufarm), S=Switch® (Syngenta New Zealand), K=Kenja® (UPL), E=Esteem® (Arxada New Zealand), AZ= ARMOUR-Zen® (Botry-Zen).

Figure 2.1. Spray application using calibrated backpack sprayer.

Botrytis cinerea infection of corollas (n=7 samples, 25 July–11 September 2023), green berries (n=4 samples, 11 September–23 October 2023) and ripe berries (n=6 samples, 6 November–11 December 2023), was determined by weekly sampling of necrotic corollas (20–50 corollas/plot), green berries (30–40 green berries/plot) and ripe berries (24 ripe berries/plot). Corollas were incubated at room temperature and 12-h photoperiod in a humidity chamber for 7 days and incidence of corollas with B.c. sporulation was counted. Green berries were surface sterilised, frozen overnight (ONFIT) and then incubated for 7 days in humidity chambers, again at room temperature and 12-h photoperiod. Surface sterilisation consisted of 1 min of 70% ethanol followed by 1 min in 1% sodium hypochlorite, 1 min of 70% ethanol and two rinses of reverse osmosis water. Ripe berries were picked into individual compartments using two 12-cell titre plates per plot and incubated for 14 days at room temperature. Ripe berries were not surface sterilised (NSS). For the berries, B.c. sporulation incidence was recorded. For all collections, hands were sanitised between sampling plots.

Data for control Treatments 1 and 2 and the 7-day grower standard (2x and 1x Switch®) Treatments 4 and 5 were pooled, as there was no statistical difference between treatments. Statistical approaches are described in the Results section, below. The full dataset (without pooled treatments) is presented in Appendix 2.

2.3 Results

Figure 2.2 is a boxplot showing the collected data from the spray trial. The numbers of observed tissues with B.c. sporulation were presented as incidence percentages, normalised by the total number of samples collected per treatment-test day combination for corolla, green berry and ripe berry. There are few outliers (plots) observed in each of the graphs. However, the incidence rate is higher in corolla compared to the green and ripe berries, especially later through the season. Differences among the treatments are also expected. Correlation between early corolla B.c. detection (25/7) and green berry (11/9) was investigated, as well as the correlation between the early corolla detection and the ripe berry (6/11). However, the incidence rate numbers were so low that no conclusion about the correlation could be achieved.

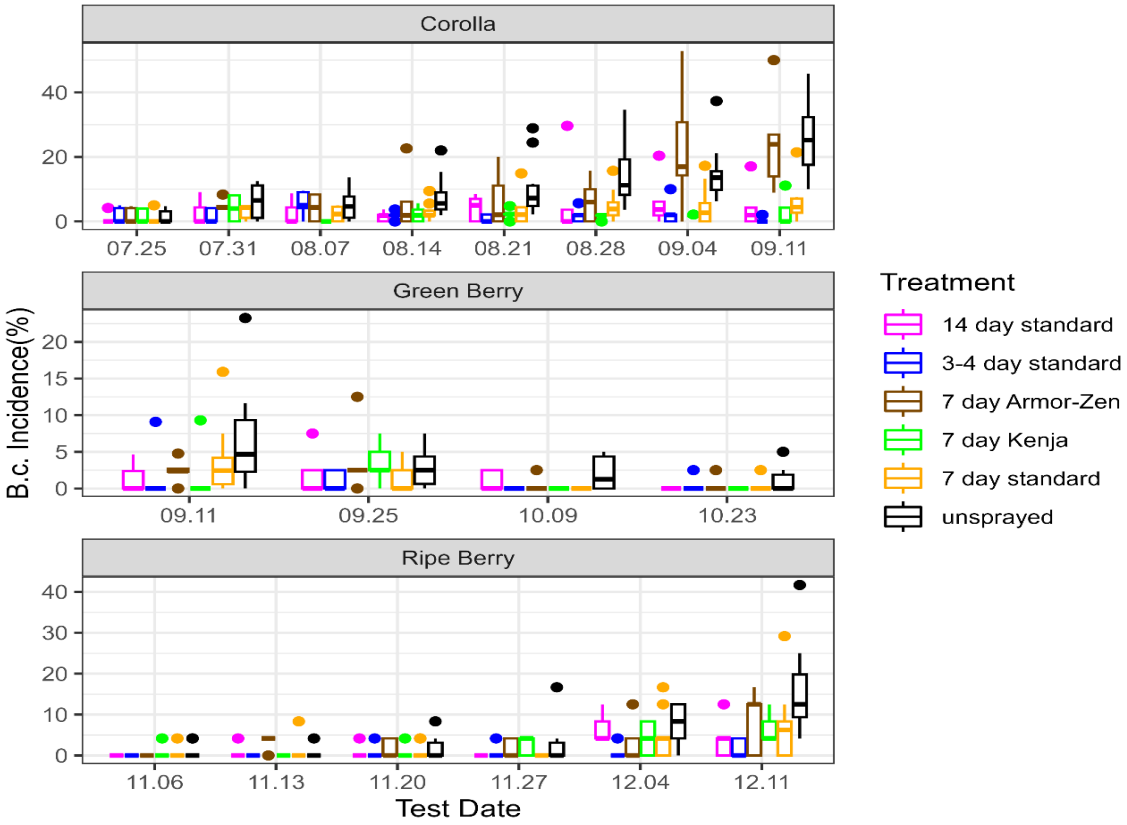

Figure 2.2. Boxplot of *Botrytis cinerea* (B.c.) incidence (percentage of B.c. observed in the total collected samples) per treatment and test date on blueberry corolla, green berry and ripe berry tissues. Test Date represents Month.Day in the 2023 production season. Box plots summarise the distribution of measurements. In these, the central line is the median, with the upper and lower quartiles shown by the upper and lower ends of the box. The distance between these two values is the interquartile range, a measure of the distribution spread. The middle 50% of data lie between the upper and lower quartiles. Location of the median relative to the upper and lower quartiles gives information about the shape of the distribution. If the median does not sit midway between these, then the distribution is skewed and hence cannot be normally distributed. The dashed lines extend out to the adjacent values. The upper adjacent value is the largest observation that is less than or equal to the upper quartile + 1.5x interquartile range, i.e. the median + 2.5x interquartile range. Similarly for the lower adjacent value. All data points beyond the adjacent values are shown individually, and these portray the extreme tails of the distribution and may be outliers.

A Generalised Linear Mixed Model (GLMM) was employed, fitted on the corolla, green berry and ripe berry datasets separately, to test the statistical significance of the treatments, using the *lme4* package in *R* (R Core Team). The response variable was the binomial ratio of the B.c. sporulation incidence recorded on each test day, to the total number of samples for that day (20–50 corollas/plot, 30–40 green berries/plot and 24 ripe berries/plot). The fixed effect factor was Treatment. The low variation in the B.c. incidence within the test days failed the GLMM model fit converge when the test day was added to the model as a fixed effect. Therefore, the test day and plot were included in the GLMM model as block effects. The averaged soluble solids content (SSC; measured as °Brix) per test day was also included in the GLMM model for ripe berries. However, there was no evidence suggesting statistical significance of SSC in B.c. incidence (*p*-value = 0.6).

The analysis was performed on the logit scale, and the mean and confidence intervals were back-transformed and are reported in Table 2.2. A chi-squared test was performed on the fitted GLMM models to identify the statistical significance of the treatment. The *p*-value for the treatment effect was statistically significant in the corolla, green berry and ripe berry models.

Table 2.2. Treatment (fixed effect) *p*-value given by the chi-squared test on the GLMM model, and the back-transformed predicted means and confidence intervals for *Botrytis cinerea* incidence in the blueberry spray trial. The multiple comparisons are based on 95% confidence intervals (CI) of the means on the logit scale. Means within a tissue type, with the same letter (A,B,C), are not statistically significantly different.

