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Controlling plant and fruit diseases in strawberry fields

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Summary

Grey mould and stem-end rot affect the strawberry industry in Australia. Losses of 10% of the crop are equivalent to a loss of $46 million across the different growing areas. The effect of different fungicides on the control of grey mould and stem-end rot affecting 'Festival' strawberry plants was studied in south-east Queensland over three years. This work followed recent changes to the use pattern for the multi-site fungicides thiram and captan, which can now be used more regularly than previously in Australia. There was a restriction on the use of captan with no more than five sprays allowed in a season. Thiram could be used only up to the first flowering. A program based on captan and thiram along with several single-site fungicides during wet weather controlled grey mould and stem-end rot. These treatments in turn provided high marketable yields.

Applications of a plant-defence promoter, plant extracts, organic acids, fatty acids, a salt, four bacteria, and a fungus were not as successful as the standard program, even when the soft fungicides were applied alternately with thiram and captan. The plant-defence promoter acibenzolar-S-methyl, organic acids, *B. subtilus* (strain *QST 713*), *B. amyloliquefaciens* and potassium bicarbonate gave intermediate control. In contrast, *B. subtilus* (strain *MBI 600*), *Streptomyces lydicus*, *Trichoderma harzianum*, potassium salts of fatty acids, and two plant extracts were ineffective. A strategy based on thiram and captan applied alternately, with the addition of single-site fungicides during wet weather provided the best control of disease, with 3 to 4% losses. Control plots had losses of 25 to 38%.

The soft chemicals were less effective than the programs based on captan, thiram and single-site fungicides. The use of the thiram and captan in rotation with other fungicides from different chemical groups in wet weather will reduce the risk of fungicide resistance and extend the useful life of registered products. This strategy has not been available to growers previously.

Keywords

Grey mould; stem-end rot; strawberry; *Botrytis cinerea*; *Gnomoniopsis fructicola*; yield; *Fragaria ×ananassa;* fungicides.

Introduction

Australia produces about 75,000 tonnes of strawberries (*Fragaria* × *ananassa*) worth $460 million each year, with cultivation distributed across all the states, except the Northern Territory. About 70% of the production is split between the two industries in Queensland and Victoria. The bulk of the remaining crop is produced in Western Australia (17%) and South Australia (9%) with small plantings in New South Wales and Tasmania. Strawberry production in southeast Queensland mainly occurs during winter in a subtropical climate, with the berries harvested from May to October. Production is affected by fruit diseases which are promoted and dispersed by direct rain contact or by high humidity (Menzel et al. 2014). These diseases are mainly grey mould (*Botrytis cinerea*) and stem-end rot (caused by *Gnomoniopsis fructicola*), with lower incidences of powdery mildew (*Podosphaera aphanis*) and black spot (caused by *Colletotrichum acutatum*). Up to 80% of the crop can be lost during periods of wet weather.

Grey mould is one of the most important diseases affecting strawberry crops and is significant in virtually all commercial growing areas around the world. The petals, stamens and pistils of the flowers are infected and then the fruit, with the infections not developing until the weather becomes favourable for the disease (Jarvis 1962; Brun and Sutton 1987; Williamson et al. 2007). The primary source of infection in the flowers is from old senescent leaves. Fungal mycelia infecting the flowers may subsequently invade the proximal end of the fruit. Secondary infections can develop on the crop when sound fruit contact decaying fruit or other infected tissues, or from conidia (spores) spread by wind, rain or machinery. The disease is promoted by temperatures of about 20^{o}C and by extended periods of rain, high humidity and leaf wetness. There are differences in the level of infection in different cultivars, although virtually all cultivars are susceptible to the pathogen.

Stem-end rot is not as important as grey mould in strawberry production, with periodic outbreaks reported in Europe and North America (Maas 1998; Santos et al. 2003; Wedge et al. 2007). In southeast Queensland, the disease often occurs after wet, windy weather. The causal fungus is considered a weak pathogen on strawberry, although it can cause considerable losses at times. Infection by the fungus often predisposes the fruit to infection by the grey mould fungus. Symptoms on the calyx of the fruit range from small lesions on the sepals to completely infected tissues that turn necrotic and brittle. Infected fruit ripen prematurely and turn pale red to brown (Gubler and Feliciano 1999). Initially, the disease is semi-systemic with few or no visible symptoms.

The two main diseases affecting strawberry crops in southeast Queensland are controlled by the application of fungicides. These must be sprayed for up to 20 weeks because of the extended production cycle in this environment and the overall wet conditions. This strategy is different from that used in other growing areas with drier conditions where a few sprays during flowering are effective (Faby 2014). There are problems with some of the products used by the industry in Australia. Some of the chemicals are ineffective under wet weather. Other chemicals have limits to the number of applications allowed in a season or may become ineffective in the long-term because of the development of resistance in the fungi (Washington et al. 1999a).

There are two main classes of fungicides used to control fungal diseases, based on their modes of action against the pathogen. The first group includes multi-site chemicals that affect several aspects of fungal growth. The second group includes single-site chemicals that affect a single aspect of fungal growth. Usually single-site fungicides are active against only one point in one metabolic pathway in a pathogen or against a single critical enzyme or protein needed by the fungus. Fungi are more likely to become resistant to these fungicides because a single mutation in the pathogen usually allows it to overcome the action of the fungicide, such as preventing it from binding to the active site in the fungus. Most of the new fungicides released in the past few decades are single-site chemicals. As a result, resistance to fungicides has become more of a concern in disease management.

In addition to the multi-site fungicides thiram and captan, single-site fungicides such as iprodione, fenhexamid, cyprodinil + fludioxonil, and penthiopyrad are used to control grey mould and stem-end rot during wet weather. These fungicides have many advantages over the multi-site fungicides, including their generally greater efficacy and lower toxicity (Grabke et al. 2014). However, as noted above, they can become ineffective as resistance develops in the fungus. Isolates of *B. cinerea* have become resistant to many single-site chemicals in strawberry fields in the United States, Germany and elsewhere (Weber 2011; Wedge et al. 2013; Fernández-Ortuño et al. 2014). There have been few studies on the resistance of the fungus in Australia. Washington et al. (1999a) found that iprodione was ineffective in two of three sites in Victoria. These results suggest that some populations of *B. cinerea* in southern Australia were resistant to the chemical more than 15 years ago.

