

EPIDEMIOLOGY OF *BOTRYTIS CINEREA* IN BOYSENBERRY (*RUBUS* SPP.)

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ABSTRACT

Potential sources of grey mould (*Botrytis cinerea*) were identified in two Nelson boysenberry (*Rubus* hybrid) gardens in 1993/94 and 1994/95. More data collection in 1996/97 confirmed and quantified inoculum sources in a Nelson and Hawkes Bay boysenberry garden. Important *Botrytis* inoculum sources were litter on the ground, desiccated primocanes and receptacles left after fruit were harvested. Minor *Botrytis* inoculum sources were dibs, leaves and dead canes at the plant base. Both spore trapping results (1993-95) and berry washings (1996/97) found that *Botrytis* inoculum peaked at harvest. A strong correlation between fruit surface contamination and latent berry infection was observed. Management strategies to reduce *Botrytis* levels are discussed.

Keywords: *Botrytis cinerea*, boysenberry, epidemiology, New Zealand

INTRODUCTION

In New Zealand, control of *Botrytis cinerea* Pers.: Fr. (grey mould) in boysenberry has been an ongoing challenge. Although, disease control with fungicides is practised, *Botrytis* has caused substantial fruit losses prior to harvest (Langford 1993). Unsatisfactory control based on fungicides may be the result of the development of resistance to commonly used compounds and/or a targeting problem where the fungicide does not come into contact with the pathogen at a vulnerable stage (Williamson and McNicol 1986). For effective *Botrytis* control (chemical and/or cultural), an understanding of the disease epidemiology is required.

The nature of the boysenberry growth habit and garden management suggest a number of potential sources of *Botrytis* inoculum. The floricanes (two year old fruiting canes) are typically trained onto wire trellises to ease management and harvest. As the floricanes are pulled from the ground, terminal tip layering (dibs) are often lifted with the cane onto the trellis. New emerging canes, called primocanes or suckers, which grow into the trellis can interfere with floricane harvesting. For this reason the early emerging suckers are usually removed by hand or sprayed (desuckered) with herbicides. Close to harvest the suckers are allowed to start growing, giving them time to produce adequate growth before winter, while not interfering with picking. After harvest (or in the winter) the floricanes are pruned off. This practise often results in a large amount of dead cane at the base of the plant. Old cane and leaves are left on the ground to be shredded.

During 1993 to 1997 we investigated potential *Botrytis* inoculum sources and their relative contribution to *Botrytis* inoculum in New Zealand boysenberry gardens. Research in the 1993/94 and 1994/95 seasons was aimed at identifying sources of *Botrytis* inoculum in two boysenberry gardens in Nelson. In 1996/97, more detailed studies were carried out qualifying inoculum sources and quantifying the potential contribution of *Botrytis* spore release from these sources in a Nelson and a Hawkes Bay boysenberry garden. Assessments of flowers and berries were carried out to determine the relationships between latent *Botrytis* infection of the fruit, spore release in the field and potential disease incidence at harvest.

MATERIALS AND METHODS

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Identification of *Botrytis* inoculum sources (1993-1995)

Experimental designs in the 1993/94 and 1994/95 seasons are described in detail by Pyke *et al.* (1994) and Stanley and Pulford (1995), respectively. Briefly, ten plots were randomly selected from two commercial boysenberry gardens (cv. Riwaka Choice) in Nelson. Standard cultural and plant protection practices were applied by each grower during the season. In the 1993/94 season, a single collection of 20 samples of each tissue type was taken at random from each plot (dibs, debris on the ground, suckers and old cane from the plant base). In the 1994/95 season, ten samples/tissue type were taken randomly from each plot at two collection times for leaf debris, cane debris and old cane from the plant base and at five collection times for dibs and suckers. *Botrytis* incidence in tissue samples was assessed after incubation under a high relative humidity at 20°C for 24 h in 1993/94 and 72 h in 1994/95. In each garden, spore trapping was carried out using a Burkhard high throughput jet spore sampler and *Botrytis* selective medium (Kerssies 1990).