Tissue	Treatment *p*-value	Treatment	Incidence Mean (%)	Incidence Lower CI (%)	Incidence Upper CI (%)	Multiple Comparison at 95%
Corolla	**<0.001**	unsprayed	9.96	6.28	15.44	A
		14 day standard	2.91	1.62	5.18	B
		7 day standard	2.75	1.64	4.56	B
		7 day Kenja®	1.52	0.8	2.84	C
		7 day ARMOUR-Zen®	8.75	5.19	14.4	A
		3–4 day standard	1.44	0.75	2.72	C
Green Berry	**0.005**	unsprayed	2.22	0.87	5.53	A
		14 day standard	0.74	0.23	2.3	B
		7 day standard	0.88	0.32	2.38	B
		7 day Kenja	0.91	0.3	2.72	B
		7 day ARMOUR-Zen	1.22	0.42	3.51	AB
		3–4 day standard	0.54	0.16	1.8	B
Ripe Berry	**<0.001**	unsprayed	2.9	1.18	6.99	A
		14 day standard	1.19	0.42	3.31	B
		7 day standard	1.31	0.51	3.33	B
		7 day Kenja	1.44	0.52	3.91	B
		7 day ARMOUR-Zen	1.78	0.66	4.7	AB
		3–4 day standard	0.39	0.11	1.35	C

The back-transformed predicted means and confidence intervals of Table 2.2 are also shown in Figure 2.3; again, means within a tissue type, with the same letter (A,B,C), are not statistically significantly different. The models were fitted for each tissue (corolla, green and ripe berries) separately: therefore, the letter groups should be interpreted independently for each tissue. However, there are consistencies in multiple comparisons among the treatments across the tissues.

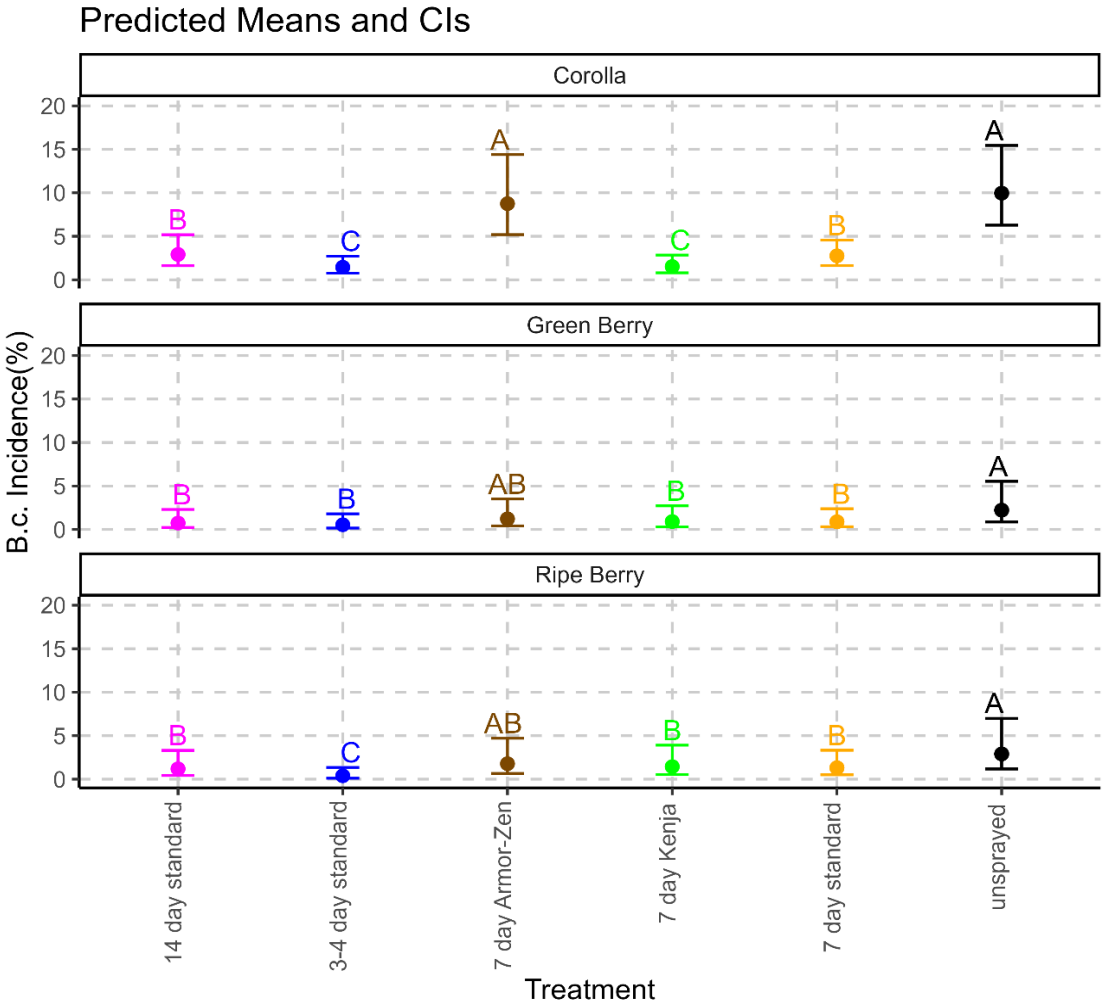

Figure 2.3**.** Predicted means and the 95% confidence intervals (CI) of the *Botrytis cinerea* incidence in blueberry tissues from the GLMM model per treatment per tissue, back-transformed from the logit scale. Means within a tissue type, with the same letter (A,B,C), are not statistically significantly different.

The overall B.c. incidence in the tissues sampled was low. Incidence of B.c. was highest in the corollas, followed by the ripe berries. The incidence rate in the unsprayed treatments was highest across all three tissue types, followed by the incidence in the 7 day ARMOUR-Zen treatment. The incidence rate was generally lowest for the 3- 4-day grower standard treatment, followed closely by the 7-day standard treatment, whereas the incidence rate in the 14-day standard treatment was slightly higher. The 7-day standard treatment produced similar results to the 7-day Kenja treatment, which is not surprising, as the Kenja treatment consisted of the 7-day standard spray application with one Kenja spray in August (Table 2.1). For corollas, the incidence seen in the 7-day Kenja treatment overall was as low as in the 3- to 4-day standard treatment, implying a potential long residual activity of this fungicide. This should be further explored, as this could also imply a long residual life on the fruit.

Botrytis cinerea incidence over time in ripe berries (not surface sterilised) is shown in Figure 2.4. This figure clearly shows the upward trend during the harvest period as well as the consistently lowest disease levels in the 3- to 4-day standard treatment. The graph also illustrates that all 7-day

treatments behaved similarly, with even the 14-day standard treatment providing similar disease control (as also shown in Figure 2.3).