Biological agents have been suggested for the control of grey mould (Elad and Stewart 2004). These include bacteria such as *Bacillus subtilis* and fungi such as *Trichoderma harzianum*. Plant-defence promoters such as acibenzolar S-methyl, various plant extracts, fatty acids and salts have also been used (Terry and Joyce 2000; Mohammadi et al. 2014; Calvo-Garrido et al. 2014; Zaker 2014). They have had a mixed effect on the control of grey mould in strawberry and other plants, and are slightly more effective when used in combination with standard fungicides. The use of this strategy can reduce the number of applications of single-site fungicides required in a season and reduce the chance of fungi developing resistance to the chemicals. Washington et al. (1999a) investigated the effect of tea tree oil and *Trichoderma* sp. on the control of grey mould and other diseases affecting strawberry plants in Victoria. The plant extract reduced the incidence of grey mould compared with that in the controls in one of three experiments. The fungus reduced the incidence of the disease in two of three experiments. However, neither of these products increased yield compared with that of the controls.

We report on the effect of fungicides on the control of grey mould and stem-end rot in strawberry plants growing in southeast Queensland. A range of standard fungicides with different modes of action, including multi- and single-site activities were assessed. We also assessed a plant-defence promoter, plant extracts, organic acids, fatty acids, a salt, four bacteria, and a fungus. Some of the treatments included weekly applications of captan and/or thiram. This work followed recent changes to the use pattern for these two fungicides in strawberry fields in Australia. The label for captan now allows for more than five applications per season. The label for thiram now allows a withholding period of two rather than seven days. The previous label for captan had a maximum of five applications in a season. The previous label for thiram had applications up to the first flowering. The current experiments were conducted over three years, with information collected on the incidence of disease in the plants and on yield.

Methodology

Three experiments were conducted in Nambour in southern Queensland (lat. 26.6^{o}S, long. 152.9^{o}E, elevation 29 m) to evaluate the effect of standard and soft or alternative chemicals on the control of grey mould and stem-end rot affecting strawberry plants.

Bare-rooted transplants of 'Festival' were planted in early April in 2012, 2013 and 2014. The transplants were grown at Stanthorpe in southern Queensland (lat. 28.6^{o}S, long. 152.0^{o}E, elevation 872 m) and had three to four leaves on each plant (Menzel and Smith 2012). The strawberry plants were grown using standard agronomic practices for this area (Menzel et al. 2014). The new plants were planted through plastic, in double-row beds 70 cm wide and 130 cm apart from the centers. The plants were spaced at

30-cm intervals within the rows, giving a density of 51,000 plants per ha. The plants were irrigated through drip-tape placed under the plastic.

There were 11 treatments in 2012, 15 in 2013, and 13 in 2014, including a nil control (Tables 1 to 3). In 2012, the first applications of the chemicals were made in late May, with the first fruit harvested in early July and the last fruit harvested in late September. In 2013, the first applications were made in late May, the first fruit harvested in mid-June and the last fruit harvested in mid-September. In 2014, the first applications were made in late May, the first fruit harvested in mid-June and the last fruit harvested in late September. Details of the different fungicide products and their sources are shown in Table 4. The treatments were applied with a Solo 15-L diaphragm-pump, knapsack sprayer with a hollow cone nozzle at 60 psi. We applied the fungicide products at 1,000 L per ha to the point of run-off.

Fruit were harvested every week for an assessment of yield (fresh weight), number of fruit per plant and defects. Mature fruit were classified as those that were at least three-quartered coloured. A record was kept of the number of fruit that were affected by rain or disease or both. Fruit that were affected by rain and disease were rated as diseased and were non-marketable. The main diseases affecting the crops were grey mould and stem-end rot, with a very low incidence of powdery mildew and black spot. The fruit were classified into only one category. The few fruit that had both grey mould and stem-end rot were rated as affected by grey mould since it was the primary disease.

The experiments were arranged in randomized blocks, with four replicate plots per treatment, and guard plots of six plants between adjacent experimental units in each row. Fruit were harvested each week from the same 20 plants in each plot. Data on yield and the incidence of grey mould and stem-end rot over the growing season were analyzed by one-way analysis of variance (ANOVA; 11 to 15 treatments × four blocks), using GenStat (version 16; VSN International, Hemel Hempstead, UK). The data on yield were transformed to $\log_{10}$ prior to ANOVA, while the data on the percentage of fruit with grey mould and stem-end rot were transformed to arcsine square root. Treatment means were back-transformed for presentation. Separate analyses were conducted for each year. Treatment means were separated by calculating least significant differences (LSDs) from the ANOVAs. The relationship between marketable yield and the incidence of disease was assessed by regression analysis using the graphics software SigmaPlot (version 12; Systat, Chicago). The regressions were fitted using the Marquart-Levenberg algorithm in SigmaPlot. The data for each year were analysed separately.

Data were collected on rainfall at the site during the experiments. In 2012, the plants were watered with over-head irrigation from 14 to 27 August to promote the development of disease during dry weather. During this time, the amount of water applied to the crop by the sprinklers was 276 mm or about 19 mm per day. In 2013, the plants were watered from 23 August to 5 September to provide another 112 mm or about 8.0 mm per day to the canopy. In 2014, the plants were watered from 11 July to 9 September to provide an additional 392 mm or about 6.4 mm per day.

Results

Marketable yields were higher in 2012 and 2014 than in 2013 (Tables 1 to 3). The main disease affecting the crop in 2012 was grey mould, with virtually no stem-end rot. In contrast, stem-end rot was important in 2013 and 2014. The level of infection in the controls due to stem-end rot was 11.3 ± 0.4% in 2013 and 5.6 ± 1.4% in 2014 (untransformed means and standard errors). The level of infection in the controls due to grey mould was 38.0 ± 0.5% in 2012, 18.8 ± 1.6% in 2013, and 19.2 ± 1.8% in 2014.

Experiment in 2012

Total rainfall over the harvest period from early July to late September was 182 mm, with some weeks completely dry and other weeks receiving more than 50 mm. Total rainfall was similar to the long-term average for Nambour of 192 mm from July to September. The over-head irrigation from mid-August to late August provided about another 300 mm of water to the canopy. The incidence of the disease tended to increase after heavy rain or over-head irrigation (data not shown).

The standard programs based on captan/thiram, applied with iprodione and fenhexamid, or cyprodinil + fludioxonil gave good control of disease (Table 1). The level of control was as good using isopyrazam alone. Azoxystrobin + difenoconazole and the plant-defence promoter acibenzolar S-methyl gave intermediate control. Potassium salts of fatty acids, and *Trichoderma harzianum* were ineffective, whereas organic acids (ascorbic + lactic + citric), potassium bicarbonate + oil (Synertrol Horti-oil), and potassium bicarbonate + oil + potassium salts of fatty acids were intermediate. None of these alternative fungicides were as effective as the more effective standard fungicides (Table 1).

