EXTERNAL CONTAMINATION OF KIWIFRUIT BY *BOTRYTIS CINEREA*: AN IMPORTANT SOURCE OF INOCULUM FOR FRUIT INFECTION

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ABSTRACT

Stem end rot of kiwifruit caused by *B. cinerea* has been responsible for significant storage losses. Epidemiological studies in 1991-3 indicated that there was a significant relationship between the size of the pathogen population in vines and the number of propagules on the fruit surface at harvest. Fruit that were adjacent to sporulating tissues in the vine canopy had significantly greater numbers of propagules per fruit compared to fruit not adjacent to inoculum sources. Up to two million colony forming units per fruit were detected, and it is suggested that the hairy fruit surface acts as a spore trap. Further studies confirmed a significant relationship between the number of *B. cinerea* propagules on the fruit surface at harvest and the amount of stem end rot in storage. We conclude that external fruit contamination by *B. cinerea* propagules is a major source of inoculum for fruit infection at harvest and during post-harvest handling. The need to reduce potential inoculum sources in the vines during fruit development is discussed.

Keywords: *Botrytis cinerea*, kiwifruit, epidemiology, inoculum sources, external fruit contamination

INTRODUCTION

Stem end rot of kiwifruit (*Actinidia deliciosa* cv. Hayward), caused by *Botrytis cinerea* (Persoon: Fries) is a major cause of post-harvest fruit loss. Direct losses in 1994 to the New Zealand kiwifruit industry were in excess of $7 million. *Botrytis* incidence is sporadic and considerable variation occurs between growing seasons, between regions and between individual orchards within a region (Pennycook 1985). Factors such as fruit maturity (Pyke *et al.* 1992; Poole and McLeod 1994), fruit water potential (Pyke *et al.* 1992) and host resistance (Poole and McLeod 1994) have been assessed for effects on disease incidence. Environmental factors, such as rain at or near harvest, were considered responsible for much of the variation (Brook 1990). Delaying cool storage of harvested fruit significantly affected the incidence of stem end rot (Beever 1992) and the environmental conditions associated with curing were also identified (Long and Bautista-Banos 1994). In the post-packing environment, rate of fruit cooling affected the incidence of stem end rot significantly (Lallu *et al.* 1992).

Brook (1990) concluded that free airborne spores at the time of harvest were the most likely source of inoculum for stem end rot infection in cool storage and that this may account for vine to vine variability. Spatial correlation analysis (Elmer *et al.* 1994) indicated that *B. cinerea* was strongly aggregated at the individual vine level. Fruit handling experiments cited by Brook (1990) demonstrated that fruit jumbled in the picking bag resulted in a higher incidence of stem end rot compared to fruit which were direct picked into fruit trays. The actual source of inoculum was not identified but it is possible that the propagules on the fruit itself caused picking wound

contamination. A method was devised to quantify the number of viable *B. cinerea* propagules on the fruit surface and up to 2 million per fruit were detected at harvest (Elmer *et al.* 1992; Fermaud *et al.* 1995). In this paper we investigated the hypotheses that *Botrytis* spores produced in vines collect on the hairy external surfaces of kiwifruit, that these spores may be transferred to picking wounds, and that, under conducive conditions, they will infect fruit and cause stem end rot.

MATERIALS AND METHODS

External fruit contamination was examined in relation to:

1) Proximity of fruit to male vines.

The effect of proximity to a significant source of inoculum (male vines) on external fruit contamination was investigated in 1991 in a Motueka experimental kiwifruit orchard trained on a T bar system where standard cultural and pest protection practices had been applied. Five fruit from the boundaries between male and female vines were sampled at random from six replicate vines. Sampling was repeated for six adjacent female vines. Each five fruit sample was washed and propagules were dislodged from the hairy outer surface by shaking vigorously for 3 minutes, first in 50 ml sterile distilled water (SDW) plus 0.05% (v/v) Tween 80 and then in 125 ml of SDW only. A sub-sample (25-50 ml) of each washing was filtered through Whatman 105 lens tissue and then vacuum-filtered through a Millipore membrane filter (pore size 5 mm). Propagules were re-suspended in 10 ml SDW plus 0.05% Tween 80. Aliquots of appropriately diluted suspensions were spread on a *B. cinerea* selective agar medium (Kerssies 1990) and then incubated in the dark for 14 days at 10°C. The number of mycelial colonies of *B. cinerea* was assessed visually. Plates were incubated at room temperature (17-24°C) exposed to natural light for a further 5 days for colony verification. Results were expressed as the mean number of viable propagules of *B. cinerea* per fruit.

