THE INCIDENCE OF *BOTRYTIS CINEREA* AND EXPRESSION OF PUTATIVE HOST DEFENCES IN GREEN- AND GOLDEN-FLESHED KIWFRUIT OF DIFFERING HARVEST MATURITY

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ABSTRACT

This study was carried out on green-fleshed *Actinidia deliciosa* cv. Hayward, the world's main commercial kiwifruit cultivar, and the recently introduced golden-fleshed *Actinidia chinensis* cv. Hort16A. The incidence of Botrytis rots was 2-fold higher in Hayward than in Hort16A, irrespective of fruit harvest maturity. Expression of chitinase activity and antifungal phenolics, in particular a compound with a R_f value of 0.56, was also higher in Hort16A than in Hayward pericarp tissue. These results suggest that genotype resistance to *B. cinerea* is greater in Hort16A than in Hayward fruit, and is associated with chitinases and phenolics. An increase in disease resistance with advanced fruit harvest maturity was more evident in Hort16A than Hayward, but did not correlate with Hort16A hydrolytic enzyme or phenolic activities, suggesting the involvement of other unidentified defence component(s). Delaying fruit harvest can be used to further augment resistance of the Hort16A cultivar to *B. cinerea*, but the mode of action remains to be determined.

Keywords: *Botrytis cinerea*, chitinases, kiwifruit, phenolics, lytic enzymes.

INTRODUCTION

Botrytis cinerea is a serious postharvest pathogen of *Actinidia deliciosa* cv. Hayward. In recent years, crop management practices that reduce pre-harvest inoculum loads have significantly lowered disease incidence in Hayward fruit in New Zealand. One such cultural practice involves delaying fruit harvest beyond the minimum acceptable harvest maturity of 6.2% total soluble solids (TSS) (Hopkirk 1992). There are no published studies comparing fruit resistance of Hayward and Hort16A to *B. cinerea*, or the efficacy of delayed fruit harvest on Botrytis rots in Hort16A. However, Hort16A leaves are less susceptible than Hayward to *B. cinerea*, suggesting a higher level of basal resistance in Hort16A (Wurms et al. 2003).

The current study was instigated to compare the effects of fruit harvest maturity on Botrytis fruit rots in Hayward and Hort16A kiwifruit cultivars. In addition, phenolics and hydrolytic enzyme activities were monitored, since these appear to be key resistance markers in other *B. cinerea*-plant interactions (Derckel et al. 1999; Wurms et al. 1999).

MATERIALS AND METHODS

Hort16A and Hayward fruits from adjacent plots were harvested at four dates (20 April, 5 May, 19 May and 2 June 2000) towards the end of the growing season to produce a range of maturities. After harvest, sepals and pedicels were removed by hand, and fruit were inoculated with a conidial suspension of *Botrytis cinerea* or were left uninoculated.

Inoculum was produced by flooding 14-28 day-old sporulating colonies of *B. cinerea* on potato dextrose agar with sterile 0.01% Tween 20, followed by filtration, haemocytometer counts and concentration adjustment to 1.73×10^6 conidia/ml. A single droplet (10 ± 0.09 µl) of this spore suspension containing 17,300 ± 155 conidia was applied to the picking scar of each inoculated fruit. Storage of aliquots of the conidial

suspension at –20ºC enabled the same batch of inoculum to be used for subsequent harvest dates, without any adverse effects on conidial viability.

After inoculation, fruit were packed into single layer cardboard export trays and placed into cold-storage. Packed fruit were stored for 12 weeks at 0ºC, then numbers of fruit with lesions were recorded. Thereafter, phenolics and hydrolytic enzymes were extracted from stem plugs (comprising the picking scar region and the underlying woody pin of tissue) and samples of pericarp-flesh taken from immediately under the stem plug. Measurement of defence mechanisms was restricted to uninoculated, non-infected fruit to avoid the complication of fungal lytic enzymes/phenolics in diseased fruit (Wurms et al. 1997). Extraction and analysis of chitinases, β-1,3-glucanases and phenolics was according to the methods described by Wurms et al. (2003), except that fruit phenolics were separated by normal-phase (NP) thin layer chromatography (TLC) rather than HPLC, since reversed-phase HPLC was ineffective in resolving individual components (data not shown). Fractions (120 µl of 1.5 g lyophilized tissue/ml methanol) of interest were separated on 0.2 mm NP-TLC plates (silica gel 60 F_{254}, EM Science, Gibbstown, NJ) and developed in 35:65 dichloromethane:ethyl acetate. R_f values of compounds of interest were calculated by:

R_f = (distance (mm) between the origin and the base of compound band) / (distance (mm) between the origin and the solvent front)

A bioassay using spores of *Cladosporium cladosporioides* overlaid on the NP-TLC plates was used to assess the fungitoxicity of phenolic fractions (Fawe et al. 1998), with zones of inhibition appearing as clear regions in a grey lawn of germinating fungal spores. *Cladosporium cladosporioides* was used as a substitute for *B. cinerea* because kiwifruit phenolic extracts tested against both fungi produced identical inhibition zones, but those produced by *B. cinerea* were extremely difficult to capture on film owing to its pale pigmentation compared to that of *C. cladosporioides* (Wurms et al. 2003).