Yield was related to the incidence of grey mould (Fig. 1), with the exception of the treatments that that were phytotoxic. These were the two treatments that included potassium bicarbonate + oil and gave lower yields than the nontreated plants despite some measure of disease control, reflecting damage to the canopy. It is possible that oil applied with the salt damaged the plants. Yield was highest with the standard programs based on captan/thiram, with iprodione and fenhexamid, or with cyprodinil + fludioxonil, and with isopyrazam applied alone (Table 1).

Experiment in 2013

Total rainfall over the harvest period from mid-June to mid-September was 153 mm. There was more than 10 mm per week in five of the fourteen weeks of fruit production. The over-head irrigation from late August to early September provided about another 100 mm of water. Total rainfall was lower than the long-term average for Nambour of 280 mm from June to September.

This season was different from the earlier one, with severe stem-end rot in 2013 and virtually none in 2012. As in the previous year, the incidence of disease tended to increase after heavy rain. The standard programs, along with isopyrazam, penthiopyrad or fluazinam alone were effective (Table 2). Dithianon, along with most of the soft chemicals alternated with captan/thiram were intermediate, and the soft chemicals alone (including *Trichoderma harzianum* and *Bacillus subtilis* strain *MBI 600*) were ineffective. The exception to these responses was the plant extract applied alternately with captan/thiram which was as effective as isopyrazam.

Yield was related to the incidence of disease (Fig. 1). Yield was high with the standard programs based on captan/thiram, with iprodione and fenhexamid, or with cyprodinil + fludioxonil, and with isopyrazam, penthiopyrad or fluazinam applied alone (Table 2).

Experiment in 2014

Total rainfall over the harvest period from mid-June to late September was 195 mm, lower than the long-term average for Nambour of 280 mm from June to September. The over-head irrigation from early July provided about another 400 mm of water.

Captan or thiram applied weekly or alternately, or used with single-site fungicides (iprodione, fenhexamid, cyprodinil + fludioxonil, and penthiopyrad) in wet weather gave good control of disease (Table 3). The program based on *Bacillus subtilis* (strain *QST 713*) mixed with captan, thiram, and multi-site fungicides, or chlorothalonil or guazatine alone were equally or nearly equally as effective as the standard programs. *B. subtilis* alone, or *B. amyloliquefaciens* alone or applied alternately with captan and thiram were intermediate. The plant extract (lupin) applied alternately with captan/thiram was intermediate, whereas the extract alone was ineffective.

There was a strong relationship between yield and the incidence of disease (Fig. 1). Yields decreased as the incidence of grey mould and stem-end rot increased. Overall, the treatments that included captan and thiram had high yields, the control low yields, and most of the other treatments intermediate yields (Table 3). The plants treated with guazatine were smaller and had smaller leaves and lower yields than expected considering the level of disease control. The fruit were also smaller and unmarketable. The applications of chlorothalonil left a white residue on the leaves, although the plants had good yields and fruit production. The residues may have accumulated because of the relatively frequent application of the chemical and appeared after about six applications of the product.

Discussion

Fungicides had a mixed effect on the incidence of grey mould and stem-end rot in the strawberry plants growing in southeast Queensland. The best control of these diseases and the best yields were generally achieved with programs based on the use of captan and thiram with the application of different single-site fungicides during wet weather.

Multi-site fungicides

Several multi-site fungicides were evaluated in the experiments conducted over three years. Captan and thiram were applied alone or alternately, whereas in other treatments they were used in combination with single-site fungicides during wet weather. The other multi-site fungicides included dithianon, chlorothalonil and guazatine and were sprayed alone. Multi-site fungicides have been used for a long time against *Botrytis cinerea* (Leroux 2004). They have a protective function against the fungus and must be applied frequently at high rates of application to be effective. In the laboratory, most of these fungicides inhibit germination of the conidia.

In the current study, the programs based on captan/thiram/single-site fungicides provided good control of disease. In year three, there was a comparison of this program with captan or thiram alone or alternately, but without the single-site fungicides. There was mixed response to the different combinations of fungicides with captan/thiram/single-site fungicides better than captan alone and captan applied alternately with thiram, but similar to thiram alone. There were problems with the other multi-site fungicides, which were less effective than the standard programs (dithianon), phytotoxic (guazatine) or left white residues on the plants (chlorothalonil).

Legard et al. (2001) showed that weekly applications of captan or thiram reduced the incidence of grey mould by more than 40% compared with the incidence of the disease in nontreated plots in Florida. Yields were increased by up to 127%. Florida produces berries during winter and has a similar climate to southeast Queensland. The two industries also share common cultivars, production technologies, pests and diseases (Whitaker et al. 2014). Later work in Florida showed that applications of captan could be replaced by the single-site fungicide fenhexamid towards the end of the growing season for good control of grey mould (Legard et al. 2005). In Louisiana in the southern United States, captan and thiram controlled grey mould and stem-end rot, although the incidence of the disease was very low even in the controls, with less than 10% losses (Wedge et al. 2007). In our study, a program based on captan/thiram with the application of single-site fungicides during wet weather was effective. Changes in the labels for the use of captan and thiram in Australia will allow more frequent applications of these two fungicides during the strawberry season. They should also reduce the number of applications of the single-site fungicides required in a season.

Single-site fungicides

We investigated the effect of five different single-site active ingredients on the incidence of grey mould and stem-end rot to determine whether these chemicals could be used to supplement the existing single-site fungicides used in southeast Queensland. Losses of about 6% were observed with isopyrazam and penthiopyrad compared with 25 to 38% in the controls. In contrast, the product that included azoxystrobin + difenoconazole (Groups 3 + 11 fungicides) was only partially effective. About 20% of the fruit were affected by grey mould compared with about 40% in the nontreated plots.

Penthiopyrad (Group 7 fungicide) has been recently registered for use on strawberry in Australia, but no more than three applications of this group of fungicides are allowed in a season. Isopyrazam is in the same group of fungicides as penthiopyrad and was equally effective. There could be difficulties in obtaining approval for this new chemical because of concerns about applying fungicides that have the same mode of action against the grey mould fungus. Penthiopyrad was developed in 2008 and registered in Florida in 2012 and in Australia in 2013. Suzuki et al. (2011) reported that the fungicide controlled grey mould in strawberry plants in a greenhouse in Japan. Their work also showed penthiopyrad prevented the germination of the conidia of the fungus for at least 24 h after inoculation and was effective for 21 days after application. They concluded that the disease was best controlled by fungicides that have a long residual life.