2) Inoculum sources in the orchard

In two similar kiwifruit orchards in the Motueka region in 1992, all potential sources of *B. cinerea* inoculum were sampled at random from ten female plots (two vines/plot) per orchard at three growth stages during the growing season. The types and proportions of host tissue sampled in each plot at petal fall, mid fruit and harvest were; leaves with necrosis (10%), wind-blown senescent shoots (5%), fruit with petals attached (5%) and fruit on the ground at harvest (25%). Prunings beneath the canopy were sampled by removal of all tissue from within a 0.5 m^2 quadrat. Host tissues were incubated in high humidity chambers for 5 days and the area of sporulation measured. The number of spores from host tissues was estimated from correlations (Elmer *et al.* 1993). The number of spores from all host tissues within plots was referred to as the total potential spore production (TPSP/plot). In the same experiment, the number of viable propagules contaminating the fruit surface was determined on three spatial replicates (lower, middle and upper canopy zones) of five fruit of similar size sampled at each growth stage. External contamination of the fruit surface was determined as described in the previous experiment.

3) Proximity of fruit to sporulating *B. cinerea* in the canopy

The effect of close proximity to infected necrotic tissues on contamination of the fruit surface at harvest was investigated further in 1992 by sampling six fruit (pedicel attached) from within 25 cm of 12 *B. cinerea* infected necrotic tissues (leaves and wind-blown senescent shoots) and six fruit which were at least 1 metre from the necrotic tissues. The sampling was repeated for 20 (two-vine) plots per orchard in two orchards. All fruit (72/plot) were placed into Plix trays and ten fruit were removed at random for estimation of external contamination. Pedicels and sepals were removed by hand from the 62 remaining fruit and these were placed in a clean paper rubbish bag and jumbled for 20 seconds. Ten fruit were removed immediately at random and the picking wounds were removed with a flame sterilised cork borer (4 mm diameter, depth 3 mm), and then transferred to small plastic vials with 3 ml SDW (plus 0.01% Tween 80). Vials were placed in an ultrasonic bath for 3 minutes to dislodge spores and then aliquots were plated on Kerssies medium and assessed as described earlier.

The remaining fruit (52) were packed in standard single layer Plix trays, sealed with a plastic liner and stored at 0°C. Stem end rot incidence was determined visually after 12 weeks.

4) Manipulation of *B. cinerea* populations in the canopy

In 1993, the potential size of the *B. cinerea* population in vines in two kiwifruit orchards, selected as before, was adjusted either by inoculation of green leaves or by removal of all potential sources of inoculum during the growing season. In a high inoculum treatment, 200 green leaves per two vine plot were inoculated with a mycelial plug at the petal fall growth stage. All inoculated leaves developed typical necrotic lesions. A low inoculum treatment was achieved by applying iprodione (Rovral FLO) at 250 g/litre to plots at full bloom and petal fall. Thereafter, all senescent and necrotic tissues were removed at 14 day intervals during the growing season. A set of untreated control plots received no fungicide, were not inoculated and senescent tissues were left in the vines. Experimental plots were separated by at least two untreated female vines to minimise interplot interference. Treatment plots were randomised within each of five replicates. The number of viable propagules on the fruit surface at harvest was determined on 10 fruit of similar size/plot, from within 1 m of the centre leader, by washing fruit as described above. One hundred additional fruit were picked at random from the same canopy regions, the pedicels and sepals removed by hand, placed in a clean paper rubbish bag and jumbled for 20 s. Immediately after jumbling fruit were packed into standard single layer Plix trays, sealed with a plastic liner, cool-stored at 0°C for 16 weeks and stem end rot incidence determined visually.

