THE ROLE OF ADHERING FLORAL TISSUES IN SCLEROTINIA DISEASE OF KIWIFRUIT

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ABSTRACT

Field trials in 10 Bay of Plenty and two Waikato orchards during 1996/98 investigated the role of adhering floral tissues in the development of sclerotinia disease of kiwifruit caused by *Sclerotinia sclerotiorum*. Petal-fall and stamen-fall extended over a period of at least six weeks. *S. sclerotiorum* was isolated from 0–53% of petals during flowering and 0–42% from adhering floral tissues on fruit. Fruit from two orchards had a significantly higher incidence of fruit scarring on fruit with adhering floral tissues compared to fruit without adhering floral tissues. The incidence of diseased fruitlets and fruit scarring was significantly reduced when floral tissues were physically removed after petal-fall. The data suggest that floral tissues are readily colonised by *S. sclerotiorum* ascospores during flowering and that disease mostly arises directly from mycelial infection originating within adhering floral tissues. This disease pathway will be exploited in developing disease management strategies.

Keywords: kiwifruit, *Sclerotinia sclerotiorum*, colonisation, disease development, fruit scarring.

INTRODUCTION

Sclerotinia disease of kiwifruit (*A. deliciosa* (A. Chev.) C. F. Liang et A.R. Ferguson, var. *deliciosa* cv. Hayward) caused by the pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary, can cause up to 5% crop loss in some years (Pennycook 1985). Disease symptoms include blight of flowers on staminate vines and pistillate vines (termed 'diseased fruitlets'), and scarring and soft rot of fruit, but there are few reports on the epidemiology of sclerotinia disease in kiwifruit. Based on field observations, Pennycook (1985) and Manning (1991) suggested floral tissues (petals and stamens) retained on fruit act as a source of fruit infection. A subsequent study showed that *S. sclerotiorum* was present in floral tissues and that disease incidence increased through December and January (Goh and Lyons 1992).

The occurrence of adhering floral tissues (AFT) on kiwifruit fruit has not been quantified, nor has the relationship between AFT and disease development been studied. In several other crops, such as bean (Sutton and Deverall 1983) and canola (Gugel and Morrall 1986), *S. sclerotiorum* infected floral tissues acted as a food base from which subsequent disease developed in adjacent healthy tissues (e.g. pods, leaves and stems). This component of the disease cycle is essential as *S. sclerotiorum* ascospores cannot directly infect healthy host tissues without external nutrients (Abawi and Grogan 1975).

With a reducing number of available fungicides and increasing organic production there is a need for a better understanding of disease development so that improved control strategies can be developed. The hypothesis tested in these experiments was that *S. sclerotiorum* within adhering floral tissues acts as a source of inoculum for infection of the permanent flower parts and developing fruit.

METHODS

Survey of AFT

The occurrence and type of AFT on 40 fruit from 20 vines spread across a block in each of two Katikati orchards (Bay of Plenty) was determined at approximately weekly intervals from 19 November 1996 (petal-fall) to 6 January 1997. Each fruit was scored for the number of petals (0, 1, 2 or 3⁺) and stamens (0–3, 4–10, 11–30 and 30⁺) that remained attached.

Incidence of *S. sclerotiorum* in floral tissues

In the same 12 orchards as above, samples of 10 petals were collected at random during mid-flowering from each of 10 vines. These were surface-sterilised in drum ethanol for 30 sec and 1% sodium hypochlorite (amended with 0.1% acetic acid and 0.1% Tween80) for 2.5 min, then rinsed in sterile distilled water and individually placed on Petri dishes of potato dextrose agar (PDA). During January, AFT from 10 fruit were collected at random from each vine, surface-sterilised and placed individually on Petri dishes of PDA. Fungal colonies growing from tissues were identified to the genus level after five and 12 days incubation at ambient temperatures on the laboratory bench. Fifty diseased fruitlets, 50 AFT from fruit with scarring symptoms and 50 AFT from fruit without scarring symptoms, were sampled from a Waikato orchard on 31 January 1997. One adhering petal from each diseased fruitlet and each of the 100 AFT were surface-sterilised as described above, placed singly on a Petri dish of PDA and then incubated on the laboratory bench. The presence of *S. sclerotiorum* was recorded after five and ten days.

