Control of flyspeck (*Schizothyrium pomi*) on *Actinidia arguta* kiwifruit

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Abstract Flyspeck, caused by *Schizothyrium pomi* (anamorph *Zygophiala jamaciensis*), is a serious cosmetic skin disorder of *Actinidia arguta* kiwifruit in New Zealand. In a study of the disease in 2009-10, the bark of all 1-year-old canes was carrying large numbers of characteristic dark fruiting bodies (thyriothecia) (flyspecks). Germinating spores and hyphae of *S. pomi* were first observed on current season's canes in December 2009. Flyspecks were first found on fruit in mid-January 2010 and their incidence and severity increased until harvest in February 2010. Sprays of trifloxystrobin or carbendazim applied pre-blossom and during flowering reduced the incidence of fruit with flyspeck at harvest by an average of 70% compared with the untreated control. A single application at either of those times was similarly effective with one exception; trifloxystrobin applied as a pre-blossom spray failed to control flyspeck. Carbendazim residues (0.02 mg/kg) were recorded in the fruit at harvest following an application during flowering.

Keywords *Actinidia arguta* kiwifruit, *Schizothyrium pomi*, flyspeck, control.

INTRODUCTION

Actinidia arguta (Siebold & Zucc.) Planch. ex Miq. (NZ Kiwiberry) is susceptible to infection by two serious fungal-induced cosmetic disorders: flyspeck, caused by *Schizothyrium pomi* (Mont. & Fr.) Von Arx (anamorph *Zygophiala jamaciensis* E.W.Mason), and sooty blotch, caused by *Peltaster fructicola* Peltaster Johnson, Sutton & Hodges and *Geastrumia polystigmatis* Batista & Farr. Fruit with minor flyspeck or sooty blotch blemishes on the skin may be sold on the domestic market but it does not meet export grade. Fruit with more severe blemishes is unacceptable for sale. Both diseases are increasing in incidence in *A. arguta* orchards in New Zealand and have a significant impact on

the export volumes. Crop losses of 30-60% were experienced in some orchards in 2009 (G. Oliver, Kiwi Produce Ltd, personal communication).

The principal disease in 2009 was flyspeck, characterised by patches of discrete dark specks on the surface of the fruit. Each speck is a thyriothecium, the fruiting body of the fungus in which asci and ascospores are formed. These can be found firmly attached to the cane, bark or the cuticle of the fruit. No control measures have been used against this disease in *A. arguta* in the past. An effective management programme for this disease is necessary to ensure ongoing viability of the industry.

Although there is considerable knowledge of sooty blotch and flyspeck on other crops worldwide, particularly on apples (Williamson & Sutton 2000; Gleason et al. 2011), little is known of the epidemiology or control of flyspeck in *A. arguta* orchards and no control fungicides are listed in the *A. arguta* crop protection programme. The aims of this study were to establish whether the vines were a source of inoculum in the orchard, to investigate when infection of the vines and fruit occurred, and to evaluate the efficacy of strategic fungicide applications that could be included in a control programme. Only those fungicides known to be effective against flyspeck on other crops (Williamson & Sutton 2000; Babadoost et al. 2004; Rosenberger et al. 2009) and with an existing label claim for *Actinidia* crops in New Zealand were included in the trial. In addition, fruit was analysed for fungicide residues at harvest to ensure that the treatment programmes would not compromise the NZ Kiwiberry industry goal of residue-free fruit production.

METHODS

The study was carried out in a block of mature *A. arguta* 'Marju Red' pergola-trained vines on a commercial orchard at Pongakawa, Bay of Plenty. The block had never received any fungicides and had sustained heavy crop losses (>40%) from flyspeck in the 2009 harvest.

Inoculum, spore release and germination

Samples of 1-year-old canes and associated new season's vegetative shoots and young fruit were picked from the unsprayed control vines on 12 October, 9 November and 9 December 2009. Using stereoscopic and transmission light microscopy (200× magnification), the parent canes were examined for the presence of thyriothecia and, following dissection, *S. pomi* spores within the thyriothecia. To gain an understanding of when infection of new growth was occurring, the surfaces of the new season canes and fruit were similarly examined for germinated spores and hyphae of the fungus.

Fungicide efficacy

The treatments applied are detailed in Table 1. The fungicides used were carbendazim (as Protek® 500 g carbendazim/litre, suspension concentrate, applied at 325 g ai/ha) and trifloxystrobin (as Flint® 500 g trifloxystrobin/kg, water dispersible granule, applied at 97.5 g ai/ha). The 'pre-blossom' application was made on 2 October 2009 when the new shoots were approximately 10 to 15 cm long and the 'flowering' application on 30 October 2009 when 50-60% of the flowers were open. The treatments were applied to single vines with four replicate vines per treatment in a randomised block design. Sprays were applied to runoff (ca 4 litres per vine, 1300 litres/ha) using a four-nozzle handgun from a Comet pump, nozzle size D4 at an operating pressure of 1379 kPa. All spraying was carried out under good drying conditions in line with standard commercial orchard practice. No other fungicides or insecticides were applied to the trial vines during the growing season.

