

Post-plant strategies for management of black root rot-related decline of perennial strawberry fields

Timothy D. Miles^{a,*}, Benjamin W. Glass^{b,1}, Roger W. Sysak^b, Annemiek C. Schilder^b

^a School of Natural Sciences, California State University Monterey Bay, Seaside, CA, USA

^b Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, USA

ARTICLE INFO

Keywords:

Fragaria × *ananassa*
Binucleate *Rhizoctonia*
Rhizoctonia fragariae
Fusarium spp.
Pythium spp.
Nematodes
Fungicides
Nutritional amendments

ABSTRACT

Perennial strawberries affected with black root rot generally decline in runner production, yield, and overall plant vigor over time. Several plant pathogens have been implicated in black root rot, including plant-parasitic nematodes, *Pythium* and *Rhizoctonia* spp. Pre-plant treatments such as a 3- to 5-year crop rotation or chemical fumigation are the main means of disease management. During 2007 and 2008, five post-plant treatments were evaluated for mitigation of strawberry black root rot in a naturally declining site in Ottawa County in Michigan. Fungicides and nutritional amendments were applied as drenches or foliar sprays. Most treatments increased runner establishment, yield, and overall plant vigor, particularly azoxystrobin and azoxystrobin + potassium phosphite. Root necrosis and incidence of root infection by binucleate *Rhizoctonia* spp. were reduced. Results were more apparent and significant in 2008 than in 2007, indicating cumulative effects over the 2 years of the trial. During 2008 and 2009, a large-scale demonstration trial was conducted with these treatments in a strawberry field with a history of black root rot in Leelanau County, MI. Improvements in bed fill and average plant weight and reductions in root necrosis and *Rhizoctonia* isolations were noted. This research represents the first post-plant chemical management strategy capable of slowing or even reversing the decline due to black root rot in established strawberry fields.

1. Introduction

In Michigan, strawberries are typically produced in perennial, matted-row plantings (Hancock, 1999). Black root rot (BRR) is a disease complex that plagues older strawberry fields and occasionally new plantings. Multiple organisms and abiotic factors have been implicated in the cause of the disease. However, *Rhizoctonia fragariae* Husain & McKeen, specifically the binucleate anastomosis groups A, K, G and I, *Pythium* spp., and the root lesion nematode *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven are generally considered the primary agents acting in concert to cause the symptoms (D'Ercole et al., 1988; Martin, 2000; Sharon et al., 2007; Wilhelm, 1987; Wing et al., 1994). *Rhizoctonia* isolates from strawberry roots in Michigan have generally been binucleate and considered to be *R. fragariae* (Glass, 2008), although most studies (Botha et al., 2003; Sharon et al., 2007) prefer to use the term “binucleate *Rhizoctonia* species” (BNR) due to unresolved taxonomy. BNR species have been associated with root rot problems in several crops outside of strawberries, including potatoes, sugar beets and turfgrass (Miles et al., 2013; Martin and Lucas, 1984).

Strawberry plants with black root rot are less vigorous and produce

fewer runners than healthy plants. Many of the main roots are black and few feeder roots are present (Wilhelm, 1987). Under dry conditions, affected plants wilt and die, resulting in bare patches in the field. Yields are diminished to the point where the planting becomes economically unsustainable. The disease can spread via infected nursery stock, movement of infested soil, or infected plant debris (Hildebrand, 1934; Strong and Strong, 1927; Wilhelm, 1987). Black root rot may significantly shorten the productive life of perennial strawberry plantings, with only 2 to 3 harvest years in some cases. In contrast, some perennial strawberry fields have been kept in production for up to 10 years (D. Gibbs, personal communication).

A common chemical treatment for prevention of black root rot is pre-plant soil fumigation, which is effective against all pathogens involved in the complex. However, because of the phase-out of methyl bromide, an effective soil fumigant used for decades, strawberry growers are looking for alternative methods of managing this disease (Batchelor, 1998; Roskopf et al., 2005). Some chemicals, such as 1,3-dichloropropene (Telone, Dow AgroSciences, Indianapolis, Indiana, USA), chloropicrin, and metam sodium (Vapam, Amvac Chemical Corporation, Los Angeles, CA, USA) have shown success as alternative

* Corresponding author.

E-mail address: tmiles@csumb.edu (T.D. Miles).

¹ Current address: Zeeland Farm Services, Inc., Zeeland, MI, USA.

Table 1

Active ingredients, trade names, manufacturers, application methods, rates, and schedules used to treat black root rot-affected strawberry plants in a small plot trial (cv. Jewel) in Ottawa County, in 2007 and 2008 and in an on-farm demonstration trial (cvs. Northeast and Earliglow) in Leelanau County, Michigan, USA in 2008 and 2009.