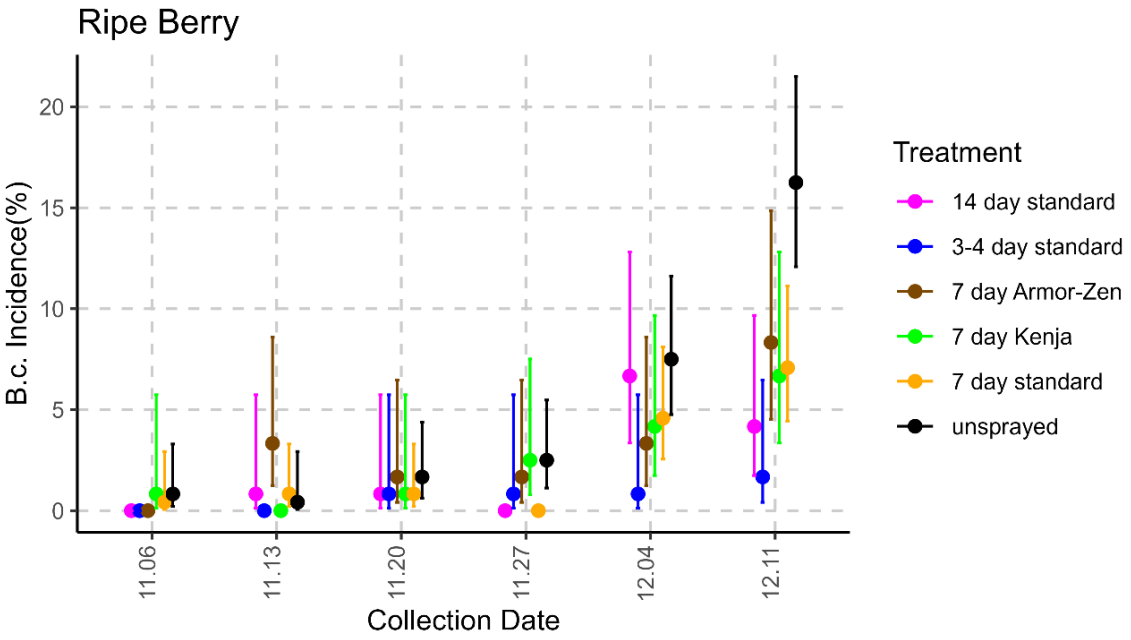

Figure 2.4. Predicted means and 95% of confidence intervals of *Botrytis cinerea* (B.c.) incidence in ripe blueberries per treatment and collection date. At each pick, berries (n=24) were sampled into individual cell trays, without surface sterilization. Overlapping confidence intervals suggest no statistically significant differences between the treatments.

2.4 Discussion

The spray trial on product efficacy and frequency of applications was carried out with cultivar 'Masena'. This was the cultivar available at the time of this experiment. 'Masena' appears to have lower B.c. susceptibility, as observed in independent grower observations during the 2023 season (n=8 growers, unpublished data). From these grower samples, B.c. infection rates of 'Masena' ripe berries was 1.3% compared with 'Eureka' 6.3%, 'First Blush' 12.2% and 'Eureka Sunrise' 13.9%. For corollas, B.c. infection rates of 'Masena' was 15.3% compared with 'Eureka' 62.7%, 'First Blush' 72.7% and 'Eureka Sunrise' 60.7%. This lower trend also held true for aborted flowers, with B.c. infection rates of 'Masena' at 48.3% compared with 'Eureka' 71.3%, 'First Blush' 67.1% and 'Eureka Sunrise' 84.6%. Please note these are observational data from tissues collected from eight growers only, during July to November 2023 (Monika Walter, unpublished data). The lower cultivar susceptibility, coupled with a dry season, resulted in overall lower pathogen infections and disease expression than experienced in previous seasons (David Holmes, pers. comm). The overall lower ripe berry infections were also noticed in the other two experiments described in this report.

For 'Masena' in a dry/low humidity season as experienced in 2023 (compared with the two previous rainy seasons), a 7-day spray interval provided adequate B.c. inoculum and berry infection control for postharvest B.c. storage rots, assuming that <10% infection rates are acceptable to growers at the end of harvest. In a dry season, for 'Masena', even a 14-day or possibly longer spray interval might be acceptable. However, a more intensive fungicide spray programme might be required for 'Eureka', 'Eureka Sunrise' and 'First Blush'. The previous two wet seasons have shown that fungicides spray intervals for those cultivars were not adequate in managing pre- and postharvest fruit loss (David Holmes, pers. comm). We recommend repeating the fungicide timing/frequency trial using more

susceptible cultivars. Evaluation of 'soft' products should also be considered. A spray programme and forecast system based on B.c. risk for indoor production could also be considered, working towards a comprehensive disease management guide that also included nutrition and cultivar susceptibility.

Fungicides protect tissues from infection, but do not eliminate colonisation of necrotic tissues. *Botrytis cinerea* berry infections occur via two main pathways: the first is flower infections via the style during bloom, and the second is surface contamination of spores on the fruit. Green, surface-sterilised, berry infections are generally indicative of stylar infections, and postharvest ripe berry storage rots are a combination of both stylar infections plus picking wound/microcrack infections from surface spore contamination during the harvest and also postharvest management processes (Auda 2021). In his thesis, Hosking (2023) reviewed that, subject to cultivar, up to 55.8% of blueberries might be lost to postharvest B.c. infections. Worldwide, B.c. crop losses of fruits and vegetables amount to US$10–100M annually (Li et al. 2018).

It is beyond the scope of this contract to provide detailed B.c. management guidelines for blueberry, but we would like to draw the readers' attention to the publications of Li et al. (2023), Hosking (2023), Auda (2021), and the comprehensive Midwest Blueberry Production Guide for general information that may also be applicable to New Zealand (https://www2.ca.uky.edu/agcomm/pubs/ID/ID210/ID210.pdf).

2.5 Recommendations

- A minimum of a 7-day spray interval for susceptible varieties is recommended. For 'Masena' in a low risk year, fewer agrichemical interventions may be required.
- Explore 'soft' alternatives for inoculum control during the season.
- Repeat the fungicide trial with a more susceptible cultivar.
- Assess spray timing, including B.c. forecast risk.

2.6 References

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3 Postharvest berry collapse

This work has been funded by BerryCo to understand the causes of postharvest berry collapse. Two hypotheses have been formulated. First, berry collapse is caused by *Botrytis cinerea* as collapsed fruit show high levels of colonisation by this pathogen. Second, berry collapse is caused by physiological/nutritional reasons. Here we try to address the relationship between fruit status (regular, soft, collapsed, pink) and *B. cinerea* colonisation via latent flower tissue infections or pre/post-harvest surface contamination.

3.1 Aim

For the Postharvest Berry Collapse study, the aim was to understand if postharvest berry collapse was of biological or physiological/other nature.

3.2 Methods

Ripe berries were collected from two grower properties (Nanric Tunnels 35 and 40, and Miro MK Block C Tunnels C and D) from 'Masena' and 'Eureka' cultivars, respectively. For each cultivar, five bushes in a single row were chosen, approximately 10 bushes apart; these were marked and given a unique plot number. From each bush, berries were picked to fill up one 125-g clamshell. Bushes were covered with white bird netting to prevent commercial picking. The fruit were then hand-sorted into different categories based on quality – pink (p), softs (s), regular (r), and collapsed (c). Where possible, for each category, berries not required for further sampling (3–6 berries) were taken for soluble solids content (°Brix) and firmness tests, using a Milwaukee MS887 refractometer and TR Turoni Fruit Hardness Blueberry Durometer, respectively. There were three picking dates: 17, 23 and 29 November 2023. Berries generally were processed within one to two days of sampling. Fruit not processed immediately were stored in the refrigerator at ±4°C

In parallel, packed (2 x 125-g clamshells) and reject fruit (approximately 750–1000 g) from the two growers were also collected from Yieldia Produce Solutions, the contracted BerryCo blueberry packhouse. Fruit were obtained on 15, 22, 27 November and 5 December 2023 from 'Masena' and 'Eureka' cultivars, if available. (Generally, packhouse fruit consists of mixed cultivar berries.) In the packhouse, berries were sorted using infrared into reject and acceptable regular fruit. Reject fruit generally consisted of pink, soft and collapsed berries. The samples brought into the lab, and again were categorised into pink (p), softs (s), regular (acceptable ripe fruit) (r), and collapsed (c). Again, for each category, berries not required for further sampling (3–6 berries) were taken for soluble solids content (°Brix) and firmness tests. Berries were processed within one to two days of sampling. Fruit not processed immediately were stored in the refrigerator at ±4°C.

Refer to Appendix 3 for the list of picking dates for orchard and packhouse samples.