The maturity experiment had a nested design with "harvest date" nested within "cultivar type". There were four boxes of 30-33 count fruit per treatment. Rot incidence, lytic enzyme activities, phenolics, and stem plug versus pericarp data were all analysed separately, but the nested design was applied in each case. Analysis of variance (ANOVA), and standard error of the mean (SEM) calculations were performed using SAS software (release 8.2). However, ANOVA statistics apply only to the main treatment effects, since comparisons of the interactions between harvest dates and cultivars on rot incidence, phenolics and lytic enzymes are not possible, owing to the hierarchical nature of the nested design.

RESULTS

Artificially inoculated Hort16A fruits had significantly fewer *B. cinerea* rots than Hayward (P=0.01) (Fig. 1a). There were also fewer naturally occurring rots in non-inoculated Hort16A than in Hayward fruits, but this difference was not significant (P=0.12) (Fig. 1b). Infection decreased markedly with later harvests of both inoculated (P<0.001) and non-inoculated (P=0.02) fruits (Fig. 1).

Glucanase activity was significantly higher (P<0.001) in stem plugs of Hayward than in Hort16A (1.39±0.34 µg glucose/min/g tissue for Hayward versus 0.05±0.02 µg glucose/min/g tissue for Hort16A), but there were no significant differences in glucanase activity in pericarp tissue (P=0.89, data not shown). Fruit harvest date had no significant effect on glucanase activity in either fruit tissue-type (P=0.37 for stem plugs and P=0.60 for pericarp tissue, data not shown).

Chitinase activity was markedly higher in pericarp tissue (P<0.001) of Hort16A than Hayward, but not in the stem plugs (P=0.90) (Figs 2a & 2b). Chitinase activity increased significantly in stem plugs (P<0.001), and in pericarp tissue (P=0.01), at the last harvest (Figs 2a & 2b).

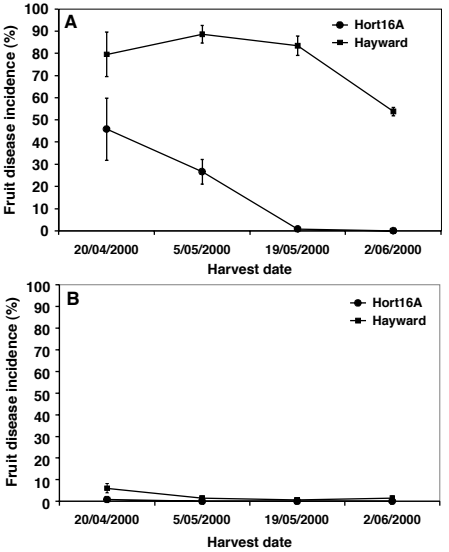

FIGURE 1: ***Botrytis cinerea*** **disease incidence (%) after 12 weeks storage at 0ºC on Hort16A or Hayward kiwifruit harvested at different stages of maturity for (a) inoculated and (b) non-inoculated fruit. Values are the mean of four boxes of 30-33 count fruit, with error bars indicating SEM.**

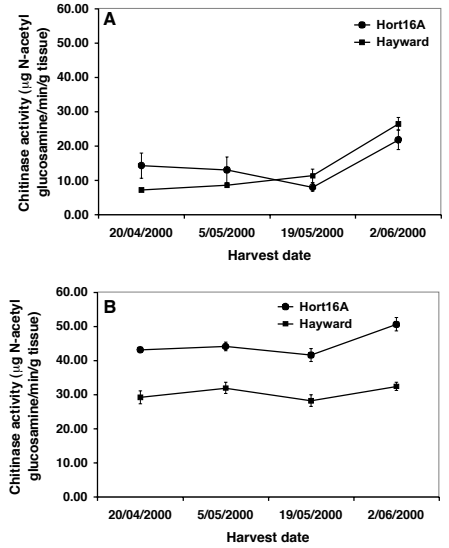

FIGURE 2: Chitinase activity (μg N-acetyl glucosamine produced/min/g fruit tissue) for Hort16A or Hayward kiwifruit harvested at different stages of maturity in (a) stem plugs and (b) pericarp tissue. Values are the mean of four extracts containing tissue pooled from 3-5 fruits, with error bars indicating SEM.

Phenolics were initially extracted from both stem plugs and pericarp tissue, but only pericarp phenolics are presented here because fungitoxicity was much greater in the pericarp than in the stem plug for both cultivars (data not shown).

A number of fungitoxic compounds with R_f values of 0, 0.56, 0.76, 0.78, 0.83 and 0.86 were present in both Hayward and Hort16A (Table 1). Concentrations of the compounds with R_f values of 0.56, 0.76 and 0.78 appeared greater in Hort16A than Hayward, as indicated by significantly larger inhibition zones using the harvest-averaged data. Compound R_f 0.56 accounted for most of the fungitoxicity (Table 1). Expression of these compounds in both cultivars remained consistent irrespective of harvest date, as shown by unchanging inhibition zone diameters (Table 1).