Active Ingredient	Trade Name and Manufacturer	Application Method	Product Rate/ha	Application Schedule ^{a,b}
Small plot trial^a				
2007				
Azoxystrobin	Abound, Syngenta Crop Protection, Greensboro, NC, USA	Drench	0.73 L	1, 5, 9
Potassium phosphite	ProPhyt, Helena Chemical Company, Collierville, TN, USA	Spray	2.34 L	1, 2, 3, 4, 5, 6, 7, 8, 9
Calcium, cobalt and zinc carbonates and microbial enzymes	Symbex 4x, Agro-K Corporation, Minneapolis, MN, USA	Drench	4.68 L	1, 5, 9
Calcium and copper phosphites	Sysstem-Cal, Agro-K, Corporation Minneapolis, MN, USA	Spray	7.02 L	1, 2, 3, 4, 5, 6, 7, 8, 9
2008				
Azoxystrobin	As above	Drench	0.73 L	1, 5
Potassium phosphite	As above	Spray	2.34 L	1, 2, 3, 4
Calcium, cobalt and zinc carbonates and microbial enzymes	As above	Drench	4.68 L	1, 5
Calcium and copper phosphites	As above.	Spray	7.02 L	1, 2, 3, 4
Demonstration trial^b				
2008				
Azoxystrobin	As above	Chemigation	0.88 L	1, 2, 3
Sodium, potassium and ammonium phosphites	Phostrol, Nufarm Americas Inc., Burr Ridge, IL, USA	Spray	4.68 L	1, 2, 3
2009				
Azoxystrobin	As above	Chemigation	0.88 L	1, 2, 3
Sodium, potassium and ammonium phosphites	Phostrol, Nufarm Americas Inc., Burr Ridge, IL, USA	Spray	4.68 L	1, 2, 3

^a Small plot trial: application dates in 2007: 1 = 23 May, 2 = 6 June, 3 = 20 June, 4 = 5 July, 5 = 18 July, 6 = 2 August, 7 = 15 August, 8 = 29 August, and 9 = 8 September. Application dates in 2008: 1 = 19 April, 2 = 6 May, 3 = 21 May, 4 = 4 June and 5 = 9 June.

^b Demonstration trial: application dates in 2008: 1 = 31 May, 2 = 19 June, and 3 = 10 September. Application dates in 2009: 1 = 17 June, 2 = 8 July, and 3 = 26 August.

fumigants (Batchelor, 1998). However, due to the cost, increased regulation and negative environmental effects of chemical soil fumigants (Batchelor, 1998; Roskopf et al., 2005), there is renewed interest in management alternatives, such as using crop rotation, cover crops, improved drainage, and biological soil disinfection methods (Ajwa et al., 2003; Martin and Bull, 2002; Martin and Hancock, 1983; Perry and Ramsdell, 1994; Shennan et al., 2014; Wilhelm, 1987). If a field has a history of BRR, the recommendation is frequently made to use a 3- to 5-year rotation out of strawberries or to find a new location for the strawberries (Funt et al., 1997; Perry and Ramsdell, 1994). However, land constraints and limitations on the location of pick-your-own fields may be reasons why strawberries cannot be grown in a different area on a given farm. In general, it is assumed that not much can be done to manage black root rot once the disease shows up in a planting. The goal of this research project was to challenge this assumption and evaluate post-plant treatments for their ability to slow or reverse plant decline in black root rot-affected strawberry fields.

Several products are labeled for use specifically against pathogenic organisms that are part of or related to those involved in the black root rot complex. The fungicide azoxystrobin (Abound, Syngenta Crop Protection, Greensboro, North Carolina, USA) is labeled as a drench in strawberries to control root rot caused by *Rhizoctonia*. Azoxystrobin is a member of the quinone outside inhibitor (QoI) class of fungicides which have broad-spectrum activity against many fungal and oomycete species (Anke, 1995). Phosphite fungicides such as potassium phosphite (ProPhyt, Helena, Collierville, Tennessee, USA) and mono- and dibasic sodium, potassium, and ammonium phosphites (Phostrol, Nufarm Americas Inc., Burr Ridge, IL, USA) are highly systemic products similar to fosetyl-Al (Aliette, Bayer Crop Science, Research Triangle Park, North Carolina, USA). They can be applied foliarly or as a pre-plant dip to strawberry roots and crowns. Since they are effective against Phytophthora diseases in strawberries, they may also have the potential to control *Pythium* spp. Potassium phosphite applied foliarly to 'Honeoye' strawberries from bloom to harvest gave excellent control of *Phytophthora cactorum* (Rebollar-Alviter et al., 2005). Furthermore, a potassium phosphite dip treatment of 'Sweet Charlie' strawberry transplants followed by a foliar application increased the percentage of

healthy plants after 24 days compared to untreated plants in a North Carolina study aimed at controlling *P. cactorum* (Louws et al., 2004). Additionally, a pre-plant dip of strawberry transplants with azoxystrobin + potassium phosphite also improved plant growth and establishment in a field with a history of black root rot in Michigan (Glass, 2008).

In addition to fungicides, it is conceivable that nutritional amendments can improve root growth and overall health of declining plants. While a product containing calcium and copper phosphites (Sysstem-Cal, Agro-K Corporation, Minneapolis, Minnesota, USA) is marketed as a foliar fertilizer, it also may have disease control activity due to its phosphite content. In addition, a fermentation product containing microbial enzymes, calcium carbonate, cobalt carbonate, and zinc carbonate (Symbex 4x, Agro K, Minneapolis, Minnesota, USA) has been claimed to boost soil microbial populations and root growth. This product is also labeled for organic production.