Association of AFT with scarring

Within one block in each of two Waikato orchards, 20 fruit with AFT and 20 fruit without AFT (no petals and less than four stamens) were chosen at random on each of 20 vines on 7 January 1997 and the incidence of fruit scarring was determined. Disease severity was determined by visually estimating the length and width of lesions. Fruit with sclerotinia scarring 100 mm² were classed as not suitable for export and were termed reject fruit.

Removal of AFT

On 7 December 1997, two weeks after mid-flowering, 10 fruit with no AFT, 10 fruit with AFT that were immediately removed by hand and 10 fruit with AFT that were allowed to remain on the fruit, were selected on each of 15 vines at one of the Waikato orchards. Fruit were selected in groups of three (one from each classification) not more than 200 mm apart. There were five groups of three fruit on each side of each vine. The fruit in each category were tagged with different coloured lengths of wool. On 7 January and 9 February 1998 the incidence of diseased fruitlets and fruit scarring was recorded. The severity of scarring symptoms was determined as described above.

All proportions were analysed by generalised linear models with logit link and errors proportional to binomial, using the statistical package Genstat. Least squares analysis was used to compare the area of scarring on fruit.

RESULTS

Survey of AFT

Petal-fall was marginally more advanced at one orchard than at the other on 19 November, although the overall timing of stamen- and petal-fall was very similar. The proportion of fruit with two or more adhering petals decreased significantly ($P < 0.001$) in both orchards between 23 November (22%) and 6 January (9%). The proportion of fruit with one adhering petal ranged from 20–25% for both orchards. From 30 November, most fruit with adhering petals (60–94%) also had adhering stamens that were grouped together and clustered in close association with the petals.

Thirty nine to 60% of fruit had adhering stamens, yet no adhering petals. Of these, 11%, 38% and 51% had 4–10, 11–30 and 30⁺ stamens, respectively, soon after fruit set. This changed considerably and by 6 January 79%, 13% and 8%, had 4–10, 11–30 and 30⁺ stamens, respectively. The proportion of fruit with no AFT increased steadily.

Incidence of *S. sclerotiorum* in floral tissues

Isolations from surface-sterilised flower petals and AFT yielded fungi from at least 13 and 15 genera, respectively. *S. sclerotiorum* was recovered from 0–53%

(mean 19%, se = 6) of petals and 0–43% (mean 11%, se = 5) of AFT, yet of these only 2% of petals and 3% of AFT yielded pure cultures of *S. sclerotiorum*. *Cladosporium* spp. were the dominant fungi on petals, whereas *Epicoccum purpureascens* (syn. *E. nigrum*) were most common on AFT. A further 3–38% of fungal isolates from both tissues types were classed as unknown. Less than 1% of all isolations produced no growth on PDA.

Forty seven of the 50 adhering petals from diseased fruitlets yielded pure cultures of *S. sclerotiorum* on PDA. Two petals yielded *S. sclerotiorum* plus other fungi, while no growth was recovered from the remaining petal. Twenty one of the 50 AFT collected from fruit with scarring symptoms yielded pure *S. sclerotiorum* cultures and a further 14 AFT yielded mixed cultures of *S. sclerotiorum* and other fungi. In contrast, only one of the 50 AFT collected from fruit without scarring symptoms yielded *S. sclerotiorum*, and all yielded mixed cultures of a range of other fungi.

Association of AFT with scarring

The incidence of fruit with scarring symptoms and the percent reject fruit was significantly ($P < 0.001$) greater on fruit with AFT than on fruit without AFT (Table 1). The incidence and mean area of scarring was significantly ($P < 0.01$) greater on fruit from orchard A compared with those from orchard B, for fruit with and without AFT respectively. The mean area of scarring was similar ($P > 0.05$) on fruit with and without AFT within an orchard (Table 1).

TABLE 1: Mean percent incidence of scarred and reject fruit and mean area of scarring, on fruit with and without adhering floral tissues (AFT) from two Waikato orchards on 7 January 1997.

Orchard	Fruit sample	Mean % scarred fruit	Mean area of scarring on scarred fruit (mm ²)	Mean % reject fruit ¹
A	With AFT	42.3 (2.5) ²	122 (8)	23.3 (2.2)
A	No AFT	7.5 (1.4)	109 (19)	3.0 (0.8)
B	With AFT	27.8 (2.3)	83 (10)	9.3 (1.5)
B	No AFT	2.8 (0.7)	62 (31)	0.5 (0.4)

¹Reject fruit are those with scar area ≥ 100 mm².