Fluazinam was developed in the early 1980s and was later released into the Japanese market as a broad spectrum fungicide (Komyoji et al. 1995). It has little curative or systemic activity. The Japanese workers showed that the chemical was active against *B. cinerea* on agar and inhibited the growth of the fungus in cucumber plants in a glasshouse. In our experiments, fluazinam was effective against the grey mould and stem-end rot fungi in southeast Queensland. The chemical reduced the incidence of disease from 30% in the controls to 4% in the treated plants. Washington et al. (1992) investigated the effect of fluazinam on the incidence of grey mould in three strawberry fields in southern Australia. The average incidence of the disease was 6% compared with 17% in the controls.

There have been no attempts to register this chemical for use on strawberry in Australia, possibly because of concerns about residues of fluazinam at harvest. Earlier work showed high residues in the fruit that were sprayed with this fungicide (Gomez, unpublished data). The concentration of fluazinam seven days after the last spray was 0.44 mg per kg. This value is higher than maximum residue limit (MRL) for fluazinam of 0.3 mg per kg in other crops such as apple after 28 days approved by the European Union (Wu et al. 2014). In these studies in apple, the concentrations of fluazinam were above the MRL up to seven days after the last spray. A seven or twenty-eight-day withholding period (WHP) is too long for commercial strawberry production.

Resistance of *Botrytis cinerea* to fungicides

Thiram was released in the early 1940s and captan about a decade later. It is generally considered that fungi have a low risk of developing resistance to these multi-site fungicides. In the current experiments, captan and thiram were effective in controlling grey mould and stem-end rot. In year three when thiram and captan were applied weekly or alternately, the percentage of fruit affected by disease was about 7% compared with about 25% in the nontreated plots. Wedge et al. (2013) investigated the resistance of thirteen isolates of *B. cinerea* to several fungicides at 0.3, 3.0 or 30.0 μM in a laboratory in Louisiana. All the isolates were resistant or intermediate to thiram at the lower concentrations and all were sensitive at the higher concentration. When the rate of application in the field (3.0 to 3.7 kg a.i. per ha) was taken into account, all the isolates were considered sensitive to thiram. A similar response occurred with captan, with all the isolates considered sensitive to the fungicide.

Iprodione was released in 1974, fludioxonil in 1990, cyprodinil in 1994, fenhexamid in the late 1990s, and penthiopyrad was released in 2008. Resistance of *B. cinerea* to the dicarboximide iprodione was reported in strawberry fields in New Zealand in the early 1980s (Beever and Brien 1983) and was widely distributed in a recent study in North and South Carolina and Florida (Grabke et al. 2014). Between 3 and 23% of isolates were reported to be low or moderately resistant in the United States. Studies from the United States and Germany have identified isolates of *B. cinerea* from strawberry fields that were resistant to several of the new single-site fungicides, including fludioxonil, cyprodinil, fenhexamid and penthiopyrad (Weber 2011; Amiri et al. 2013, 2014; Fernández-Ortuño et al. 2014; Li et al. 2014). Some isolates were resistant to up to five or six different fungicides. Monitoring following the first use of penthiopyrad in Florida in 2013 indicated that resistant populations appeared soon after (Amiri et al. 2014).

In some cases, resistance to a particular fungicide in the United States followed the long-term repeated use of the chemical in a strawberry field. However, in other cases, resistant isolates appeared in fields that had never been sprayed with that particular chemical. These results suggested cross-contamination from nearby strawberry plants or other hosts. Research in Florida has shown that the grey mould fungus can be introduced into commercial fields with the new strawberry transplants (Oliveira et al. 2013).

There have been limited studies of the resistance of *B. cinerea* collected from strawberry fields in Australia to the single-site fungicides released in the last 15 years. There was an earlier study that showed some resistance to iprodione (Group 2), pyrimethanil (Group 9) and fenhexamid (Group 17) in samples collected from four strawberry fields in southern Queensland (Gomez, unpublished data). However, the significance of these findings for broad-scale resistance across the industry is unclear since only 20 samples were collected. It was also not determined whether the resistant isolates were common. In the current study, penthiopyrad was applied alone and appeared to be effective. Iprodione, cyprodinil + fludioxonil, and fenhexamid were applied with captan and thiram. Earlier work showed that isolates of *B. cinerea* were resistant to iprodione in southern Australia (Washington et al. 1992).

B. cinerea is likely to develop resistance to these single-site fungicides in southeast Queensland because production in this area is intensive with most of the fields located within 50 km of each other. This can increase the movement of isolates from fields sprayed with different fungicides. The season is also long, with extended periods of flowering and fruiting, and extended periods of wet weather. Up to 20 sprays may be applied each season in some fields. The lack of routine monitoring of the fungus is likely to contribute to the development of multiple resistance across the industry. The current recommendation for the use of single-site fungicides indicates a maximum of three sprays per season for each class of chemicals. The continued use of single-site fungicides, applied as mixtures or applied alternately can lead to resistance to several fungicides (Li et al. 2014). Strategies to overcome this scenario include more frugal use of single-site fungicides, more frequent use of multi-site fungicides, and close attention to clean planting material and sanitation during fruit production. The industry should also consider more frequent monitoring for resistance to the main chemicals used to control grey mould.

Alternative fungicides

A range of soft or alternative fungicides was assessed in our study. These included a plant defence promoter, two plant extracts, organic acids, fatty acids, a salt, four bacteria, and a fungus. In some of the treatments, the soft fungicides were applied alternately with captan/thiram or mixed with captan/thiram/single-site fungicides.

When the alternative fungicides were applied alone, the plant-defence promoter acibenzolar-S-methyl, organic acids and potassium bicarbonate + oil were intermediate. Potassium salts of fatty acids, the two plant extracts, *Streptomyces lydicus* and *Trichoderma harzianum* were ineffective. There was a mixed response with the other bacteria. *B. subtilis* (strain *QST 713*) and *B. amyloliquefaciens* were intermediate in 2014, whereas *B. subtilis* (strain *MBI 600*) was ineffective in 2013. Alternating captan/thiram with *B. subtilis*, *Streptomyces* or *Trichoderma* in 2013 improved the level of control compared with that achieved with the bacteria or fungus alone. The same response occurred when captan or thiram were applied alternately with *B. amyloliquefaciens* in 2014. However, the level of control with these combinations did not match that obtained with captan/thiram/single-site fungicides. The only alternative treatment that gave good control of disease was *B. subtilis* mixed with captan/thiram/single-site fungicides. In most cases, the control achieved with the combined treatments was probably due to the residual effect of the multi-site fungicides. Washington et al. (1999b) demonstrated that thiram applied every two weeks gave good control of grey mould in Victoria, and was sometimes equal to thiram applied every week.