To develop recommendations for increasing the longevity and profitability of perennial strawberry plantings, we investigated the effectiveness of post-plant fungicide and fertilizer treatments in reversing black root rot-related decline.

2. Materials and methods

2.1. Post-plant treatment efficacy trial

A commercial strawberry field (cv. Jewel) with typical black root rot symptoms in Hudsonville (Ottawa County), Michigan, USA, was selected for the trial. The soil type was a Granby loamy sand-lake plain soil with the top 28 cm consisting of loamy sand. The field had been established in 2005 with transplants purchased from a commercial nursery in Michigan and was showing severe decline symptoms. Preliminary tests indicated that the site had high levels of needle nematodes [*Longidorus elongatus* (de Man) Thorne & Swanger] as well as several fungal root pathogens (*Rhizoctonia*, *Cylindrocarpon* and *Fusarium* spp.). The trial was established on 23 May 2007 in a randomized complete block design with four replications per treatment. Each treatment plot consisted of three 3.05-m rows, spaced 1.07 m apart. All

rows were treated but only the center row was used for data collection. Five different treatments were applied as either a foliar spray or soil drench (Table 1). Foliar sprays were applied every 2 weeks at 467.5 L/ha with an R&D Research CO₂ cart-styled sprayer (R&D sprayers, Opelousas, Louisiana, USA) equipped with six 3-L bottles, a twin gauge Norgren pressure regulator set at 379 kPa, and a single XR TeeJet 8002VS nozzle on a 1.5-m spray boom. Drenches were applied to individual plots by hand, using 3.8-L jugs at a rate equivalent of 18,700 L/ha. Control plots remained untreated. All plots received the standard practices of the grower throughout the season.

In 2007, a hand aerator (Swisher AE-48, Warrensburg, MO, USA) was used to aerate the soil to a depth of 5–8 cm to ensure that the drenches would reach the root zone. The aerator was used only during the first application so as to not damage fruit in subsequent applications. In 2008, treatments were applied to the same plots as in 2007 in order to determine cumulative effects of the treatments on plant growth and yield.

Bed fill ratings were taken on 23 May, 19 June, 23 July, and 14 September in 2007, and on 14 May and 12 June in 2008 as described below. Harvests occurred on 13, and 27 June in 2007; and on 12 and 19 June in 2008. On 30 July 2007 and 19 June 2008, two representative plants from each plot were dug up to assess fresh and dry plant biomass, root health, and conduct pathogen isolations as described below.

2.2. On-farm demonstration trial

In 2008 and 2009, a large-scale, on-farm demonstration trial was conducted to assess if selected treatments would be effective when applied through an irrigation system. A perennial, 4-year-old strawberry field about 1 ha in size and in the early stages of black root rot decline was chosen in Lake Leelanau (Leelanau County), Michigan, USA. The soil was a sandy loam and the field was evenly divided into two cultivars: Northeast and Earliglow. Both are early-season cultivars and moderately susceptible to black root rot (LaMondia, 2004; Particka and Hancock, 2005). Affected plants had black roots and young roots with brown lesions, reduced numbers of feeder roots, and reduced plant size. There were three treatments: an untreated control, Abound (azoxystrobin), and Abound plus Phostrol (mono- and dibasic sodium, potassium and ammonium phosphites) arranged in a randomized complete block design where the cultivars were treated as blocks. Treatments were applied to six-row-wide strips of each cultivar. Abound was applied through the irrigation system and Phostrol was applied as a foliar spray (Table 1). The field received three applications of each during the growing season on 31 May, 19 June, and 10 September in 2008, and on 17 June, 8 July, and 26 August in 2009. The irrigation system utilized 1.2-cm nozzles at 413 kPa with high volumes of water (2.1–3.1 cm per hectare). Foliar sprays were applied using a spray boom with three hollow-cone nozzles per row at 561 liters/ha at 896 kPa.

2.3. Plant evaluation

Bed fill was visually assessed several times during the season on a 1 to 5 scale, with 5 = runners healthy and establishing well, full bed; 4 = fewer runner plants than in 5, runner size and establishment otherwise good; 3 = thinning evident; 2 = few runners, mother plants evident; and 1 = few to no runners establishing, few to no mother plants.

For measuring yield, the interior meter of the center row of each plot in the small-plot trial was marked and all ripe berries were picked at each harvest date and combined to calculate total yield for each year (two harvests were completed per year in June, separated by 1 week). In the demonstration trial, within each large treatment plot, there were three areas (3-m-long bed sections in two rows in the front and back of the plot) where measurements were taken from all six locations and considered subsamples throughout this experiment. Berries were

counted at the time of harvest and weighed on location. The berries were picked without regard to size or condition. Average berry weight was calculated by dividing total yield by the total number of fruit harvested. Average yield per hectare was calculated by taking the yield per meter of row and multiplying that by the number of row meters per hectare, based on a row spacing of 1.07 m.