Berries of all categories were incubated at room temperature in humidity chambers for 7 days with and without surface sterilisation. If only a few berries were obtained for a particular category, then the berries were surface sterilised. Surface sterilisation consisted of 1 min of 70% ethanol followed by 1 min in 1% sodium hypochlorite and two rinses of reverse osmosis water. Trays were checked regularly and fruit showing rots were removed during the incubation period, to prevent nesting. The main rot observed was *Botrytis cinerea* (B.c.), with a few berries showing e.g. *Aspergillus, Penicillium*

or *Phomopsis* spp. Fungal rots were identified by morphotyping. As there were only a few, these other rots have been ignored in the data analyses.

A subsample of 4–6 surface sterilised berries (all categories) were then each cut in half aseptically and scored for interior browning using the scale of Moggia et al. (2017). One half of each of four berries then was placed into a sterile cell of a 12-cell titre plate. The other half was plated onto potato dextrose water (PDA) amended with antibiotics or put into fixative (for future microscopy). Berries in titre plates were wetted (3 puffs/plate) with reverse osmosis water mist to maintain humidity. Berries in the titre and on the agar plates were also monitored regularly and fungi were morphotyped (Figure 3.1). Again, if mycelial growth was observed, this was predominately caused by B.c.

Statistical methods are embedded in the Results section (below).

Figure 3.1. Humidity chambers with surface sterilised and non-surface sterilised blueberry fruit, titre plates with surface-sterilised fruit, and agar plates with surface-sterilised fruit.

Cell structure

The microscopy of collapsed, soft and regular berries with and without *Botrytis cinerea* is still to be done. The work is planned for March/April 2024, in agreement with BerryCo to jointly select the appropriate berry samples (n=12 from over 200 berries stored).

3.3 Results

Berry collapse

The data were summarised by removing samples for which the data were not available. There were few symptoms observed in the dataset apart from those caused by B.c.; including symptoms caused by *Cladosporium, Penicillium, Aspergillus*, *Epicoccum, Rhizopus* and yeast species. These symptoms were all labelled as “Other”, to reduce variation. The numbers of samples with no growth, with B.c.,

and with other symptoms were counted for each trial (Orchard or Yieldia), assessment method (Agar or Titre), berry type (collapse, soft, pink, or regular), and cultivar (primarily ‘Eureka’ and ‘Masena’). Cultivars could not be differentiated when fruit were collected in the packhouse, and therefore the ‘cultivar category’ consisted of “mixed” (regular fruit), “premium” (large berries) and “River Run” (lower grade of regular fruit, but still acceptable) categories. Figure 3.2 shows the symptom percentages normalised by the total counts in each category. Most of the B.c. incidence was observed in collapsed tissue, and possibly most in ‘Masena’ and mixed cultivars.

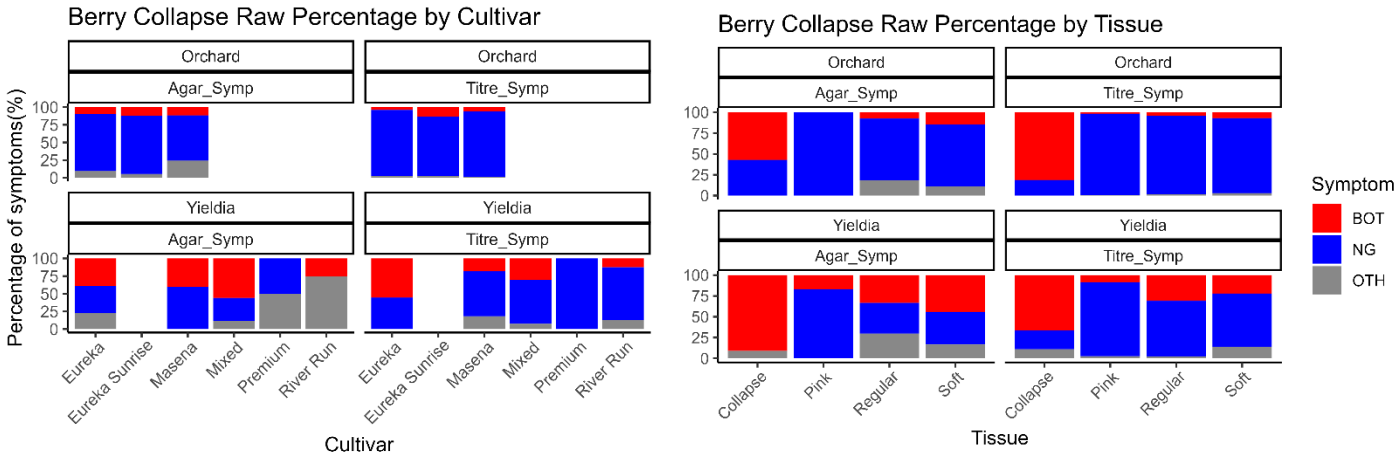

Figure 3.2. Percentage of *Botrytis cinerea*-caused (BOT) or other (OTH) symptoms, and no growth (NG), of the total blueberry samples assessed by cultivar and by tissue. Other pathogens/microorganisms colonising the berry or agar included *Cladosporium, Penicillium, Aspergillus*, *Epicoccum, Rhizopus* and yeast species.

The cultivar and tissue effects on B.c. incidence were investigated by employing a Generalised Linear Model (GLM) on the binomial counts of B.c. incidence out of total samples for which data were recorded (missing data removed). The fixed effects were cultivar, tissue and the cultivar/tissue interaction, in addition to the growers as the block effect. The model was fitted separately for Orchard and Yieldia, and for Titre and Agar methods. It is notable that the GLMM model failed to converge by fitting the growers as the block effect. Table 3.1 gives the *p*-values of the chi-squared tests on the fixed effects. The analysis was performed on the logit scale, and the predicted means and 95% confidence intervals were back-transformed. Figure 3.3 shows berries in a titre plate and on agar. Figure 3.4 illustrates the predicted means and confidence intervals of Table 3.1.

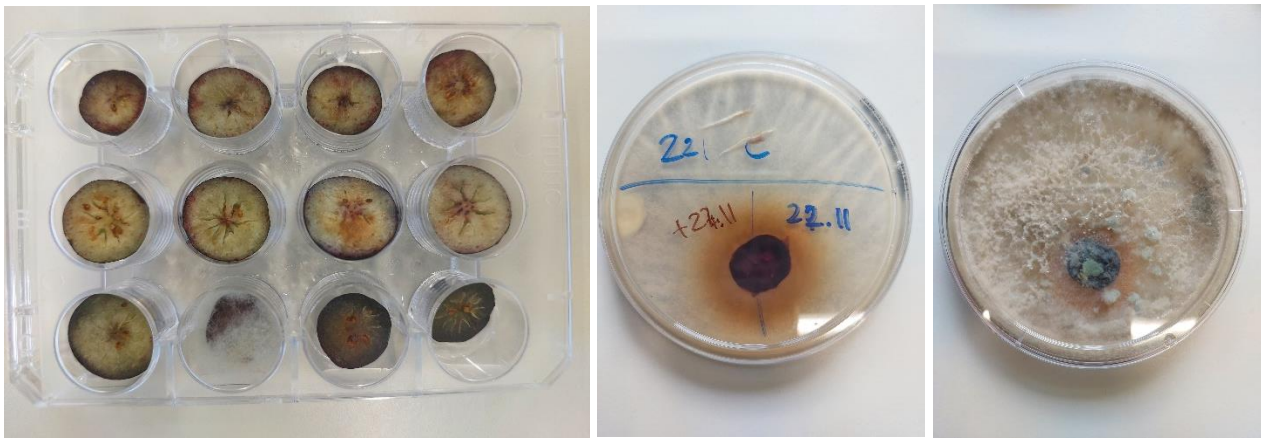

Figure 3.3**.** Surface-sterilised blueberries, cut in half and incubated in titre plates (left) and on agar (middle and right, bottom and top view of the same plate). A few berries in the titre plate show internal browning, with one berry showing mycelial growth of *Botrytis cinerea.* The agar plate shows colonisation by *B. cinerea,* and a *Cladosporium* isolate.