On 14 December 2009 and 14 January 2010, 20 fruit clusters on each of the untreated control vines were examined *in situ* for symptoms of sooty blotch or flyspeck using an Opti-VISOR® (3× magnification). These *in situ* assessments were used to identify the approximate time of year when flyspecks were first visible on the fruit.

On 26 January 2010, 10 fruit clusters per plot were picked without conscious bias and examined in the laboratory for flyspeck. Flyspeck severity was rated as either 'light' (flyspecks covering an area <0.5 cm² on the fruit surface) or 'severe' (flyspecks covering an area >0.5 cm² on the fruit surface). Fruit with 'light' flyspeck would be acceptable for sale, but only on the domestic market; fruit with 'severe' flyspeck would not be acceptable for sale. A further 10 fruit clusters per plot were picked without conscious bias on 10 February 2010, approximately 1 week prior to the commercial harvest, and similarly graded using the 'light' and 'severe' categories.

Residues

On 10 February 2010, a second fruit sample was picked from each of the plots treated with both trifloxystrobin and carbendazim as well as from

the untreated control plots. Samples were double bagged and frozen within 20 min of harvest before being despatched, still frozen, to Hill Laboratories Limited in Hamilton, for analysis for chemical residues.

Analysis

The effect of spray treatment on the percentage of fruit with flyspeck and the percentage of fruit with severe flyspeck was compared using a one-way Analysis of Variance (ANOVA). Percentage data of fruit with flyspeck were angular transformed before analysis. If the ANOVA revealed a statistically significant treatment effect, Tukey's HSD adjustment was used in the multiple comparison to identify which means were significantly different from one another. The analysis was performed using the General Linear Model Procedure (PROC GLM) in SAS 9.2. Raw data means are presented in the results.

RESULTS

Sooty blotch was not detected on any fruit at the trial site in 2009-10, and so the following results all relate to flyspeck infection.

Inoculum, spore release and spore germination

The bark of all 1-year-old canes, from which the current season's growth and fruit were developing, carried large numbers of thyrothecia (up to 30 per cm²) from the previous season. Dissection of thyriotheceia in October 2009 revealed immature and mature asci and typical two-celled ascospores of *S. pomi*. Germinating spores and hyphae of *S. pomi* were first observed on the surface of new season's vegetative shoots and young fruit in November 2009.

Fungicide efficacy

No flyspeck symptoms were found on the fruit during the *in situ* check on 14 December 2009, but 10 of the 1033 fruit inspected on the untreated control vines had flyspeck on 14 January 2010. All flyspeck found was in the 'light' category; no fruit had 'severe' flyspeck.

The laboratory assessments on 26 January 2010 revealed that 31% of the fruit from the

untreated control vines had flyspeck symptoms (light and severe categories combined), and of those, 6% had flyspeck in the 'severe' category (Table 1). Vines that had received a pre-blossom application of trifloxystrobin also had a high proportion of fruit with flyspeck (11.2%) relative to the other spray treatments, but only one fruit showed severe flyspeck symptoms. All other fungicide treatments resulted in significantly fewer fruit with flyspeck symptoms (0 to 3.6%) than on the untreated control vines ($P=0.001$).

There was a marked increase in fruit with flyspeck symptoms over the 2-week period between the January and February assessments (Table 1). At harvest on 10 February 2010, 99% of the fruit from the untreated control vines had flyspeck, with 89% showing 'severe' symptoms. Trifloxystrobin applied only at pre-blossom resulted in a similar proportion of symptomatic fruit, nearly two-thirds of which were classed as 'severe'. All other fungicide treatments significantly reduced the proportion of fruit with flyspeck relative to the untreated control ($P<0.0001$), with the greatest reduction of 75% being achieved when trifloxystrobin was applied pre-blossom, followed by carbendazim at flowering.

Residues

No residues of trifloxystrobin or carbendazim were detected in fruit harvested on 10 February 2010 following a single application of carbendazim pre-blossom followed by a single application of trifloxystrobin during flowering. When the order of the sprays was reversed, that is, trifloxystrobin applied pre-blossom and carbendazim applied during flowering, no trifloxystrobin residues were detected, but carbendazim was detected at 0.02 ± 0.008 mg/kg.

DISCUSSION

Flyspeck has a very wide host range that includes trees and shrubs surrounding orchards, all of which can act as reservoirs of infection (Williamson & Sutton 2000). Observations made during this trial have shown that in *A. arguta* kiwifruit orchards, a principal source of inoculum

Table 1 Mean percentage of *Actinidia arguta* ‘Marju Red’ kiwifruit showing flyspeck symptoms (all symptoms or severe symptoms only) on 26 January and 10 February 2010 following the application of chemical treatments applied as sprays pre-blossom (PB) and/or during flowering (FL) in October 2009.