At the end of the season, plants in the small plot and demonstration trials were removed from each plot for determination of fresh and dry plant biomass, root health, and pathogen isolation. In the small plot trial, 2 representative plant samples were in 2007 and 2008 were collected per treatment and 10 root pieces were plated as described below (i.e. 2 plants per plot and 4 replications). In the demonstration trial, a bed fill rating was conducted and representative plant samples were collected in October (5 plants per treatment in 2008 and 10 plants per treatment in 2009). Root health was rated on a 1 to 5 scale with 1 = roots mostly black to dark brown, no finely branched roots; 2 = same as 1, except for one or two finely branched roots; 3 = half of roots light brown and finely branched; 4 = two-thirds of roots light brown and finely branched, 5 = all roots light brown and finely branched (Fig. S1). After the rating, the crown was cut in half just above the uppermost adventitious roots and fresh weights were obtained for both foliage and roots. The foliage and roots were then dried for 48 h at 80 °C to obtain dry weights.

After taking fresh root weight, 1 gram of root tissue was removed for fungal isolations from each of the plants. The roots were surface sterilized with 20% bleach solution (1% NaOCl) for 2 min and then rinsed twice in sterile water for 1 min. The roots were blotted dry on sterile paper towels, and ten 6-mm root pieces were cut and placed on water agar in Petri plates for the small plot trial. For the demonstration trial, 25 root pieces (5 per plant) were collected from harvested plants in 2008 and 50 root pieces (5 per plant) in 2009. Plates were incubated at room temperature (22–23 °C) and examined daily for fungal growth for up to 8 days. If growth was observed, the fungi were sub-cultured on potato dextrose agar and subsequently identified based on culture appearance and morphology (Barnett and Hunter, 1998; Barron, 1968; Domsch et al., 1980). The frequency of specific fungi recovered was calculated based on the total number of fungi that grew out of individual root pieces.

In the demonstration trial, plant-parasitic nematode populations in the plots were assessed by the Michigan State University Diagnostic Services Clinic in 2009. Analysis was conducted on a subset of roots and surrounding soil from the harvested plants. Plant-parasitic nematodes were extracted from 100 cm³ soil using a modified centrifugation/flotation procedure (Jenkins, 1964) and from 1.0 gram of root tissue using a shaker technique (Bird, 1971). Identification was to genus and the nematodes were counted using a dissecting microscope at 40X magnification.

2.4. Statistical analysis

Statistical analyses were performed using the ANOVA when possible and mean separation functions (Fisher's protected least significant difference test at $P \leq 0.05$) of the StatGraphics statistical computer program (StatPoint Inc., Warrenton, VA, USA) after analyzing the equality of variance. If needed, data were log-transformed prior to analysis. For the demonstration trial, subsamples within the plots were averaged for both cultivars (Northeast and Earliglow) and the averaged numbers were used to run ANOVA tests on plant fresh weight, and plant dry weight using the StatGraphics statistical computer program. For categorical variables such as bed fill and root health a Chi-Square test of independence was conducted. Results from this tests show if treatment classifications are independent by each category. Therefore if $P \leq 0.05$, the observed row for a particular case is related to its treatment effect.



Fig. 1. Appearance of 3-year-old 'Jewel' strawberry beds subjected to various post-plant treatments for management of black root rot in 2007 and 2008 in Ottawa County, Michigan, USA. Photographs were taken at bloom on 14 May, 2008 (A–C) and during harvest on 12 June, 2008 (D–F). Untreated control (A, D), azoxystrobin drench (B, E), and azoxystrobin drench + potassium phosphite foliar spray (C, F).

3. Results

3.1. Post-plant treatment efficacy trial

In 2007, visual differences in bed fill were observed among treatments in the small plot trial. However, these were not statistically different due to high variability within the trial. Treatments with azoxystrobin tended to have the highest ratings. In 2008, however, there were obvious and statistically significant differences between treatments in bed fill early in the season and at harvest (Fig. S2.). Chi-Square tests of independence showed the following levels of significance by date in 2007 on May 23 ($P = 0.797$), June 19 ($P = 0.663$), July 23 ($P = 0.392$), and September 14 ($P = 0.448$) and in 2008 on May 14 ($P = 0.067$) and June 12 ($P = 0.058$). Rows treated with azoxystrobin or azoxystrobin + potassium phosphite had the highest bed fill ratings while the untreated control continued to have a poor stand throughout the season (Fig. 1). At harvest, the bed fill ratings in all treatments were statistically higher than in the control with the exception of potassium phosphite. No additional bed fill ratings were taken after harvest because the planting was plowed under by the grower.

Even though trends towards higher yields were apparent in most treatments in 2007, differences in yield, average berry weight and total number of berries were not statistically significant (Fig. 2). However, in 2008 there was a significant increase in yield and total berry number in all treatments except potassium phosphite, compared to the untreated control. The average yields of some treatments (azoxystrobin,

azoxystrobin + potassium phosphite, and the calcium, cobalt, and zinc product) were two to three times higher than that of the untreated control (Fig. 2). In 2008, the equivalent yield per hectare in the azoxystrobin treatment was calculated to be 16,916 kg, whereas the untreated control only yielded an estimated 4393 kg/ha.