Main *Botrytis* inoculum sources (1996/97)

In the 1996/97 season, two commercial boysenberry gardens, one in Hawkes Bay (cv. semi-thornless Hawkes Bay late) and one in Nelson (cv. Riwaka Choice) were selected. In both gardens, ten plots were selected at random. Plot length was 7.5 m along the row and plot depth was 0.5 m measured from the center wire into the row. Standard cultural and plant protection practices were applied by each grower during the season.

Host tissues identified in the previous studies (1993 to 1995) were sub-sampled at six different times in the Nelson garden and at four different times in the Hawkes Bay garden over the growing season. Sampling commenced on 29 August 1996 (mid winter) and terminated on 14 January 1997 (harvest). Tissues were sampled as available and consisted of litter on the ground, leaves with necrosis, damaged suckers, dibs, dead canes within the plant base and receptacles of harvested fruit.

Litter samples were taken at random using two quadrats/plot (0.04 m²/quadrat). All litter within the quadrats was collected. In the canopy, green leaf samples were collected randomly using three quadrats/plot (0.04 m²/quadrat). All leaves within the quadrat were harvested to the centre wire. Necrotic area on each leaf was estimated visually as a proportion of the total leaf area. The number of total suckers, damaged suckers, dibs and dead cane were counted in each plot at each sampling time and 10% of each tissue type was collected. At late harvest, approximately 30 receptacles/plot were collected and the total berry number estimated by counting all fruit within a known area. The inoculum potential of the various tissues collected was measured using the procedures of Elmer *et al.* (1995). In summary, samples were incubated under high humidity at room temperature for 5 days. *Botrytis* inoculum potential was measured by visually estimating the sporulating area (mm²).

Flower and fruit infection studies (1996/97)

From each plot 20 flowers or berries (flowers-berries) were collected randomly at each sampling time to determine the effect of flower-berry contamination by *Botrytis* on latent fruit infection. Ten flowers-berries were examined for *Botrytis* surface contamination using a modification of methods described previously (Elmer *et al.* 1995). The ten flowers-berries were washed by shaking them vigorously for 5 min in 20 ml sterile distilled water amended with 0.05% Tween 80 (tween water). A 10 ml aliquot was removed and centrifuged at 3000 rpm for 2 min. The supernatant was decanted and the spore pellet resuspended in 1 ml tween water, vortexed and pipetted (250 ml/plate) onto four petri dishes containing Kerssies medium. Petri dishes were assessed for *Botrytis* colony formation after 14 days of incubation at 18°C in the dark.

The remaining ten flowers-berries/plot were used to determine latent *Botrytis* flower-berry infection by surface sterilising with 0.3% sodium hypochlorite (Wilson) for 5 min, rinsing twice in tap water, incubating for 5 days under high humidity at room temperature and assessing for *Botrytis* incidence. To assess the contribution of latent infection to potential berry rot, at late harvest in Hawkes Bay and early and late harvest in Nelson, 20 ripe fruit/plot were sampled at random individually into sterile plastic containers (Labserv P354) and assessed for *Botrytis* incidence after 5 days of incubation at 20°C under high humidity.

Data from the 1993 to 1995 seasons are expressed as *Botrytis* incidence (%) /tissue type. Data for each year were analysed separately using the PROC MIXED procedure in the SAS statistical package. Data from 1996/1997 season are expressed qualitatively and quantitatively as *Botrytis* inoculum potential/tissue type/plot. These data were subject to logarithmic transformation before analysis of variance and the application of Fisher's Least Significant Difference Test using Systat.

RESULTS

Identification of *Botrytis* inoculum sources (1993-1995)

Initial investigations in the 1993/94 season revealed no significant differences between gardens with respect to *Botrytis* incidence of various tissue samples (Table 1). Dibs showed the highest *Botrytis* incidence for both gardens. In the 1994/95 season, the dibs again showed highest *Botrytis* incidence in Garden 1, whilst all inoculum sources were of equal importance in Garden 2.