TABLE 1: Diameter (mm) of fungitoxic zones of compounds from fruit pericarp extracts of Hayward and Hort16A kiwifruit of different maturity. Compounds were separated on silica TLC plates overlaid with a *Cladosporium cladosporiodes* spore suspension. The position of compounds on the plate is given by the R_f value. Individual values are the mean ± SE of three plates, and the overall mean for all harvest dates is presented.

	Harvest Date				
Cultivar	20/04/00	5/05/00	19/5/00	20/6/00	Mean
Hayward					
$R_f = 0$	2.0 ± 2.0	5.0 ± 2.5	2.3 ± 2.3	7.7 ± 0.3	4.3 ± 1.1
$R_f = 0.56$	10.7 ± 3.2	12.3 ± 1.2	16.3 ± 2.2	15.0 ± 2.9	13.6 ± 1.3
$R_f = 0.76$	2.0 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 1.2	1.1 ± 0.5
$R_f = 0.78$	3.7 ± 2.0	3.0 ± 1.5	3.0 ± 1.7	4.3 ± 0.7	3.5 ± 0.7
$R_f = 0.83$	5.3 ± 0.9	5.3 ± 2.7	4.0 ± 2.1	2.3 ± 2.3	4.3 ± 1.0
$R_f = 0.86$	4.3 ± 0.9	6.7 ± 0.3	4.0 ± 2.1	6.0 ± 1.0	5.3 ± 0.6
Hort16A					
$R_f = 0$	4.7 ± 2.3	0.0 ± 0.0	3.3 ± 3.3	2.7 ± 2.7	2.9 ± 1.2
$R_f = 0.56$	14.0 ± 4.7	15.0 ± 2.0	22.0 ± 1.2	16.7 ± 1.8	17.1 ± 1.6
$R_f = 0.76$	2.7 ± 1.5	5.0 ± 0.0	5.3 ± 0.3	2.3 ± 1.2	3.7 ± 0.6
$R_f = 0.78$	4.7 ± 2.6	6.0 ± 1.2	6.7 ± 0.9	6.3 ± 0.7	6.0 ± 0.7
$R_f = 0.83$	5.0 ± 2.5	4.0 ± 4.0	5.3 ± 1.5	4.0 ± 2.1	4.6 ± 1.0
$R_f = 0.86$	4.7 ± 2.4	7.5 ± 0.5	5.7 ± 1.8	5.7 ± 1.5	5.7 ± 0.8

DISCUSSION

Constitutively-expressed genotype resistance against *B. cinerea* appears to be greater in Hort16A than Hayward, since disease incidence was higher in Hayward than in Hort16A fruits, regardless of artificial inoculation or not (Fig. 1). This correlated positively with the expression of greater chitinase activity (Fig. 2) and higher concentrations of antifungal phenolic compounds, especially a fungitoxic phenolic compound with a R_f of 0.56 (Table 1) that was greater in Hort16A than Hayward pericarp tissue. *Botrytis cinerea* first enters the fruit through the stem plug, and its growth through this tissue is probably impeded by both physical and, to a lesser extent chemical, factors (Sharrock & Hallett 1992). Once the fungus breaches the stem plug, pericarp defence mechanisms act as the next line of defence, with increased chitinase activity and antifungal phenolics creating a more inhibitory environment in Hort16A pericarp. Since fruits from each cultivar were harvested from separate adjacent plots, differences in rot incidence and putative defence compounds could be attributable to the location rather than the cultivar. However, this appears unlikely given that the same trends in rot incidence and expression of defence compounds have been observed in Hayward and Hort16A fruits harvested from different orchards, where environmental variation is likely to be much greater than that existing between two adjacent plots on the same orchard (Wurms 2003).

Developmentally-related resistance also appears to exist in Hort16A kiwifruit, since there were fewer *B. cinerea* rots with later fruit harvests (Fig. 1). Higher resistance in later-harvested fruit is already well documented for Hayward fruit (Pennycook & Manning 1992; Pyke et al. 1993), and Wurms (1996) observed that an increase in chitinase activity with delayed harvest dates was evident after cold-storage, but not at the time of harvest. However, chitinase and glucanase activities did not appear to be associated with the reduction in rots in late harvests of Hort16A fruit, suggesting that other as yet unidentified mechanisms may be operating in developmentally-related Hort16A resistance.

Harvest date did not have a marked influence on phenolic profiles or their fungitoxicities for either cultivar. One explanation might be that total polyphenol concentrations do not alter considerably during ripening of *Actinidia* fruit (Fuke & Matsuoka 1984). In addition, biochemical changes occurring during fruit ripening usually make the fruit more palatable to animals to favour seed dispersal, hence the accumulation of often bitter-tasting phenolic compounds that can be toxic to animals is unlikely (Derckel et al. 1998).

This study has established that delaying fruit harvest can reduce *B. cinerea* disease incidence in Hort16A and has identified at least two factors (phenolics and chitinases) likely to contribute to the greater constitutive resistance to *B. cinerea* expressed by Hort16A fruits. This information will contribute to the development of an environmentally benign and multi-facetted approach to disease control.

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