In 2007, whole plant fresh and dry weight, and root dry weight were statistically higher in the azoxystrobin treatment than in the untreated control (Table 2). While most other treatments showed improvements, these were not statistically significant. In 2008, whole plant fresh weight and root dry weight were significantly higher in the azoxystrobin + potassium phosphite treatment than in the untreated control. Root dry weight had also improved significantly in the potassium phosphite treatment. Most of the treatment effect appeared to be due to increases in root mass rather than in foliage.

In 2007, differences were observed in root health, particularly in plants treated with azoxystrobin or potassium phosphite (Fig. 3), but these were not statistically significant. However, in 2008, all treatments were statistically better than the untreated control in terms of their root health rating, with the azoxystrobin and azoxystrobin + potassium phosphite treatments having the highest root health rating compared to the untreated control (Fig. 3).

In 2007, *Fusarium* spp. were the most common fungi isolated from roots, followed by *Rhizoctonia*, *Cylindrocarpon*, and *Pythium* spp. (Table 3). The lowest recovery of *Rhizoctonia* spp. occurred from plants treated with azoxystrobin + potassium phosphite. *Trichoderma* spp., which are considered beneficial fungi, were also isolated at low levels. In 2008, *Rhizoctonia* spp. were predominant, followed by *Fusarium*, *Cylindrocarpon*, and *Pythium* spp. Additionally, *Trichoderma* spp. were commonly recovered in all treatments except the untreated control. Furthermore in 2008, all treatments had a lower isolation frequency of *Rhizoctonia* spp. than the untreated control, with azoxystrobin and azoxystrobin + potassium phosphite-treated plants having the lowest *Rhizoctonia* root infection incidence (Table 3).

3.2. On-farm demonstration trial

In the on-farm demonstration trial in 2008, the treatment of azoxystrobin + phosphites resulted in higher ratings for bed fill, whole plant fresh weight, and whole plant dry weight compared to the untreated control (Supplemental Table 1). Azoxystrobin by itself also increased whole plant dry weight compared to the untreated control but not as much as azoxystrobin + phosphites. The root health ratings, though numerically better in the fungicide treatments, were not significantly different based on a Chi-Squared test of independence. In 2009, an infestation of cyclamen mite (*Phytonemus pallidus* (Banks)) as identified by MSU entomologists in the field resulted in considerable variability in plant growth in the field and no consistent effect of the treatments was observed in bed fill or plant fresh weight, although a trend towards somewhat higher ratings for the fungicide treatments was observed. In both 2008 and 2009, however, *Rhizoctonia* was recovered less frequently from plant roots in the azoxystrobin and azoxystrobin + potassium phosphite treatments compared to the untreated control (Table 4).

In the demonstration trial in 2009, the following plant-parasitic nematodes were found: root lesion (*Pratylenchus penetrans*), juvenile root knot (*Meloidogyne hapla*), dagger (*Xiphinema americanum*) and needle (*Longidorus* sp.) nematodes (data not shown). Root lesion nematodes were the most common and ranged from 0 to 25 (average 6.5) per 100 cm³ of rhizosphere soil. Root lesion nematodes in the roots ranged from 5 to 445 (average 64) per gram of root tissue. There was no significant effect of treatment on nematode counts.

4. Discussion

In this study, post-plant applications of fungicides and nutritional amendments were evaluated for their efficacy in slowing or reversing