5) The degree of *B. cinerea* contamination and stem end rot

In 1994, we investigated the relationship between external fruit contamination and stem end rot further by using an isolate of *B. cinerea* (BC48) resistant to carbendazim-based (MBC) fungicides. Fifty-six fruit were obtained from each of six replicate vines in a Bay of Plenty orchard that had no history of MBC application. Isolate BC48 was applied by dipping each fruit up to the "shoulder" in a freshly-made spore suspension in SDW (plus 0.01% Tween 80) derived from 14 day old cultures grown on V8 juice agar. The concentration was adjusted to apply $1x10^3$, $1x10^4$, $1x10^5$ and $1x10^6$ spores/fruit. Fruit were dried overnight, and a sub-sample of 10 fruit was removed at random for estimation of the number of viable propagules contaminating the fruit surface as described earlier. The Kerssies selective medium was amended with 100 mg/litre carbendazim to allow growth of only the viable BC48 propagules. The frequency of MBC resistant isolates in the orchard population was also assessed, and found to be not significantly different from zero. Pedicels and sepals were removed from the remaining fruit by hand and 46 fruit per treatment were placed in a clean dry picking bag, jumbled 10 times and a sub-sample of 10 fruit removed at random for estimation of BC48 propagule numbers on the picking wounds. The remaining fruit (36) were packed into standard single layer Plix trays, sealed with a plastic liner and the incidence of stem end rot determined after 12 weeks storage at 0°C. The presence of BC48 in diseased fruit was confirmed by isolation and growth on malt extract agar (MEA) amended with 100 mg/litre carbendazim.

RESULTS

Fruit from female vines immediately adjacent to male vines had significantly ($P<0.01$) more viable propagules on the external fruit surfaces at petal fall (mean = 12,550/fruit) compared to fruit adjacent to other female vines (mean = 374/fruit).

There was abundant inoculum at petal fall as indicated by the TPSP values (Table 1), coinciding with the presence of senescing floral tissues. There was a significant ($P<0.01$) reduction in TPSP during the season. Viable propagules were detected on the fruit surface at all three growth stages, with significant ($P<0.01$) variation between sites and orchards and a significant ($P<0.01$) decline at harvest. There were significant ($P<0.05$) correlations between TPSP and the number of viable propagules on the fruit surface at individual sites at the petal fall and harvest assessments, but not at the mid-fruit assessment.

TABLE 1: The correlation between total potential spore production (TPSP/ plot) and the number of viable *B. cinerea* propagules/cm^2 of fruit surface at three growth stages.

Growth stage	Spore production/plot[1]	Viable propagules /fruit surface[2]	Correlation[3] of spore production and viable propagules/ fruit surface
Petal fall	18.58 ($117x10^6$)[4]	6.60 (735)	0.53*
Mid Fruit	17.25 ns[5] ($31x10^6$)	6.65 (772)	0.06ns
Harvest	16.96 *** ($23x10^6$)	5.43** (228)	0.55*
LSD (P<0.01)	0.737	0.851	
LSD (P<0.001)	1.277	1.135	

[1] $\log_n$ total potential spore production (TPSP)
[2] $\log_n$ number of viable propagules/cm^2
[3] Pearson correlation coefficient (r value)
[4] Back-transformed values.
[5] ns = P>0.05, *, **, *** significant at P< 0.05, P< 0.01 and P< 0.001 respectively.

The number of viable propagules on the fruit surface at harvest was significantly (P<0.05) greater on fruit sampled adjacent to inoculum sources ($\log_n$ 9.74) compared to fruit sampled at least 1 m from sources of inoculum ($\log_n$ 8.87). After simulating harvest practices by jumbling fruit in a bag, significantly (P<0.01) more viable *B. cinerea* propagules were detected on the picking wound from fruit sampled adjacent to inoculum sources ($\log_n$ 2.19) compared to fruit sampled from the non-adjacent sites ($\log_n$ 1.63). However, stem end rot incidence was not significantly (P>0.05) different between the fruit sampled from the two different sites in the canopy (adjacent $\log_n$ 1.22; non-adjacent $\log_n$ 1.46).