Table 3.1. *Botrytis cinerea* incidence on ripe blueberries: back-transformed predicted means and 95% upper (UCI) and lower (LCI) confidence intervals generated by the GLM model. The chi-squared test *p*-values for the grower, cultivar, tissue and the cultivar/tissue interaction are reported. NA=not available. Berries were surface sterilised

	Method	Cultivar	Tissue	Grower *p*-value	Cultivar *p*-value	Tissue *p*-value	Interaction *p*-value	Mean (%)	LCI (%)	UCI (%)
Yieldia (packhouse)	Titre	‘Eureka’	Regular	0.586	0.032	0.001	0.381	55.56	32.74	76.25
		‘Masena’	Collapse					33.33	4.19	85.1
		‘Masena’	Pink					0	*NA*	*NA*
		‘Masena’	Soft					25	3.25	76.81
		Mixed	Collapse					99.98	*NA*	*NA*
		Mixed	Pink					0	*NA*	*NA*
		Mixed	Regular					25	6.17	62.83
		Mixed	Soft					0.09	*NA*	*NA*
		Premium	Regular					0	*NA*	*NA*
		River Run	Regular					0	*NA*	*NA*
	Agar	‘Eureka’	Regular	0.325	0.286	0.040	0.581	38.89	19.33	62.82
		‘Masena’	Collapse					100	*NA*	*NA*
		‘Masena’	Pink					0	*NA*	*NA*
		‘Masena’	Soft					50	5.44	94.56
		Mixed	Collapse					100	*NA*	*NA*
		Mixed	Pink					25	3.14	77.44
		Mixed	Regular					50	11.72	88.28
		Mixed	Soft					1.28	*NA*	*NA*
		Premium	Regular					0	*NA*	*NA*
		River Run	Regular					0.15	*NA*	*NA*
Orchard	Titre	‘Eureka’	Collapse	0.514	0.022	<0.001	0.602	50	5.82	94.18
		‘Eureka’	Pink					0.93	0.13	6.33
		‘Eureka’	Regular					5.54	2.66	11.2
		‘Eureka’	Soft					4.88	1.84	12.31
		‘Eureka Sunrise’	Collapse					100	*NA*	*NA*
		‘Eureka Sunrise’	Pink					8.7	2.18	28.94
		‘Eureka Sunrise’	Regular					6.67	1.67	23.11
		‘Eureka Sunrise’	Soft					20.69	9.59	39.09
		‘Masena’	Collapse					83.32	51.81	95.87
		‘Masena’	Pink					0.9	0.13	6.13
		‘Masena’	Regular					2.48	0.8	7.42
		‘Masena’	Soft					4.46	1.67	11.36
	Agar	‘Eureka’	Collapse	0.237	0.840	0.001	0.318	25	3.32	76.39
		‘Eureka’	Regular					7.03	3.16	14.9
		‘Eureka’	Soft					12.76	5.37	27.4
		‘Eureka Sunrise’	Collapse					100	*NA*	*NA*
		‘Eureka Sunrise’	Pink					0	*NA*	*NA*
		‘Eureka Sunrise’	Regular					4.76	0.66	27.31
		‘Eureka Sunrise’	Soft					22.73	9.75	44.46
		‘Masena’	Collapse					100	*NA*	*NA*
		‘Masena’	Regular					8.8	4.21	17.48
		‘Masena’	Soft					11.95	4.97	26.03

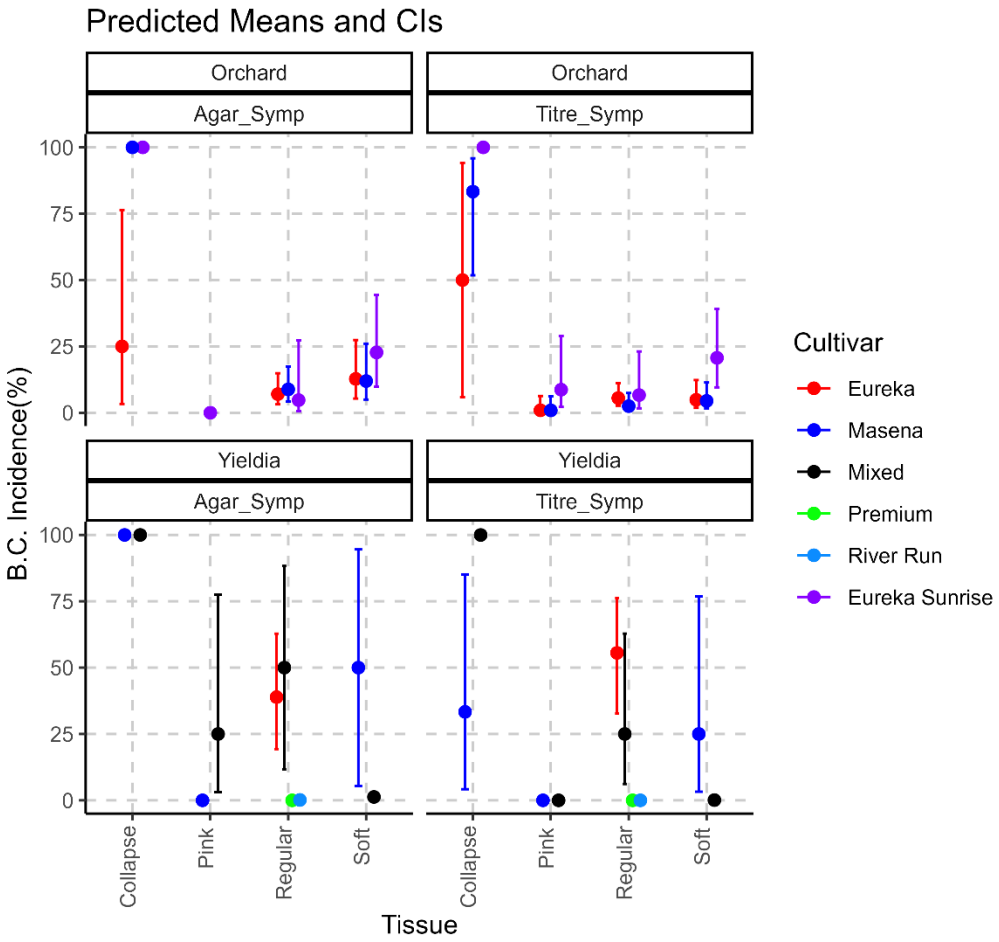

Figure 3.4. Back-transformed predicted means and 95% confidence intervals of *Botrytis cinerea* (B.c.) incidence observed for each blueberry cultivar/tissue combination.

Since the number of samples in each cultivar/tissue combination was unbalanced with low variation (see Section 3.2), there is considerable uncertainty associated with the B.c. predicted means by the GLM model. There was no evidence suggesting difference among the growers, nor for the cultivar/tissue interaction. However, there was evidence suggesting differences in B.c. incidence among the tissues, for both the Orchard/Yieldia and Titre/Agar assessment methods. These results strengthen acceptance of the hypothesis that the B.c. incidence rate is higher in collapsed tissue, and probably lowest in pink.

Finally, to investigate the relationship between Agar and Titre samples, we ran a random forest classification algorithm. The aim was to investigate how well symptoms on agar (*Botrytis cinerea*-caused (BOT), other (OTH) symptoms, or no growth (NG)) could be predicted by the cultivar, tissue, packhouse or orchard, Most importantly, how well could symptoms on agar by predicted by the titre symptoms? If the Titre and Agar methods resulted in relatively similar symptom detection, then the classification by Agar should have been highly predictive of the Titre method. However, the random forest algorithm reported 30.1% classification error, mostly by misclassifying the B.c. and other symptoms compared with the no-growth of symptoms (64% and 95% misclassification accordingly). Interestingly, the algorithm did not indicate that the Titre-recorded symptoms as a strong classifier of the Agar-recorded symptoms. The strongest classifier was reported to be the fruit tissue. This conclusion could be due to the high variability between the replicates and but more likely due to the

low numbers of B.c. or other symptoms detected compared with the No Growth data. More data points will be required to ascertain the relationship between titre and agar methods.