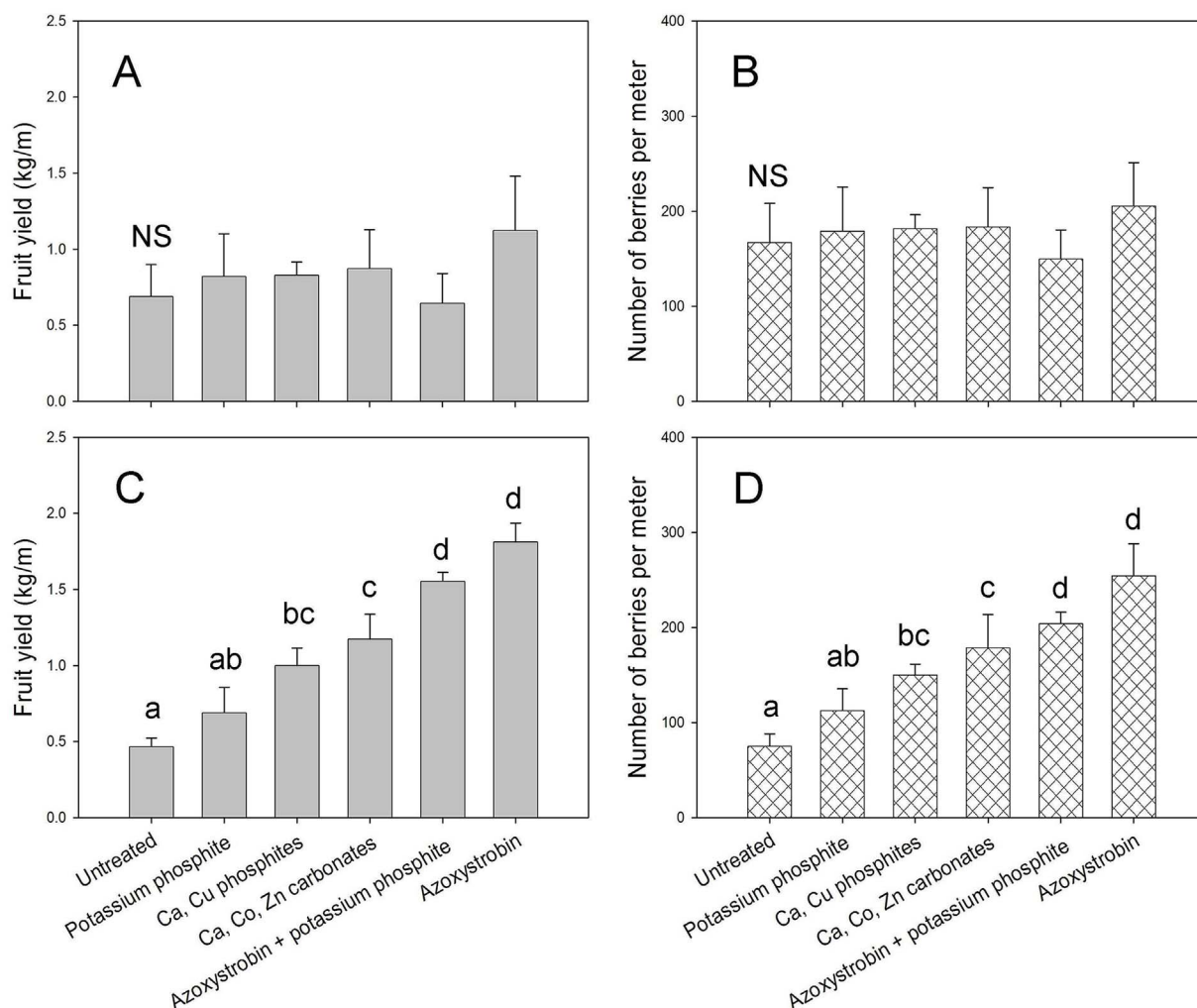


Fig. 2. Effects of post-plant treatments on yield of 'Jewel' strawberries declining due to black root rot in Ottawa County, Michigan, USA in 2007 and 2008. A) Total fruit yield (kg) per meter of row in 2007; B) total number of berries per meter of row in 2007; C) total fruit yield (kg) per meter of row in 2008; D) total number of berries per meter of row in 2008. Treatments with the same letter are not significantly different according to Fisher's protected least significant difference test ($P = 0.05$) ($n = 4$); NS = not significant.¹ Treatments: azoxystrobin (Abound) - drench; potassium phosphite (ProPhyt) - foliar spray; calcium, cobalt, zinc carbonates (Symbex 4x, also contains microbial enzymes) - drench; calcium and copper phosphites (System-Cal) - foliar spray.

black root rot (BRR)-related decline in established strawberry fields. Products were aimed at the root system, either as drenches or as foliar sprays of highly systemic products. The most effective treatments for slowing or reversing the decline of strawberries due to black root rot were azoxystrobin or azoxystrobin + potassium phosphite or other phosphites, resulting in increased plant size and more runner establishment, as evidenced by significantly higher bed fill ratings, higher yield and improved root health compared to the untreated control. Other drench treatments, such as calcium, cobalt, zinc carbonates and calcium and copper phosphites also resulted in significantly higher yield, bed fill, and root health. In addition, significantly fewer fungi, particularly *Rhizoctonia* spp., were isolated from root pieces in treatments that included azoxystrobin. The treatments did not appear to affect plant-parasitic nematodes in the roots or rhizosphere soil. Thus, the effect of the fungicides may be explained by protection of new roots from fungal infection, and recovery of plant growth and yield due to improved root health. The effect appears to be cumulative and consecutive years of treatment may be needed to regain productivity once plant decline has set in. The large-scale demonstration trial in Leelanau County also showed root health improvement, suggesting that scaling up the treatments to farm level works but there may be challenges with evenness of treatments applied via overhead sprinkler irrigation, and other pests like the cyclamen mite may mask treatment effects.

This approach to managing BRR in perennial strawberry fields may have significant benefits to Michigan producers. BRR is one of the most difficult challenges facing commercial strawberry producers in perennial strawberry-growing states and yield losses of 50% or more are common in affected fields (Particka and Hancock, 2005). This is due to the fact that most strawberry growers in Michigan utilize a matted row perennial cropping system where BRR pathogens build up in the planting (Wilhelm, 1987). Soil fumigation has been widely recommended to control BRR in annual plantings, perennial plantings, and nurseries of strawberries (Cal et al., 2004; Particka and Hancock, 2005). However, it is generally assumed that management of black root rot is not possible after planting and the only choice is to plow up the planting once it has become unproductive (Ellis et al., 2006). The soil drench approach with azoxystrobin described in this study is the first attempt to solve this problem. Also of note is that this is already labeled for use in strawberries using various application methods (e.g. pre-plant root dip).