Spore trapping over two seasons in the two Nelson gardens resulted in considerable variability in the number of colonies counted during the two seasons from day to day and between gardens. The number of colonies counted early in the 1994/95 season at Garden 2 was unusually high, but the number trapped dropped during mid-season. The number of colonies measured at the two gardens peaked at harvest in January in both seasons in Garden 1 and in 1993/94 in Garden 2 (Fig. 1).

TABLE 1: Mean *Botrytis* incidence (%) detected on various host tissues sampled from two gardens in Nelson over two growing seasons.

	1993/94		1994/95	
	Garden 1	Garden 2	Garden 1	Garden 2
Host tissues				
Suckers	17	10	17	19
Dibs	70	81	37	20
Dead cane	9	10	3	34
Debris (leaves and canes)	7	6	4	18
ANOVA variables				
Garden	ns		***	
Material	***		***	
Garden*Material	ns		***	

ns = not significant different ($P < 0.05$)

*** = highly significant different ($P < 0.001$)

Main *Botrytis* inoculum sources (1996/97)

Total inoculum potential of *Botrytis* was greater ($P < 0.01$) in the Nelson boysenberry garden than in the Hawkes Bay garden. However, in both gardens *Botrytis* total inoculum potential followed similar trends as no interactions between sites and time could be detected ($P > 0.05$). Total inoculum potential of *Botrytis* fluctuated, with two peaks at flowering and harvest. The main tissues contributing to *Botrytis* inoculum were litter, suckers and receptacles (Fig. 2). Throughout the season in both gardens, dibs and dead cane contributed less than $7 \text{ mm}^2/\text{plot}$ to the average (7750 mm^2) total inoculum potential of *Botrytis* (Fig. 2). Leaves in Hawkes Bay harboured more *Botrytis* inoculum than leaves in Nelson ($P < 0.001$), because leaves in Hawkes Bay were damaged by herbicide drift. The relatively high *Botrytis* inoculum potential for Hawkes Bay compared with Nelson at mid season was due (99.4%) to the contribution of leaves (Fig. 2).

Flower and fruit infection studies (1996/97)

During the growing season the number of *Botrytis* colonies from conidia washed off boysenberry flowers-berries increased ($P < 0.001$; Fig. 3). Similarly, latent *Botrytis* infection