Disease Expression. Only regular (healthy) tissue was tested in the orchard samples. To investigate the difference in B.c. incidence among the cultivars, a GLM model was fitted on the binomial ratio of the affected samples to the total, for both surface-sterilised and non-surface-sterilised methods. The fixed effects were cultivar and growers. There was evidence suggesting difference between cultivars (*p*-value <0.001), but no evidence suggesting difference among the growers (*p*-value = 0.80 for non-sterilised and 0.99 for surface-sterilised, respectively). The analysis was performed on the logit scale with the multiple comparison test was applied with 95% confidence. The predicted means and confidence intervals are reported in Table 3.2.

Table 3.2. Back-transformed predicted means and 95% upper (UCI) and lower (LCI) confidence intervals of the *Botrytis cinerea* incidence predicted by cultivar (the orchard trial) and tissue (the Yieldia (packhouse) trial). Ripe berries were incubated in humidity chambers as is (not surface sterilised) or after surface sterilisation. Means within a column, with the same letter (A,B,C etc.), are not statistically significantly different.

Effect			Not surface sterilised				Surface sterilised			
			Mean (%)	LCI (%)	UCI (%)	Letter Group	Mean (%)	LCI (%)	UCI (%)	Letter Group
Orchard	Cultivar	'Eureka'	21.96	18.82	25.45	A	5.18	3.9	6.85	A
		'Eureka Sunrise'	28.66	21.07	37.68	A	10.8	6.51	17.41	B
		'Masena'	7.66	5.77	10.1	B	1.28	0.72	2.26	C
Yieldia (Packhouse)	Fruit Tissue	Collapse	95.48	70.58	99.47	A	56.36	42.03	69.71	A
		Pink	9.37	5.39	15.78	B	11.19	7.43	16.5	B
		Premium	2.23	0.26	16.55	B	12.88	5.93	25.73	BC
		Regular	9.54	5.11	17.12	B	14.03	9.61	20.04	BC
		Riverrun	12.88	5.67	26.69	B	22.75	12.85	37.03	CD
		Soft	32.78	27.44	38.61	C	34.49	28.68	40.8	D

Several tissue types were tested in the Yieldia trial. However, the cultivars were mainly mixed, with only two 'Masena' samples. Since the term "cultivar" could severely increase the uncertainty in prediction, only the effect of tissue type was investigated in the Yieldia trial. A GLM model was fitted to the binomial ratio of the affected samples to the total number of samples, with tissue type and grower as the fixed effects. There was evidence suggesting difference in tissue types for surface-sterilised and non-sterilised methods (both *p*-values <0.001), as well as in growers for the surface-sterilised method (*p*-value <0.001), but there was no evidence suggesting difference between growers for non-sterilised fruit (*p*-value = 0.13). The analysis was performed on the logit scale and the predicted means and 95% confidence intervals are given in Table 3.2. These results are also demonstrated in Figure 3.5.

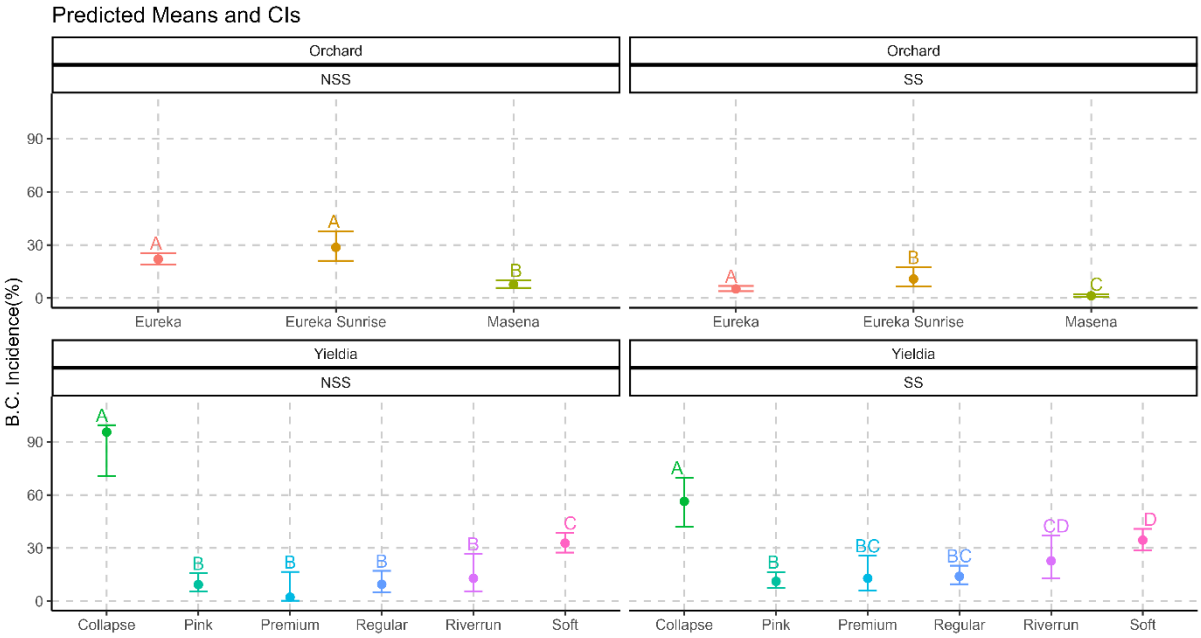

Figure 3.5. Back-transformed predicted means and 95% confidence intervals of *Botrytis cinerea* (B.C.) incidence by blueberry cultivar for the Orchard trial and by fruit tissue for the Yieldia (packhouse) trial. Means with the same letter (A,B,C etc.), are not statistically significantly different. SS = surface-sterilised, NSS = non-surface-sterilised.

The disease expression trial provided evidence that 'Eureka Sunrise' had the highest B.c. incidence rate, followed by 'Eureka' and then 'Masena'. It also showed that the B.c. rate was highest in collapsed tissues, followed by soft and then Riverrun fruit.

The highest correlation between the surface-sterilised and non surface-sterilised methods was for the mixed cultivars in Yieldia on regular (healthy) tissue (correlation coefficient = 0.63). The correlation between the sterilisation methods were generally positive on regular tissue in 'Eureka' and 'Masena', albeit relatively low (both coefficients = 0.34). The correlation coefficient between the sterilisation methods was particularly low and negative for 'Eureka Sunrise' regular tissue (coefficient = −0.24), and also negative for soft tissue of mixed cultivars (coefficient = −0.43) and pink (unripe) berries (coefficient = −0.45). In general, the correlation coefficients were not high enough to recommend the non surface-sterilised method as a strong predictor of the surface-sterilised method outcomes.

3.4 Discussion

As expected, B.c. incidence was highest in collapsed fruit, followed by soft, River run, regular, premium and pink (or unripe) fruit. However, not all collapsed fruit expressed B.c., especially if surface sterilized. For Orchard samples, surface sterilization overall reduced ripe berry infections upon incubation. This clearly indicates that spore contamination and berry injuries during harvest and postharvest management increase the risk of B.c. infection. For packhouse samples, this was not the case: other than for collapsed berries, B.c. disease expression was similar between surface-sterilised and non-surface-sterilised fruit. Surface sterilisation did not reduce disease incidence as it did in the Orchard samples, meaning that infections already probably have established beyond the outer skin layer or picking scar. This might be explained by the duration from picking to processing in the packhouse, which can take up to three days (David Holmes, pers. comm.), and by postharvest damage from mechanical impact. Blueberries are very susceptible to mechanical damage (Moggia

et al. 2017). Damage, combined with B.c. spores being able to germinate and penetrate the host cells during that time, will allow the pathogen to colonise the berry particularly if the cool-chain is broken and/or condensation occurs. Cappellini & Ceponis (1977) reported that the picking scar is the principal locus of infection in harvested blueberry fruit. Spores germinate between 0 and 25°C and when humidity is above 93% (Botden & Kooten 2002).