In the current study, none of the microbes were effective as the standard treatment with captan/thiram/single-site fungicides even when they were applied alternately with captan or thiram. There has been much research on the biological control of plant diseases. However, the results are often inconsistent when these microbes are applied in commercial fields (Alabouvette et al. 2006). There are two main reasons why these agents may fail. A single strain of the bacteria or fungi may not control all the isolates present in the population. Environmental conditions in the field also may not be optimum for the growth of the biocontrol agent. Biological strategies have been successful for plants in greenhouses where control of the environment is possible (Hyakumachi 2013). In contrast, there have been few success stories with crops in open fields. Elmer and Reglinski (2006) reviewed the effectiveness of biological agents in the control of grey mould in grapevines. They concluded that the responses in the field have been inconsistent compared with the responses in glasshouses or laboratories. The total world market for fungicides is about $10 billion annually, but less than 1% of the total market is for biologicals (Hirooka and Ishii 2013).

Conclusions

Research over three years in southeast Queensland showed that a program based on the multi-site fungicides captan and thiram along with several single-site fungicides during wet weather controlled grey mould and stem-end rot in strawberry plants. These treatments in turn provided high marketable yields. Applications of alternative fungicides were generally not as successful as the standard programs, even when the soft fungicides were applied alternately with thiram and captan. The plant-defence promoter acibenzolar-S-methyl, organic acids, *B. subtilus* (strain *QST 713*), *B. amyloliquefaciens* and potassium bicarbonate + oil gave intermediate control. In contrast, *B. subtilus* (strain *MBI 600*), *Streptomyces lydicus*, *Trichoderma harzianum*, potassium salts of fatty acids, and two plant extracts were ineffective.

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Table 1 Effect of fungicides on the incidence of grey mould and marketable yield from 'Festival' strawberry plants at Nambour in 2012. Data are the means of four replicates per treatment, and have been back-transformed. Thiram was sprayed fourteen times, captan four times, iprodione four times, fenhexamid once, and cyprodinil + fludioxonil five times. All the other products were sprayed fourteen times. Potassium bicarbonate was applied with an oil (Synertrol Horti-oil) that was phytotoxic to the strawberry plants. Means in a column followed by different letters are significantly different ($P = 0.05$). The incidence of stem-end rot in the plants was very low. A plus sign indicates that the chemicals were applied in a mixture. The ampersand indicates that the chemicals were applied at different times during the season. Captan and thiram were applied separately at different times during the season. The controls were sprayed only with water.

Treatment	Grey mould (% of fruit)	Marketable yield (g per plant)
Control	38a	780cd
Captan/thiram, with iprodione & fenhexamid	4e	1557a
Captan/thiram, with cyprodinil + fludioxonil	3e	1443a
Isopyrazam	5e	1418a
Azoxystrobin + difenoconazole	19d	1006b
Acibenzolar S-methyl	25cd	909bc
Organic acids (ascorbic + lactic + citric)	30bc	696d
Potassium bicarbonate + oil	25cd	513e
Potassium salts of fatty acids	33ab	799cd
Potassium bicarbonate + oil + potassium salts of fatty acids	20d	248f
Trichoderma harzianum strain *Td 81b*	33ab	829c

Table 2 Effect of fungicides on the incidence of grey mould and stem-end rot, and marketable yield from 'Festival' strawberry plants at Nambour in 2013. Data are the means of four replicates per treatment, and have been back-transformed. In the standard programs, thiram was sprayed 12 times, captan four times, iprodione three times, fenhexamid twice, and cyprodinil + fludioxonil five times. In the treatments with the soft chemicals, thiram and captan were sprayed four times each, and the *Bacillus subtilis*, *Streptomyces lydicus* and plant extract eight times. In the treatments with *Trichoderma harzianum*, thiram and captan were sprayed three times each, and the fungus ten times. All the other treatments were applied 16 times. Means in a column followed by different letters are significantly different (P = 0.05). A plus sign indicates that the chemicals were applied in a mixture. The ampersand indicates that the chemicals were applied at different times during the season. Captan and thiram were applied separately at different times during the season. The controls were sprayed only with water.

Treatment	Grey mould & stem-end rot (% of fruit)	Marketable yield (g per plant)
Control	30ab	343h
Captan/thiram, with iprodione & fenhexamid	4hi	656a
Captan/thiram, with cyprodonil + fludioxonil	3i	615ab
Isopyrazam	7fg	596abc
Penthiopyrad	5gh	536bcd
Fluazinam	4hi	676a
Dithianon	20c	429fg
Bacillus subtilis strain *MBI 600*	30ab	325hi
Bacillus subtilis strain *MBI 600* alt. with captan/thiram	12de	513cde
Streptomyces lydicus	30ab	374gh
Streptomyces lydicus alt. with captan/thiram	13d	503def
Extract from rhubarb weekly	26b	283i
Extract from rhubarb alt. with captan/thiram	9ef	440ef
Trichoderma harzianum strain *Td 81b*	32a	362h
Trichoderma harzianum strain *Td 81b* (2 weeks) alt. with captan/thiram (1 week)	16cd	449ef

Table 3 Effect of fungicides on the incidence of grey mould and stem-end rot, and marketable yield from 'Festival' strawberry plants at Nambour in 2014. Data are the means of four replicates per treatment, and have been back-transformed. In the treatment with thiram alternated with captan, thiram was sprayed nine times and captan eight times (weekly applications). In the standard program with single-site fungicides, thiram and captan were sprayed two times, iprodione three times, fenhexamid three times, cyprodinil + fludioxonil three times, and penthiopyrad four times. In the treatment with the *Bacillus subtilis* mixed with thiram/captan/single-site fungicides, the fungicides were applied as in the standard treatment, with a total of 16 sprays. In the treatment with the plant extract, thiram and captan were sprayed four times each and the extract nine times. In the treatment with *B. amyloliquefaciens*, thiram and captan were sprayed four times each and the bacteria nine times. Chlorothalonil and guazatine were sprayed 17 times. In the treatment with *B. subtilis* alone, the bacteria were applied 16 times. In the treatment with plant extract alone, the extract was applied 17 times. In the treatment with *B. amyloliquefaciens* alone, the bacteria were applied 17 times. Means in a column followed by different letters are significantly different ($P = 0.05$). A plus sign indicates that the chemicals were applied in a mixture. The ampersand indicates that the chemicals were applied at different times during the season. Captan and thiram were applied separately at different times during the season. The controls were sprayed only with water.