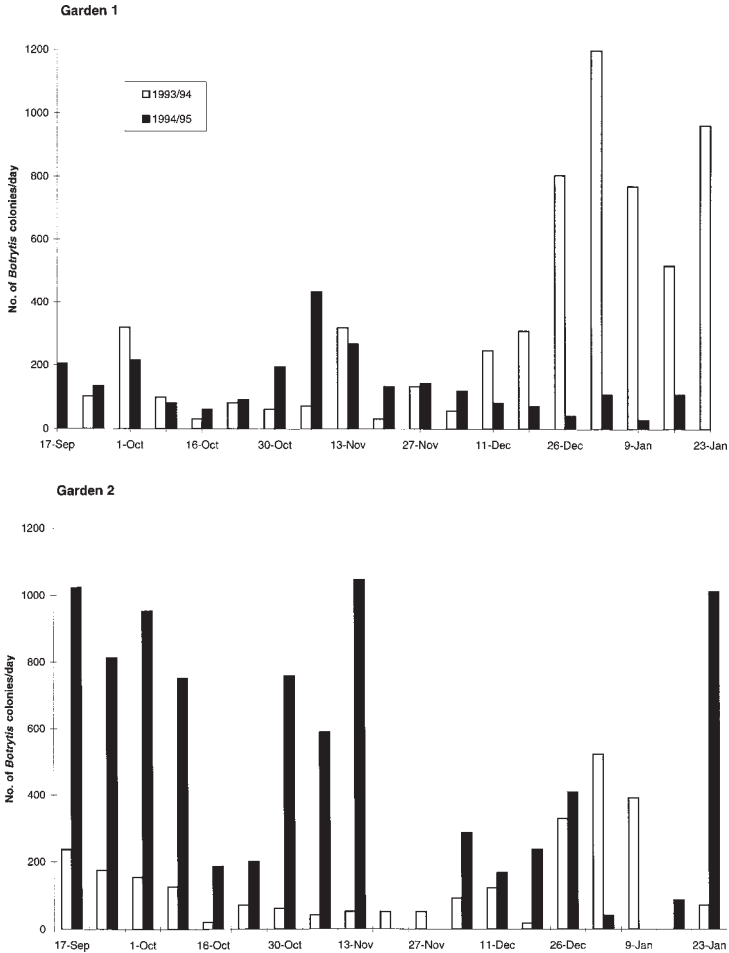


FIGURE 1: Number of *Botrytis* spores trapped throughout two season in two boysenberry Gardens in Nelson. (Spore traps operated for 5 min at each of four times/day at a rate of 750 litres of air/min. Each data point represents daily totals averaged weekly).

of flowers-berries increased ($P < 0.001$) between flowering and harvest (Fig. 3). Latent infection and surface contamination of flowers-berries were correlated ($r = 0.512$; $P < 0.001$). Latent infection and potential *Botrytis* berry rot were also correlated ($r = 0.616$; $P < 0.001$) (Fig. 4) with latent infections contributing on average 92% of the potential berry rot. A weak correlation between *Botrytis* total inoculum potential and surface contamination of flowers-berries was observed ($r = 0.212$; $P < 0.05$) indicating that *Botrytis* total inoculum potential may reflect the actual size of *Botrytis* populations in the field.

DISCUSSION

Botrytis incidence on plant tissues varied between the 1993/94 and 1994/95 seasons and between the two gardens (1994/95). This may be the result of climatic parameters

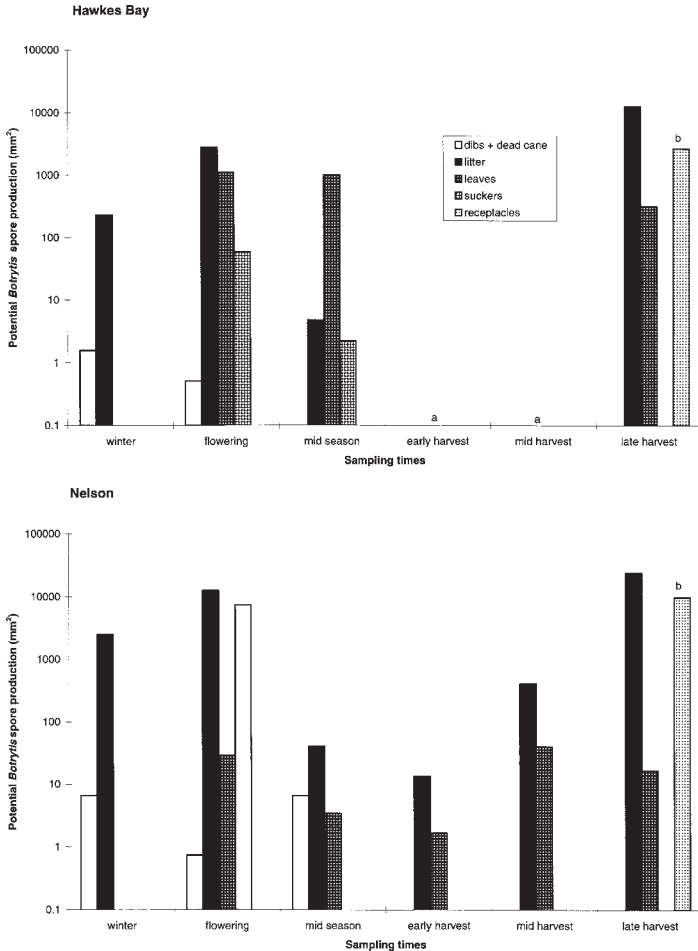


FIGURE 2: Seasonal trends of *Botrytis* inoculum potential/plot on a range of host tissues sampled during the 1996/97 season in Nelson and Hawkes Bay boysenberry gardens. (a = not sampled; b = receptacles sampled at late harvest only).

and/or changed garden management practices which are known to affect *Botrytis* epidemiology (Marois 1996). For example, the reduced incidence of *Botrytis* on dibs in 1994/95 was probably the result of changed pruning practices which involved cutting dibs out during the winter pruning (Stanley and Pulford 1995). The new strategy was adopted in 1994/95 after the 1993/94 findings by Pyke *et al.* (1994) on the high *Botrytis* incidence of dibs. Therefore, in 1994/95, only a low number of very small dibs could be found and these dried out more rapidly.