²Figures in parentheses are standard error (n = 400).

TABLE 2: Mean percent incidence of diseased fruitlets and scarred fruit and mean area of scarring, on fruit with no natural adhering floral tissues (AFT), fruit with removed AFT and fruit with retained AFT in a Waikato orchard during 1997/98.

Treatment	Mean % diseased fruitlets		Mean % scarred fruit		Mean area of scarring on scarred fruit (mm ²)	
	7 January		7 January	9 February	7 January	9 February
No natural AFT	0.7 (0.6) ¹	0 (0)	0 (0)	-	-	-
Removal of AFT	5.3 (1.8)	2.6 (1.3)	2.0 (1.1)	27 (11)	93 (34)	
Retained AFT	15.9 (2.6)	17.9 (2.7)	19.2 (2.8)	21 (5)	40 (13)	

¹Figures in parentheses are standard errors (n = 150).

Removal of AFT

During the removal of AFT, small (<20 mm²) soft rot lesions were observed on several fruit at the point of contact between AFT and the ovary and/or sepals. The incidence of diseased fruitlets and scarred fruit was significantly reduced ($P < 0.001$) on fruit that had AFT removed compared to those with retained AFT (Table 2). There

were no scarred fruit, and only one diseased fruitlet (0.7%) from fruit which initially had no natural AFT. The mean area of scarring on diseased fruit was not significantly different ($P>0.05$) between fruit with removed and retained AFT, on 7 January and 9 February. Although there was an increase in the mean area of scarring on fruit between assessment dates, this was also non-significant ($P>0.05$).

DISCUSSION

Petals and stamens are integral components of floral biology, but serve no purpose for the plant after pollination and fertilisation. A significant proportion of Hayward kiwifruit fruit retained petals and stamens for at least 6 weeks after anthesis and the level of this appeared to be determined within two weeks of petal-fall. *S. sclerotiorum* was one of many fungi isolated from petals during flowering and from AFT on fruit, suggesting these tissues are readily colonised. Because *S. sclerotiorum* was the dominant species isolated from petals on diseased fruitlets and from AFT from fruit with scarring symptoms, there appears to be a close association between the development of disease and the presence of floral tissues colonised by *S. sclerotiorum*. Further, the higher incidence of sclerotinia scarring on fruit with AFT and the significantly reduced incidence of diseased fruitlets and scarring on fruit when AFT were removed, strongly suggests it is fruit with AFT that are at risk of developing disease. Related research showed that on average 90% of 519 fruit in these orchards that had sclerotinia scarring also had AFT within 5 mm of the lesion (Hoyte 2000).

Ascospores are the only possible source of primary infection of floral tissues (Abawi and Grogan 1975). It is suggested from this research that: (i) *S. sclerotiorum* ascospores colonise kiwifruit petals and stamens during flowering and petal-fall, (ii) diseased fruitlets develop from secondary spread of *S. sclerotiorum* mycelium in petals and stamens immediately after flowering and (iii) sclerotinia scarring on fruit largely results from secondary disease spread originating from AFT. Therefore, disease risk is related to the colonisation of floral tissues by *S. sclerotiorum*, reinforcing the need for fungicide applications to be timed during flowering. Whether these floral tissues are only colonised during flowering or colonisation also takes place later in the season is not certain.

The presence of AFT on fruit may be an inherent trait of Hayward kiwifruit or related to the colonisation of these tissues by fungi. Rainfall during flowering has been observed to increase the retention of petals (H. Pak, pers. comm.). If there is a genetic basis for the failure of petals and stamens of kiwifruit flowers to cleanly abort at petal-fall, then new and existing cultivars that have less AFT may be less prone to sclerotinia disease. Fruit of the cultivar Hort16A (Zespri Gold™) have been observed to have a very low incidence of AFT (H. Pak, pers. comm.). Phenotypic variation in the incidence of AFT needs to be quantified within *Actinidia* spp and this feature may able to be utilised in plant breeding programmes.

Cultural control options, such as use of air-blast sprayers to dislodge AFT, need to be validated under experimental conditions. Biological control of sclerotinia (Elmer *et al.* 1999) can also be targeted at this pathway of disease development. Further research on the effects of fungicide timing on colonisation of kiwifruit floral tissues and disease incidence is also recommended. There is potential to develop an integrated disease management system for sclerotinia to reduce fungicide use and crop losses.

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