Treatment	Grey mould & stem-end rot (% of fruit)	Marketable yield (g per plant)
Control	25a	871h
Captan weekly	9de	1349bcde
Thiram weekly	6efg	1660a
Captan alt. with thiram	8def	1413abc
Captan/thiram initially, with single-site fungicides during wet weather	3g	1585ab
Chlorothalonil	6efg	1514ab
Guazatine	8de	955gh
Bacillus subtilis strain *QST 713*	14bc	1175ef
Bacillus subtilis strain *QST* 713 with captan/thiram initially, and single-site fungicides during wet weather	5fg	1413abcd
Extract from lupin	23a	912gh
Extract from lupin alt. with captan/thiram	12bcd	1202cdef
Bacillus amyloliquefaciens	15b	1072fg
Bacillus amyloliquefaciens alt. with captan/thiram	10cde	1175def

Table 4 Details of the different fungicides used in the evaluation of chemicals for the control of grey mould and stem-end rot in strawberry plants at Nambour in 2012, 2013, and 2014. The different groups are based on their mode of action as suggested by the Fungicide Resistance Action Committee (FRAC). The numbers and letters are used to distinguish the fungicide groups according to their cross-resistance behaviour.

Chemical	FRAC code	Trade name	Concentration of active ingredient	Concentration of sprayed product	Source
Captan	Group M4 (multi-site)	Captan	900 g/kg	1.25 g/ L	Crop Care
Thiram	Group M3 (multi-site)	Thiram	800 g/kg	1.5 g/L	Barmac
Iprodione	Group 2	Rovral	250 g/L	1 ml/L	Bayer
Fenhexamid	Group 17	Teldor	500 g/L	1 ml/L	Bayer
Cyprodinil + fludioxonil	Groups 9 +12	Switch	375 + 250 g/kg	0.8 g/L	Syngenta
Azoxystrobin + difenoconazole	Groups 3 + 11	Amistar Top	200 + 125 g/L	1 ml/L	Syngenta
Isopyrazam	Group 7	-	125 g/L	1.2 ml/L	Syngenta
Acibenzolar-S-methyl	Group P1 (Host plant-defence induction)	Actigard	500 g/L	0.2 g/L	Syngenta
Ascorbic + lactic + citric acids	Not classified (NC)	Citrex	23 g/L (ascorbic)	2 ml/L	Colin Campbell Chemicals
Potassium bicarbonate + Synertrol Horti-Oil	Not classified (NC)	Eco-carb	950 g/kg + 905 g/L	5 g/L + 2 m/L	Organic Crop Protectants
Potassium salts of fatty acids	Not classified (NC)	Eco-protector	194 g/L	20 ml/L	Organic Crop Protectants
Trichoderma harzianum strain *Td 81b*	Group F6	Nemesis	1×10^{11} cfu/g	0.1 g/L	Metcalf Industries
Penthiopyrad	Group 7	Fontelis	200 g/L	1.75 ml/L	Dupont
Fluazinam	Group 29	Shirlan	500 g/L	1 ml/L	Farmoz
Dithianon	Group M9 (multi-site)	Delan	700 g/kg	0.5 g/L	Nufarm
Bacillus subtilis strain *MBI 600*	Group F6	Clarity	5.5×10^{10} cfu/g	0.4 g/L	Becker Underwood
Streptomyces lydicus	Group F6	Actinovate	1×10^{7} cfu/g	0.5 g/L	Organic Farming Solutions
Extract from rhubarb	Not classified (NC)	Kobe	100 ml/L	2 ml/L	Colin Campbell Chemicals
Chlorothalonil	Group M5 (multi-site)	Bravo	500 g/L	2.5 ml/L	Syngenta
Guazatine	Group M7 (multi-site)	Panoctine	400 g/L	0.5 g/L	Nufarm
Bacillus subtilis strain *QST 713*	Group F6	-	7.3×10^{9} cfu/g	0.5 g/L	Bayer
Bacillus subtilis strain *QST 713* with captan & thiram		-	-	3 ml/L	Bayer
Extract from lupin	Not classified (NC)	-	200 g/L	3 ml/L	Colin Campbell Chemicals
Extract from lupin, with captan & thiram		-	-	10 ml/L	Colin Campbell Chemicals
Bacillus amyloliquefaciens	Group F6	Loli-pepta	1×10^{8} cfu/ml	10 ml/L	Bio-film Crop Protection