Studies in 1996/97 were designed to measure the relative contribution of various plant materials harbouring *Botrytis* to the total inoculum potential of *Botrytis* in boysenberry gardens from winter to harvest. Our studies have identified several important

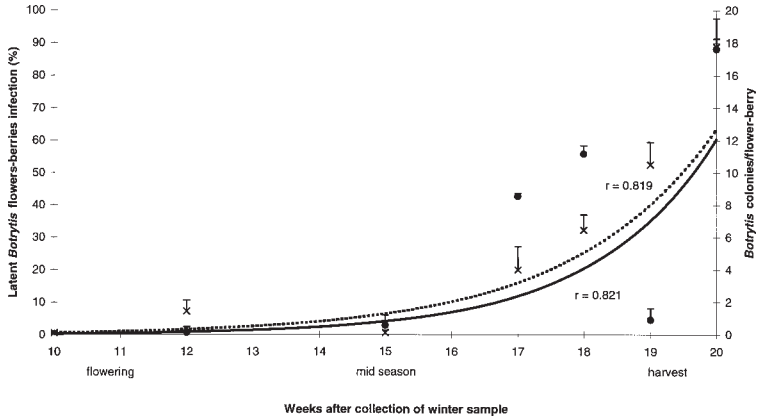


FIGURE 3: Increase in latent *Botrytis* flower-berry infection (—+—) and increase in *Botrytis* colonies washed off the flower-berry surface (---x---) during the 1996/97 season in Nelson and Hawkes Bay boysenberry gardens.

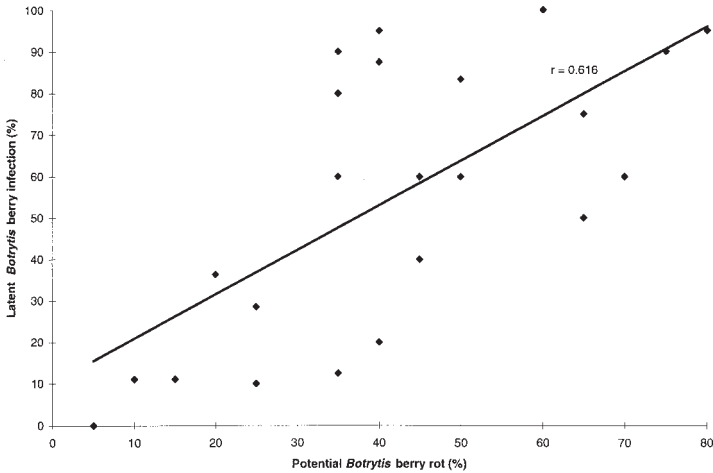


FIGURE 4: Correlation between potential *Botrytis* berry rot and latent *Botrytis* berry infection at harvest in Nelson and Hawkes Bay boysenberry gardens (1996/97).

Botrytis inoculum sources. These were litter on the ground, primocanes burnt off by desuckering herbicides and receptacles left in the canopy after fruit were harvested. Less important *Botrytis* inoculum sources were dibs, leaves and dead canes at the plant base. Because of seasonal growth habits and garden management practices, not all tissue types were available at each sampling time (eg suckers and receptacles). Under current management strategies, dibs, dead cane in the plant base and necrotic leaves in the vines

were less important compared with litter, suckers and receptacles, with regard to their contribution to the total inoculum potential of *Botrytis*.