The number of viable *B. cinerea* propagules on the fruit surface at harvest was significantly (P<0.05) greater on fruit sampled from the high inoculum and untreated plots compared to the low inoculum plots (Table 2). Stem end rot incidence was also significantly (P<0.05) higher in the high and control treatments compared to the low inoculum treatment. Stem end rot incidence and the number of viable propagules on the fruit surface at harvest were significantly (P<0.001) correlated. There were also highly significant (P<0.001) correlations between the number of viable BC48 propagules on the fruit surface at harvest and the number detected on picking wounds (r=0.868), and between the number of viable BC48 propagules on the fruit surface at harvest and the incidence of stem end rot from which BC48 was isolated (r=0.832).

TABLE 2: The relationship between *B. cinerea* inoculum in vines and the number of viable *B. cinerea* propagules on the fruit surface at harvest and subsequent stem end rot in cool store (1994).

Treatment	Mean ($\log_n$) number of viable propagules/fruit	Stem end rot[1] ($\log_n$)	Correlation (r value) between number of viable propagules/fruit and stem end rot
Low inoculum	6.10a[2]	0.89a	
High inoculum	8.02b	2.41b	0.430 ***
Untreated	8.05b	1.71b	
LSD (P<0.05)	1.45	0.71	
LSD (P<0.01)	1.90	0.95	

[1] Visually assessed after 16 weeks at 0°C
[2] Numbers followed by the same letter do not differ significantly at P<0.05 (LSD test)

DISCUSSION

Results from epidemiological studies in 1993 (Elmer *et al.* 1994) confirmed that inoculum production was highly variable and that *B. cinerea* incidence on host tissues was very focal. We detected large numbers of viable *B. cinerea* propagules on the external fruit surfaces of kiwifruit, and demonstrated a link between both the proximity to inoculum and the total size of the *B. cinerea* population at harvest to the number of viable propagules on the fruit surface at harvest. Proximity to male vines may represent a greater hazard for fruit contamination because of the large inoculum source found on male flowers and dispersal of that inoculum. The accumulation of spores on the external fruit surface of kiwifruit has not been reported, but *B. cinerea* propagules on the smooth surface of pear fruit were regarded as an important source of inoculum for fruit infection during post-harvest handling (Spotts 1985). We propose that the hairy surface of the Haywood cultivar of *Actinidia deliciosa* acts as a spore trap and that spores accumulate on the hairy surface during the growing season. Some preliminary evidence (Elmer, Whelan and Pyke, unpublished) suggested that there was substantially less accumulation on smooth-skinned types. The mechanism of adhesion to the hairy fruit surface is not known but positive electrostatic charges found on the spores of a basidiomycete fungus were reported by Gregory (1957). A similar phenomenon may occur in the *Botrytis*/kiwifruit system and research to investigate the occurrence of an electrostatic charge on the hairy fruit surface which aids adhesion of *Botrytis* spores is in progress. The relation between fruit contamination and storage rot was variable. This may be explained in part by the conditions for infection immediately after picking scar contamination, and in the initial stages of cool storage when humidity is important for infection. The detection of significant differences is also made more difficult by the overall low frequency of stem end rot in many experiments with kiwifruit.

The links between external fruit contamination, picking wound contamination and stem end rot constitute a potential risk to producers, but knowledge of this also provides an opportunity for control. Reductions in the number of viable propagules on fruit at harvest resulted in significant reductions in the number of viable propagules contaminating the picking scar of the fruit at harvest, and a significant reduction in stem end rot incidence in cool-store. Removal of inoculum sources during the growing season has the potential to be an effective disease control strategy, especially in non-chemical or reduced-chemical growing systems. This strategy is also compatible with the NZKMB strategy of reducing pesticide residues on fruit (Martin pers. comm.).

Pak and Manning (1994) suggested that the potential size of *B. cinerea* populations in the canopy may be useful as a predictive tool for disease management. The picking wound is the major site of infection for stem end rot (Pennycook 1985). Transfer of spores to the picking wound by air-borne conidia from the canopy is possible, but our experiments conducted in 1994 with an MBC resistant isolate of *B. cinerea* showed highly significant correlations between the number of viable propagules on the fruit surface at harvest, dispersal to the picking wound and subsequent stem end rot in storage. It may be argued that there is a more direct link between spores present on the fruit surface and stem end rot than between canopy spore production and stem end rot. Thus, estimation of the number of viable propagules on the fruit surface at harvest has potential as a predictive tool for decision-making in disease control.

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