Several alternative control strategies to methyl bromide are currently being considered for soilborne pathogens of strawberries including soil solarization, anaerobic soil disinfestation, biological control, crop rotations, and fungicide applications. While soil solarization has been shown to be effective against *Phytophthora cactorum* in strawberries (Porrás et al., 2007), this method is unlikely to be of use in

Table 2
Effects of post-plant drench and spray treatments on plant weight of perennial strawberries (cv. Jewel) declining due to black root rot in Ottawa County, Michigan, USA in 2007 and 2008.

Treatment ^a	Whole plant fresh weight (g)	Whole plant dry weight (g)	Foliage dry weight (g)	Root dry weight (g)
2007				
Untreated	42.23 ab ^b	16.05 a	2.02 ns	14.03 ab
Azoxystrobin	117.63 c	44.08 b	6.44	37.64 c
Azoxystrobin + potassium phosphite	81.26 bc	31.23 ab	5.35	25.89 bc
Potassium phosphite	60.75 ab	26.79 a	3.44	23.35 ab
Calcium and copper phosphites	36.81 a	19.02 a	7.04	11.98 a
Calcium, cobalt, zinc carbonates	68.38 ab	26.43 a	2.91	23.52 ab
2008				
Untreated	39.80 a	14.81 ns	5.74 ns	9.07 a
Azoxystrobin	53.64 ab	19.47	2.85	16.62 abc
Azoxystrobin + potassium phosphite	76.24 b	27.00	3.37	23.62 c
Potassium phosphite	72.24 ab	27.27	3.85	23.42 bc
Calcium and copper phosphites	41.76 a	14.73	2.39	12.34 ab
Calcium, cobalt, zinc carbonates	63.09 ab	20.93	4.91	16.02 abc

^a Treatments: azoxystrobin (Abound) - drench; potassium phosphite (ProPhyt) - foliar spray; calcium, cobalt, zinc carbonates (Symbex 4x, also contains microbial enzymes) - drench; calcium and copper phosphites (System-Cal) - foliar spray.
^b Values in columns followed by the same letter are not significantly different according to Fisher's Protected Least Significant Difference Test ($p = 0.05$) ($n = 4$); ns = not significant.

the Midwest due to the lack of high soil temperatures. Anaerobic soil disinfestation has been shown to control a wide range of soil-borne pathogens and nematodes in strawberry including *Fusarium oxysporum* f. sp. *fragariae*, *Pythium* and *Rhizoctonia* species (Shennan et al., 2014) and may have promise for black root rot but needs to be evaluated in cooler climates. For biological control, *Trichoderma* spp. inoculations on strawberries have been shown to provide reductions *P. cactorum* populations, and in leather rot incidence (Porrás et al., 2007). Also, a number of microbial isolates have been extensively screened for possible biocontrol agents, but so far success has been minimal due to the lack of reproducibility with the isolates (Martin and Bull, 2002). Also, researchers studying Verticillium wilt of strawberries have found that using broccoli-strawberry crop rotation system could be an economically viable option (Subbarao et al., 2007). However, a follow up study saw that these reductions had no significant effect on *Pythium* populations (which are a part of the BRR complex) so it is unclear if these rotations will aid in control (Njoroge et al., 2009).

Drench treatments have been shown to be effective in other systems, for instance, *Rhizoctonia solani* in sugar beets was effectively controlled by the fungicide penthiopyrad (Liu and Khan, 2016) applied as in-furrow and soil drench applications; however foliar applications were not effective. This was presumably because penthiopyrad needed to be in close proximity to the pathogen for disease control (Liu and Khan, 2016). Considering the success of the treatments incorporating azoxystrobin drenches in a post-plant disease management program, it may be useful to understand the longevity and dynamics of azoxystrobin in these soils in order to time treatments more optimally. Research has shown that after 21 days there is significant biotic breakdown of the chemical and that fungal communities in soil can be significantly altered (Adetutu et al., 2008). Furthermore, investigating possible fungicide resistance seems important because in other fungi significant resistance has been reported for azoxystrobin and other FRAC 11 fungicides primarily due to single amino acid changes (i.e. F129L and G143A) in the cytb gene of the mitochondrial genome (FRAC, 2017).