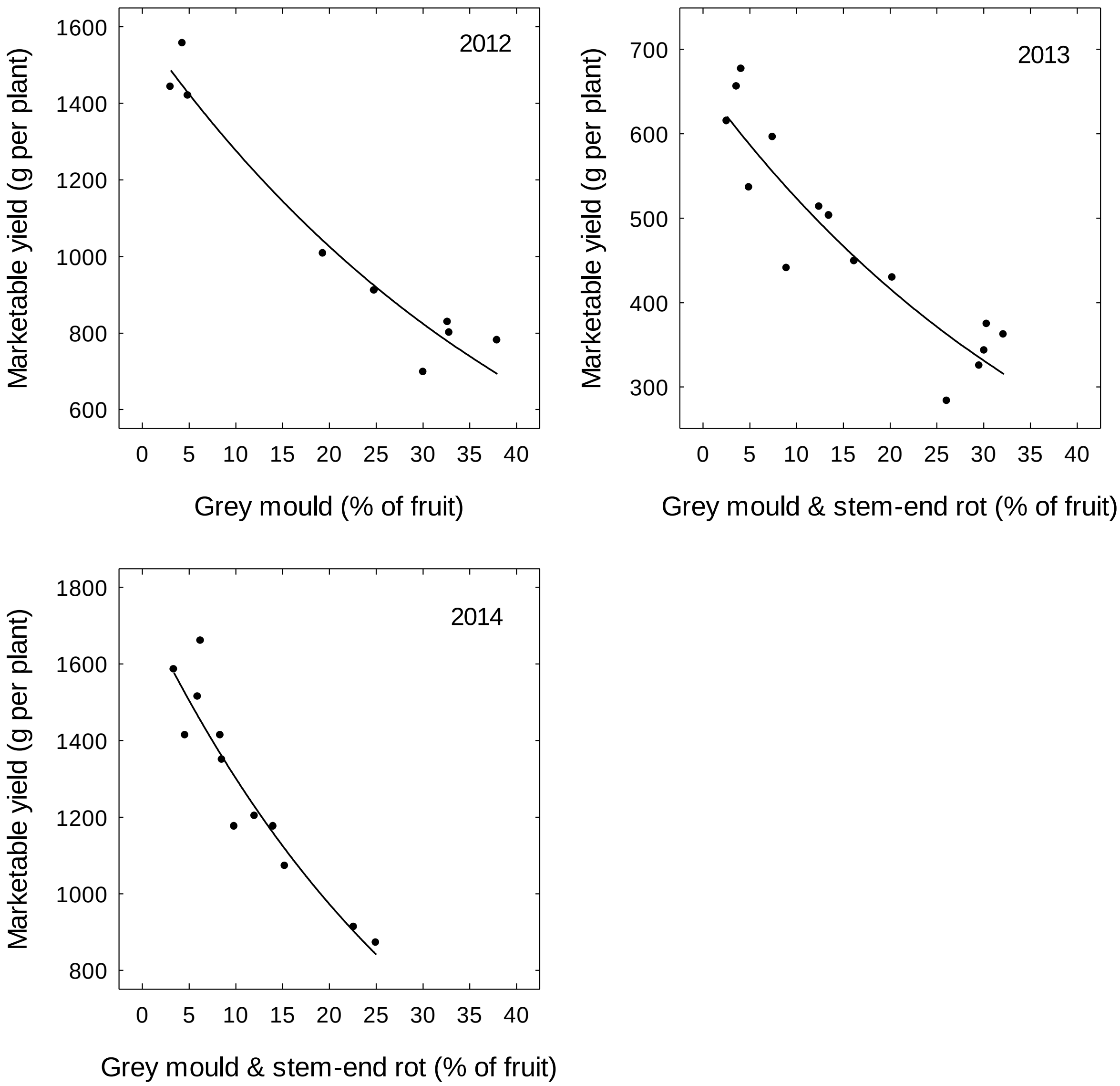

Fig. 1 Relationship between marketable yield and the incidence of grey mould and stem-end rot in strawberry plants at Nambour in 2012, 2013, and 2014. The data for the two treatments that included potassium bicarbonate + oil in 2012 and the data for guazatine in 2014 were excluded from the analyses because they were phytotoxic. For 2012, yield = 1588 × exp. (-0.02 × disease) (N = 9, R^2 = 0.93). For 2013, yield = 658 × exp. (-0.02 × disease) (N = 15, R^2 = 0.82). For 2014, yield = 1740 × exp. (-0.03 × disease) (N = 12, R^2 = 0.89). There was virtually no stem-end rot in 2012. The few fruit that had both grey mould and stem-end rot in the other years were rated as affected by grey mould since it was the primary disease.

Outputs

Publications

Gomez A (2012). The China strawberry symposium from a plant pathology perspective. Simply Red (Queensland Strawberry Industry Promotions Council) 26:8.

Gomez A (2012). Integrated disease management in strawberry plants. Department of Agriculture, Fisheries and Forestry, 4 pp.

Gomez A (2014). Disease management and fungicide use guide for winter strawberry production in Queensland. Department of Agriculture, Fisheries and Forestry, 4 pp.

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Menzel CM, Gomez A, Smith L (2012). Controlling grey mould in strawberry crops on the Sunshine Coast. Department of Agriculture, Fisheries and Forestry, 2 pp.

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Menzel CM, Gomez A, Smith L (2013). Evaluating chemicals for the control of fruit diseases affecting strawberry plants. Simply Red (Queensland Strawberry Industry Promotions Council) 31:4–6.

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Menzel CM, Gomez A, Smith L (2014). Improving the control of grey mould and stem-end rot in strawberry plants in southern Queensland. Simply Red (Queensland Strawberry Industry Promotions Council) 35:4–5.

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Menzel CM, Gomez A, Smith L (2014). Better control of fruit diseases in southern Queensland. Simply Red (Queensland Strawberry Industry Promotions Council) 36:8–9.

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Menzel CM, Gomez A, Smith L, Mertely J, Seijo T, Peres NA (2013). Improving the management of plant and fruit diseases affecting strawberry production in Queensland and Florida. Simply Red (Queensland Strawberry Industry Promotions Council) Special Edition 2:1–20.

Menzel CM, Gomez A, Smith L, Mertely J, Seijo T, Peres NA (2015). Strategies for improving the control of plant and fruit diseases affecting strawberry fields in Queensland and Florida. Simply Red (Queensland Strawberry Industry Promotions Council) Special Edition 3:1–20.

Vorpagel B, Gomez A (2015). Captan limits relaxed. Good Fruit & Vegetables 26 (8):18.

Vorpagel B, Gomez A (2015). Improved strawberry disease control with Captan 900WG use-limit removed. Fruit & Vegetable News March, p. 20.

Field days and seminars

Queensland Strawberry Growers' Association Meetings. The QSGA holds four meetings a year at Glasshouse Mountains on the Sunshine Coast. Over the past three years, members of the project team have provided updates on progress in plant and fruit diseases to local producers. Printed reports were provided to the growers at many of these meetings. Information was also provided to growers in Bundaberg at meetings in 2013 and 2014.

Queensland Strawberry Growers' Field Day. A field day was hosted by QSGA for the industry on the Sunshine Coast at Wamuran in July 2013. About 150 commercial strawberry growers attended the day, which included displays from commercial operators such as machinery suppliers, chemical companies, fertilizer companies, and pest scouts, etc. Information on disease management was presented to industry.

Berry industry conferences/field days. Apollo Gomez presented overviews of the research on plant and fruit diseases at the industry meetings held in Queensland and Victoria in 2012 and 2014. The conferences attracted about 100 commercial growers and trade exhibitors.

Strawberries Australia Inc. Levy Papers' Meeting. Members of the project team provided a summary of the research to industry at a Strawberries Australia Inc. meeting held at Nambour in 2012. Approximately 50 growers from around the country attended the field day. A flyer summarising the research was presented to the attendees.

International Horticultural Congress. Apollo Gomez presented a paper on the effect of different fungicides on the incidence of fruit diseases at this international meeting held in Brisbane in August 2014.

Outcomes

Three experiments were conducted to investigate different chemicals used to control plant and fruit diseases affecting strawberry fields in Queensland. The results of the research showed that a program based on the multi-site fungicides captan and thiram with several single-site fungicides during wet weather was highly effective in controlled grey mould and stem-end rot. This treatment in turn provided high marketable yields.

Applications of alternative fungicides were not as successful as the standard program, even when the soft fungicides were applied alternately with thiram and captan. The plant-defence promoter acibenzolar-S-methyl, organic acids, *B. subtilus* (strain *QST 713*), *B. amyloliquefaciens* and potassium bicarbonate + oil gave intermediate control. In contrast, *B. subtilus* (strain *MBI 600*), *Streptomyces lydicus*, *Trichoderma harzianum*, potassium salts of fatty acids, and two plant extracts were ineffective.