In Hawkes Bay most suckers were removed either mechanically and/or chemically throughout the season, explaining the low contribution of this tissue type to the total inoculum potential of *Botrytis*/plot. In contrast, all suckers in Nelson were removed after flowering. The high *Botrytis* peak at flowering stresses the importance of *Botrytis* control on suckers, particularly if suckers have been burnt off by a herbicide, as the dying tissues provided an ideal environment for *Botrytis* development. *Botrytis* could be controlled chemically by ensuring that botryticides are targeted directly onto suckers. Where suckers are mechanically pruned, suckers should be removed and shredded finely to ensure rapid tissue break down. Early sucker control prior to and during flowering needs to be carefully managed to prevent *Botrytis* build up and flower infection.

Receptacles of picked fruit are an ideal substrate for *Botrytis* colonisation. Because of continual harvesting over 4-6 weeks, fungicide applications to prevent colonisation of receptacles is limited due to potential contamination of fruit with fungicide residues. The only botryticide registered in New Zealand with less than 2-3 days withholding period for export boysenberries is iprodione. However, the development of resistance in *Botrytis* populations to iprodione (Langford 1993) limits its regular use as a protective spray during harvest.

The fluctuations in *Botrytis* inoculum potential of the litter during the season may be driven by environmental conditions, garden management practises and/or changes of litter composition during the season. For example, the warm and wet conditions in spring may trigger the over wintering inoculum to develop. Dry summer conditions and the drying of host tissues and/or fungicide applications during flowering and the resulting run off onto the ground may explain the decline in *Botrytis* inoculum. The increase at harvest may be a result of dropped fruit and *Botrytis* infected mummified berries on the ground. Litter management may be an important tool for *Botrytis* control. In boysenberry gardens all potential *Botrytis* sources usually will end up on the ground. Prunings are generally shredded and left in the aisles between the rows or raked around the plant base. Enhanced litter degradation and therefore decomposition of *Botrytis* sources using suitable organic and/or inorganic amendments may decrease *Botrytis* contamination in the litter. Various compost applications and inorganic fertilisers have been successfully used to enhance the decomposition of over wintering black spot in apple leaf litter (Tränkner 1993). Alternatively, boysenberry growers could employ sanitary measures by removing all prunings and thus *Botrytis* inoculum sources from the garden (Jarvis 1962a).

After flowering, there was an increase in total *Botrytis* inoculum potential in the garden. Associated with this, not only the number of *Botrytis* conidia increased on the surface of the growing berry, but also latent berry infection increased. Accumulation of *Botrytis* spores on fruit surfaces has been reported on kiwifruit (Elmer *et al.* 1995). To date, latent infection of *Rubus* has been associated primarily with flower infection of the styles (Dashwood and Fox 1988). Our research suggests that boysenberry styles remain susceptible to *Botrytis* colonisation throughout the development of the drupelets right up to harvest. This is supported by Williamson *et al.* (1987). They reported that although the rate of *Botrytis* decay was reduced when flowers were inoculated at a later growth stage (growth stage 5, green drupelets formed), berries remained susceptible to *Botrytis* infection. Unfortunately, the post harvest decay was based on unsterilised fruit only, therefore the contribution of latent infection to postharvest rot is unknown.

The high proportion of latent infection to total potential berry rot (92%) suggests that external contamination of the fruit surface itself did not cause berry rot. This is in accordance with findings by Jarvis (1962b) who attributed only 1% of infections in the field to conidia germinating in water films on fruit surfaces of raspberry and strawberry fruit.

At any time during the growing season *Botrytis* populations in a boysenberry garden will be affected by substrate availability and favourable weather conditions for disease development. *Botrytis* control strategies should be targeted to protect tissues from colonisation by the pathogen and/or remove potential plant materials harbouring the

disease from the garden. This is particularly important in the light of our the findings, that with an increase in *Botrytis* conidia on surfaces of flowers-berries, latent fruit infection also increased. Considering that 92% of potential berry rot was attributed to latent infection, *Botrytis* accumulation on the flowers-berries should be prevented. Future research is required to further investigate the role of styles in the process of development of latent infections and their susceptibility to *Botrytis* colonisation from flowering to harvest. Further, the effect of garden management practises, in particular litter management on *Botrytis* build up, dispersal and/or decomposition needs to be examined.

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