Treatment	26 January 2010		10 February 2010	
	All flyspeck	Severe flyspeck	All flyspeck	Severe flyspeck
Untreated control	30.9 a ¹	6.1 a	98.6 a ¹	88.6 a
Trifloxystrobin PB	11.2 ab	0.3 b	80.5 ab	50.4 ab
Trifloxystrobin at FL	1.3 b	0 b	38.5 c	16.8 bc
Carbendazim PB	3.6 b	0.4 b	50.2 bc	19.1 bc
Carbendazim at FL	0.9 b	0 b	46.5 bc	13.4 bc
Trifloxystrobin PB, Carbendazim FL	0 b	0 b	24.7 c	7.0 c
Carbendazim PB, Trifloxystrobin FL	1.6 b	0.4 b	47.2 bc	21.6 bc
P-value	0.001	0.001	<0.0001	<0.0001

¹Mean values within a column followed by the same letter are not significantly different.

for flyspeck appears to be the vines themselves. The causal pathogen of flyspeck overwinters on the previous season's canes as the visible flyspecks that are the future fruiting bodies (thyriothecia) of the fungus. Maturation of *S. pomi* spores within the thyriothecia was observed in early spring, with both immature and mature spores being present and released by October. The experimental protocol did not include multiple observations over the season, therefore the full period over which the thyriothecia mature and release spores could not be defined. However, because thyriothecia containing asci of different stages of maturity were observed, it is likely that spores would mature and be released over a period of weeks in the early part of the season, as is the case with this organism in apple orchards (Williamson & Sutton 2000). In the orchard used for this study, all overwintering canes were carrying extremely high numbers of thyriothecia. This would subsequently translate to large numbers of ascospores being released within centimetres of new season's vegetative shoots and the young fruit.

Ascospores germinate on any new growth (leaf, stem, petiole or fruit) under wet conditions and hyphae ramify across the plant surface (Williamson & Sutton 2000). The first signs of spore germination and hyphal growth on new season's stems and fruit of *A. arguta* were observed

in November. However, no flyspeck symptoms were observed on the fruit until January, with a significant increase in numbers of flyspecks on the fruit over the following 2 weeks. This suggests that, although the fungus may be present on the fruit and plant surfaces of *A. arguta* from early in the season, it lives on the surface as a network of hyphae invisible to the naked eye until January, at which time it starts to form visible thyriothecia. Observations in the present study have shown that the pattern of development of the disease on *A. arguta* is similar to that previously described on apples (Grabowski & Wrona 2004; Mayr et al. 2010) where it was concluded that epiphytic colonisation of the fruit surface by the pathogen can occur throughout the fruit growing period, but the time to the appearance of visible thyriothecia can range from a few weeks to several months depending on the environmental conditions, in particular, humidity and rainfall.

The fungicides used in this trial have previously been shown to control sooty blotch and flyspeck in apple orchards when applied as non-consecutive sprays at intervals throughout the growing season (Williamson & Sutton 2000; Babadoost et al. 2004; Rosenberger et al. 2009). The two test fungicides in this trial, carbendazim and trifloxystrobin, were both effective in reducing *S. pomi* infection of the fruit

when applied before fruit set, especially when trifloxystrobin was applied pre-blossom and carbendazim at flowering. A single application of carbendazim pre-blossom or at flowering was similarly effective, as was trifloxystrobin applied at flowering. However, residue analyses showed that the application of carbendazim during flowering resulted in carbendazim residues in the fruit at harvest. In order to meet the NZ Kiwiberry industry goal of zero residues, carbendazim should not be applied beyond the pre-blossom period, with flowering applications restricted to trifloxystrobin. It is possible that a postharvest application of carbendazim may be equally as effective as a flowering application and this alternative spray timing should be explored in future studies.

The high inoculum load in the experimental *A. arguta* block combined with the fact that no fungicides had previously been applied, meant that achieving a high degree of control within the first season would be difficult with even the most effective fungicides. Nevertheless, the results from this study have shown that one or two fungicide applications in the spring can reduce the numbers of *S. pomi*-infected fruit by approximately 50% and, more importantly, the numbers of 'severely' affected fruit by approximately 75%. While not measured in this trial, it could be expected that a reduction in thyriothecia numbers on the fruit would be matched by a corresponding reduction of thyriothecia on the overwintering canes. The spore numbers produced in the vicinity of new growth in spring 2010 could be expected to be significantly lower than in 2009, with a corresponding increase in the apparent efficacy

of the fungicide programme. Thus, the inoculum production capacity within the canopy should be progressively reduced with successive seasons of fungicide use, with a parallel reduction in crop loss from flyspeck.

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