For the Orchard samples, berries were picked into a clamshell and handled carefully, resulting in an average of 5.7% B.c. rot incidence for the three cultivars, compared with Riverrun, regular and premium fruit from the packhouse with 16.6%—or approximately a three-fold increase.

Not all soft or collapsed fruit expressed B.c. or other pathogens. This implies that collapsed fruit are not caused solely by pathogens; other causes play important roles. Maturity and firmness at picking, nutrition, and postharvest mechanical damage and handling processes may all contribute (Moggia et al .2017; Li 2023; Li et al. 2018).

3.5 Recommendations

- Investigate postharvest management, including mechanical damage and cool-chain conditions and its impact(s) on berry collapse.
- Explore the relationship between fruit firmness at picking and postharvest berry collapse.
- Determine B.c. spore and other pathogen loadings before and after picking, as well as before and after grading, to understand the effects of microbial loading on postharvest shelf life.
- Analyse the relationships between nutrition, berry firmness, berry collapse and susceptibility to pathogens.
- Analyse the effects of cultivar and cell structure on berry collapse.

3.6 References

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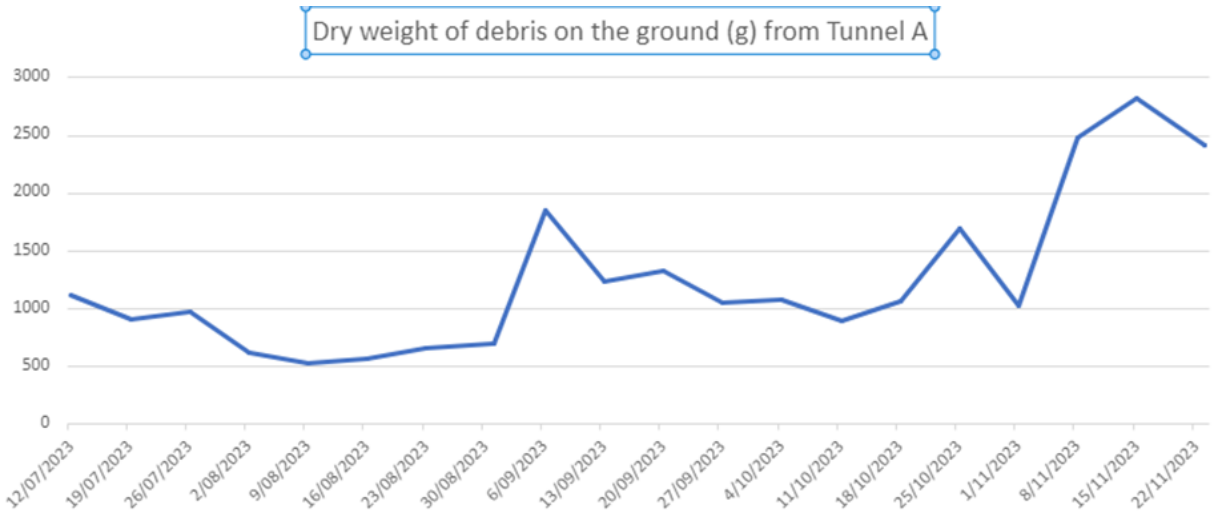
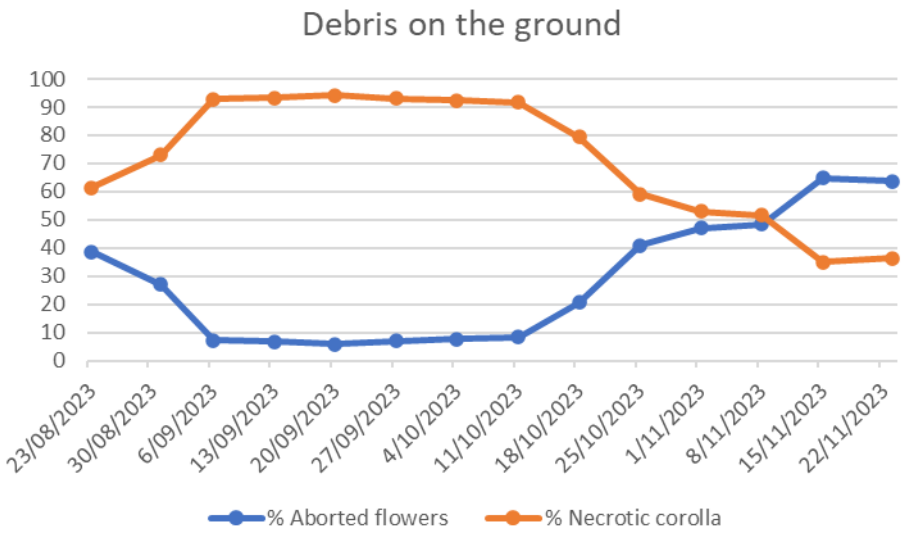
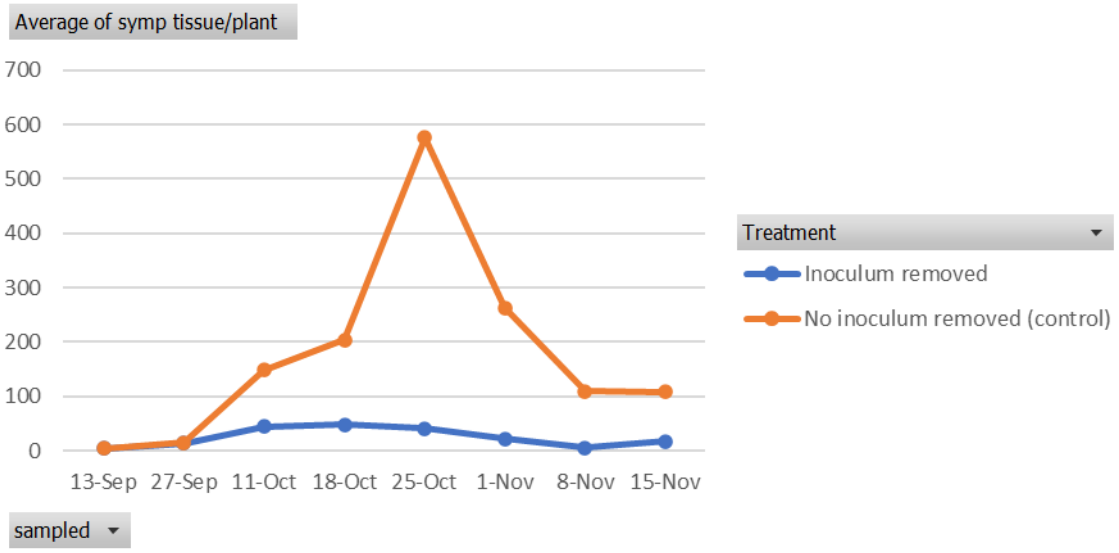
Appendix 1. Nanric inoculum management: supporting graphs