Our results showed that a program based on captan/thiram with the application of single-site fungicides during wet weather was effective. Average losses with this strategy were about 3 to 4% compared with more than 25% on nontreated control plots. Changes in the labels for the use of captan and thiram in Australia will allow more frequent applications of these two fungicides during the strawberry season. They should also reduce the number of applications of the single-site fungicides required in season. Strawberry growers have more certainty in controlling fungal diseases and managing fungicide resistance, with the removal of the restrictions on the use of captan and thiram. The use of the two multi-site fungicides in rotation with other fungicides from different chemical groups will reduce the risk of fungicide resistance occurring and extend the life of registered products. This strategy has not been available to growers previously.

Evaluation, Discussion and Recommendations

Some of the treatments were effective in controlling grey mould and stem-end rot, some were intermediate, and some were ineffective (Table 5).

Table 5 Effectiveness of the different fungicides for the control of grey mould and stem-end rot in strawberry plants at Nambour in 2012, 2013, and 2014. The different groups are based on their mode of action as suggested by the Fungicide Resistance Action Committee (FRAC). The numbers and letters are used to distinguish the fungicide groups according to their cross-resistance behaviour.

Chemical	FRAC code	Efficacy
Captan	Group M4 (multi-site)	Effective
Thiram	Group M3 (multi-site)	Effective
Iprodione	Group 2	Unknown, product not applied alone
Fenhexamid	Group 17	Unknown, product not applied alone
Cyprodinil + fludioxonil	Groups 9 +12	Unknown, product not applied alone
Azoxystrobin + difenoconazole	Groups 3 + 11	Intermediate
Isopyrazam	Group 7	Effective, but in the same group as penthiopyrad
Acibenzolar-S-methyl	Group P1 (Host plant-defence induction)	Intermediate
Organic acids (ascorbic + lactic + citric)	Not classified (NC)	Intermediate
Potassium bicarbonate + Synertrol Horti-oil	Not classified (NC)	Intermediate, but was phytotoxic with applied oil
Potassium salts of fatty acids	Not classified (NC)	Ineffective
Trichoderma harzianum strain *Td 81b*	Group F6	Ineffective
Penthiopyrad	Group 7	Effective
Fluazinam	Group 29	Effective, but potential issues with residues above accepted MRLs
Dithianon	Group M9 (multi-site)	Intermediate
Bacillus subtilis strain *MBI 600*	Group F6	Ineffective
Streptomyces lydicus	Group F6	Ineffective
Extract from rhubarb	Not classified (NC)	Ineffective
Chlorothalonil	Group M5 (multi-site)	Effective, but potential issues with white residues on the leaves and fruit
Guazatine	Group M7 (multi-site)	Effective, but phytotoxic
Bacillus subtilis strain *QST 713*	Group F6	Intermediate
Extract from lupin	Not classified (NC)	Ineffective
Extract from lupin, with captan & thiram		Intermediate
Bacillus amyloliquefaciens	Group F6	Intermediate

None of the multi-site fungicides appear to be potential replacements for captan or thiram, the two industry standards. Dithianon was ineffective, guazatine was phytotoxic, and chlorathalonil left a white residue on the plants. No potential new single-site fungicides were identified during the research. Isopyrazam was effective but it is in the same group of fungicides as penthiopyrad (Group 7). It is not likely to be registered for use on strawberry plants growing in Australia. Fluazinam was effective but there are concerns about possible residues of the chemical in the fruit. None of the soft chemicals were as effective as the standard treatments with captan and thiram. They are not likely to be successful in areas with regular wet weather.

To summarise, the best option for commercial strawberry growers is to use thiram and captan, with the single-site fungicides such as iprodione, fenhexamid, penthiopyrad, and cyprodinil + fludioxonil applied during wet weather.

Botrytis cinerea is likely to develop resistance to these single-site fungicides in south-east Queensland. Strategies to overcome this scenario include more frugal use of single-site fungicides, more frequent use of multi-site fungicides, and close attention to clean planting material and sanitation during fruit production. The industry should also consider more frequent monitoring for resistance to the main chemicals used to control grey mould.

We have clearly demonstrated the effectiveness of captan and thiram used with the application of single-site fungicides during wet weather. We have also assisted research which has led to changes in the label for the use of these two multi-site fungicides on strawberry plants in Australia. Previously, there was a restriction on the use of captan, with no more than five sprays per season. Thiram was allowed to be used only up to the first flowering. The changes in the labels for captan and thiram now allow the use of these two chemicals every fortnight. Strawberry producers now have a protectant program for the control of grey mould and stem-end rot. The application of single-site fungicides can be restricted to periods of wet weather, extending the life of these chemicals. This strategy is likely to delay the development of resistance in the grey mould fungus to these fungicides. There is low risk of the fungus developing resistance to captan and thiram. A suggested spray program for commercial strawberry producers on the Sunshine Coast is shown in the Appendix.

Grey mould and stem-end rot are major diseases affecting the strawberry industry in Australia. A loss of 10% of production is equivalent to a loss of $46 million to producers in all states. Reducing these losses to 5% would save the industry $23 million.

Scientific Refereed Publications

Menzel, C.M., Gomez, A., Smith, L.A., 2015. Control of grey mould and stem-end rot in strawberry plants growing in a subtropical environment. Australasian Plant Pathology (In preparation).

Intellectual Property/Commercialisation

No commercial IP generated.

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Appendix

A guide for the use of fungicides to control plant and fruit diseases affecting strawberry plants growing on the Sunshine Coast. If conditions favour the development of disease, apply iprodione (Group 2 fungicide), fenhexamid (Group 17), cyprodinil + fludioxonil (Groups 9 and 12) or pyrimethanil (Group 9). Use no more than three sprays per season of the same group of fungicides. Use no more than two consecutive sprays of the same group of fungicides. Cyprodinil and pyrimethanil are in the same group of fungicides (Group 9). Penthiopyrad (Group 7) can be used to control both grey mould and powdery mildew. Myclobutanil (Group 3) and trifloxystrobin (Group 11) can be used to control powdery mildew but are not recommended for the control of grey mould.

	Week 1	Week 2	Week 3	Week 4	Week 5
April			Cyprodinil + fludioxonil	Penthiopyrad	
May	Myclobutanil & thiram	Captan	Thiram	Trifloxystrobin & captan	Penthiopyrad
June	Captan	Thiram	Myclobutanil & captan	Trifloxystrobin & thiram	
July	Captan	Thiram	Penthiopyrad	Myclobutanil & thiram	Captan
August	Thiram	Myclobutanil & captan	Trifloxystrobin & thiram	Captan	
September	Thiram	Captan	Thiram	Captan	
October	Thiram	Captan	Thiram	Captan	