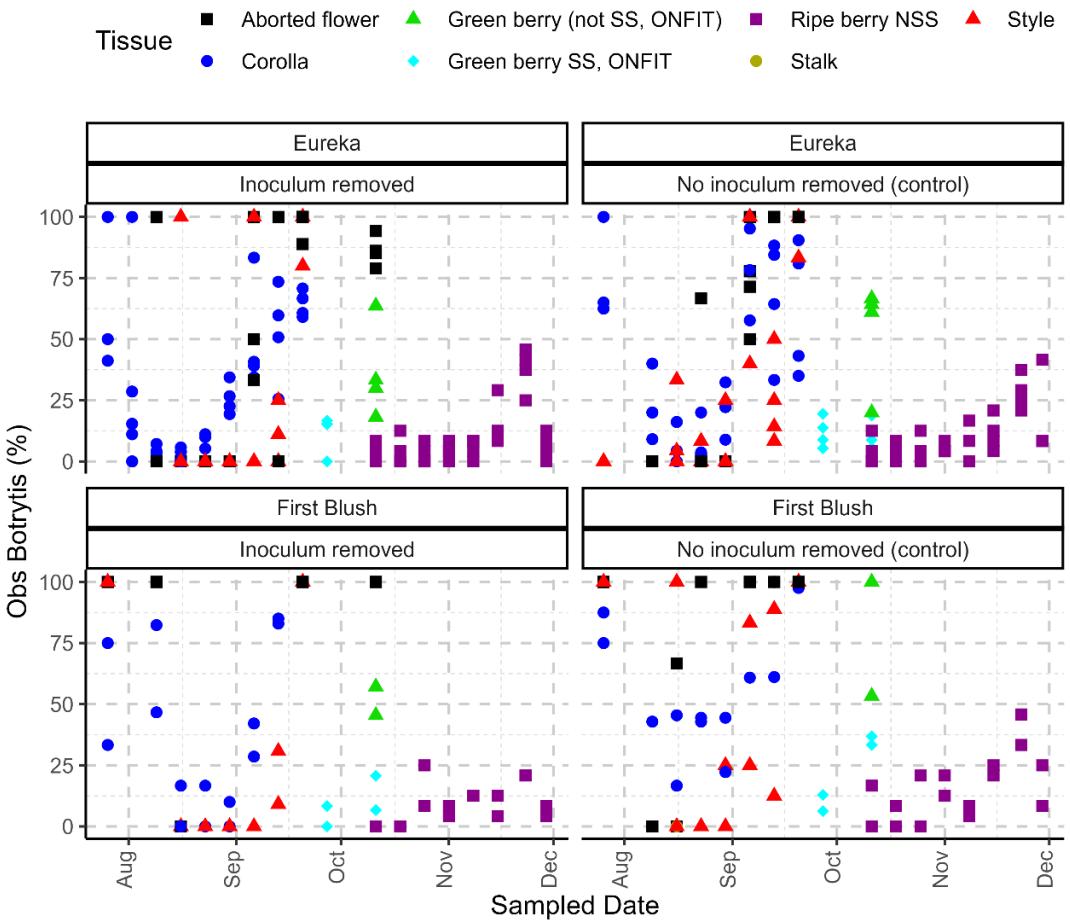
Total amount of debris (dry weight, g) on the ground from 50 m of Tunnel A length, or 247 blueberry plants consisting of 'Eureka' and 'First Blush'. Note: debris composition changed over time. Initial weights were driven by leaf drop. Around 30 August 2023, corollas started dropping in great numbers, and from 8 November berry drop became apparent.



Proportion of aborted blueberry flowers and necrotic corollas in the sweepings of the ground in Tunnel A (inoculum removed).



Counts of symptomatic tissues per blueberry plant in Tunnel A (inoculum removed) and Tunnel B (control). With the onset of harvest, symptomatic tissues dropped onto the ground because of the disruption/shaking of the plants by the pickers. The symptomatic tissues consisted mainly of aborted flowers.



Overview of data and tissues monitored for *Botrytis cinerea* colonisation for blueberry cultivars 'Eureka' and 'First Blush' in Tunnel A (inoculum removed) and Tunnel B (control). SS = surface sterilised, NSS = not surface sterilised, ONFIT = green berries were frozen overnight and then incubated to break down the natural fruit resistance to *Botrytis cinerea.*

Appendix 2. Lanvale spray trial: supporting tables

Chemical	Rate per 100 L
Pristine®	50 g
Captan®	160 mL
Lokit® (for Captan)	40 mL
Switch®	80 g
Kenja®	37.5 mL
ARMOUR-Zen®	100 mL
Esteem®	100 mL

Treatment (fixed effect) p-value given by the chi-squared test on the GLMM model, and the back-transformed predicted means and confidence intervals for *Botrytis cinerea* incidence in the blueberry spray trial. The multiple comparisons are based on 95% confidence intervals of the means on the logit scale. Means within a tissue type with the same letter (A,B,C etc.), are not statistically significantly different. Results are presented for all treatment plots without pooling of data for the two unsprayed treatments and the 7-day standard with one and two Switch® applications

Tissue	Treatment *p*-value	Treatment	Incidence Mean (%)	Incidence Lower CI (%)	Incidence Upper CI (%)	Multiple Comparison at 95%
Corolla	<0.001	unsprayed (1)	9.23	5.51	15.07	A
		14 day standard + 1 Switch®	2.8	1.56	4.98	B
		7 day standard	2.13	1.17	3.84	BC
		7 day Kenja®	1.46	0.78	2.73	C
		7 day MIRAVIS®	8.37	4.97	13.78	A
		3-4 day standard	1.38	0.73	2.6	C
		unsprayed (2)	9.74	5.81	15.88	A
		7 day standard + 2 Switch	3.2	1.8	5.63	B
Green Berry	0.003	unsprayed (1)	2.65	1	6.86	A
		14 day standard+ 1 Switch	0.75	0.24	2.32	BC
		7 day standard	0.58	0.18	1.88	B
		7 day Kenja	0.92	0.31	2.72	BC
		7 day MIRAVIS	1.24	0.43	3.52	ABC
		3–4 day standard	0.56	0.17	1.82	B
		unsprayed (2)	1.86	0.68	4.99	AC
		7 day standard + 2 Switch	1.24	0.43	3.5	ABC
Ripe Berry	<0.001	unsprayed (1)	2.17	0.84	5.5	A
		14 day standard+ 1 Switch	1.22	0.44	3.32	AB
		7 day standard	0.89	0.31	2.53	BC
		7 day Kenja	1.48	0.55	3.91	AB
		7 day MIRAVIS	1.82	0.69	4.7	AB
		3–4 day standard	0.4	0.12	1.37	C
		unsprayed (2)	3.82	1.54	9.16	D
		7 day standard + 2 Switch	1.82	0.69	4.7	AB

Appendix 3. Berry collapse

Berry Collapse – Orchard Sample Collection

Grower	Variety	Date Sampled
1	Sunrise	11/11/2023
1	Eureka	11/11/2023 17/11/2023 23/11/2023
1	Masena	11/11/2023 17/11/2023 23/11/2023
2	Eureka	11/11/2023 17/11/2023 23/11/2023
2	Masena	11/11/2023 17/11/2023 23/11/2023

Berry Collapse – Packhouse Sample Collection

Grower	Class	Variety	Arrived At Yeildia	Processed at Yeildia
2	General Reject	Masena	*	21/11/2023
1	Other Reject	Mixed	20/11/2023	22/11/2023
			23/11/2023	24/11/2023
			24/11/2023	27/11/2023
			4/12/2023	5/12/2023
1	Soft Reject	Mixed	20/11/2023	22/11/2023
			23/11/2023	24/11/2023
			24/11/2023	27/11/2023
			4/12/2023	5/12/2023
1	River Run	Mixed	20/11/2023	22/11/2023
			24/11/2023	27/11/2023
			4/12/2023	5/12/2023
1	Premium	Mixed	20/11/2023	22/11/2023
			24/11/2023	27/11/2023
			4/12/2023	5/12/2023

* Date unknown

Orchard and Packhouse sampling collection times showing a typical blueberry plant and reject bins, respectively. Slides by Annie Reid, summer student PFR/BerryCo..

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