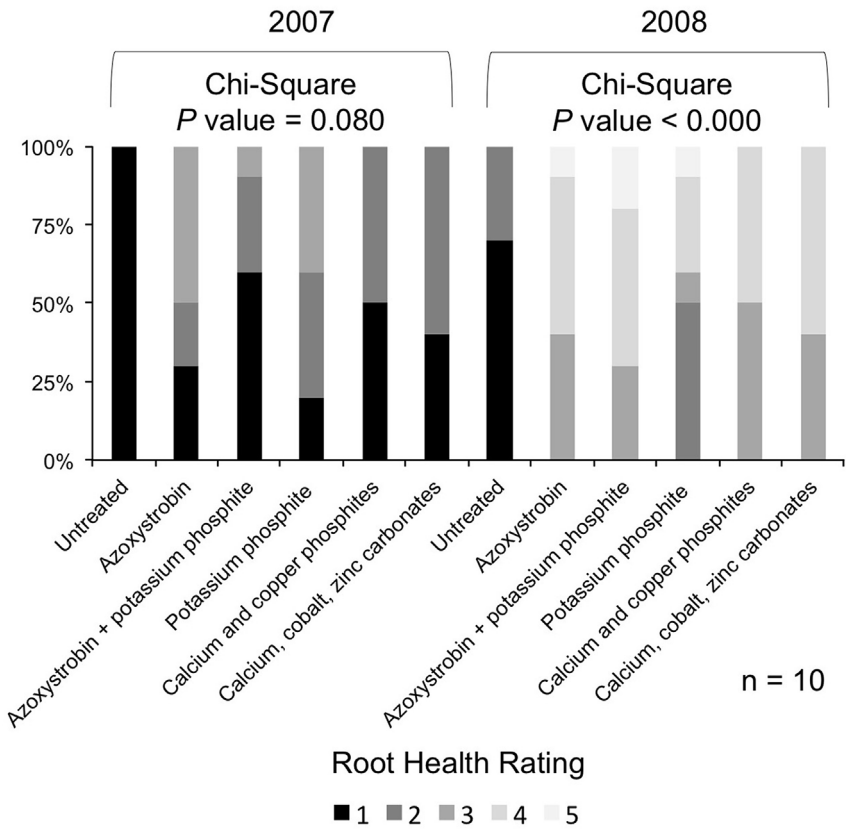


Fig. 3. Effects of post-plant drench and spray treatments on root health of perennial strawberries (cv. Jewel) declining due to black root rot in Ottawa County, Michigan, USA in 2007 and 2008. Rating scale (1–5) used for root health ratings for strawberry black root rot studies in Michigan. 1 = roots mostly black to dark brown, no finely branched roots; 2 = same as 1, except for one or two finely branched roots; 3 = about half of roots light brown and finely branched; 4 = about two-thirds of roots light brown and finely branched; 5 = most roots light brown and finely branched. Chi-Square test of independence demonstrates differences are treatment dependent.

Table 3

Isolation frequency of fungal genera from diseased roots of 'Jewel' strawberries treated with post-plant fungicides and nutritional amendments in Ottawa County, Michigan, USA in 2007 and 2008.

Treatment ^a	Total Number of Fungi Isolated ^b	Isolation Frequency of Fungal genus (%) ^b					
		<i>Rhizoctonia</i>	<i>Cylindrocarpon</i>	<i>Fusarium</i>	<i>Pythium</i>	<i>Trichoderma</i>	Other ^c
2007							
Untreated	43	11.6	11.6	41.9	2.3	2.3	30.2
Azoxystrobin	44	20.5	20.5	29.5	4.5	0.0	25.0
Azoxystrobin + potassium phosphite	54	3.7	7.4	50.0	0.0	0.0	38.9
Potassium phosphite	60	21.7	5.0	31.7	6.7	1.7	35.0
Calcium and copper phosphites	42	35.7	16.7	21.4	4.8	2.4	21.4
Calcium, cobalt, zinc carbonates	42	7.1	7.1	26.2	16.7	0.0	42.9
2008							
Untreated	46	47.8	4.3	15.2	4.3	0.0	28.3
Azoxystrobin	39	7.7	7.7	10.3	5.1	12.8	56.4
Azoxystrobin + potassium phosphite	49	4.1	2.0	14.3	14.3	12.2	59.2
Potassium phosphite	52	19.2	17.3	11.5	9.6	3.8	38.5
Calcium and copper phosphites	61	23.0	13.1	4.9	1.6	14.8	42.6
Calcium, cobalt, zinc carbonates	35	22.9	14.3	22.9	0.0	20.0	20.0

^a Treatments: azoxystrobin (Abound) - drench; potassium phosphite (ProPhyt) - foliar spray; calcium, cobalt, zinc carbonates (Symbex 4x, also contains microbial enzymes) - drench; calcium and copper phosphites (System-Cal) - foliar spray.

^b Fungal isolation frequency calculated based on total number of fungi isolated from 80 root pieces per treatment.

^c Other category includes: *Acremonium*, *Alternaria*, *Botrytis*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Epicoccum*, *Gliocladium*, *Humicola*, *Mucor*, *Nectria*, *Penicillium*, *Phoma*, *Phomopsis*, *Sphaerodes*, *Streptomyces*, and *Tetracladium* spp.

Table 4

Frequency of fungal genera isolated from diseased roots of 'Northeast' or 'Earliglow' strawberries subjected to post-plant treatments in a large-scale demonstration trial conducted in Leelanau County, Michigan, USA in 2008 and 2009.

Treatment ^a	Cultivar	Total Number of Fungi Isolated ^b	Isolation Frequency of Fungal genus (%) ^b					
			<i>Rhizoctonia</i>	<i>Cylindrocarpon</i>	<i>Fusarium</i>	<i>Pythium</i>	<i>Trichoderma</i>	Other ^c
2008								
Untreated	Northeaster	11	45.5	9.1	18.2	0.0	0.0	27.3
Azoxystrobin	Northeaster	9	22.2	0.0	11.1	0.0	0.0	66.7
Azoxystrobin + phosphites	Northeaster	13	23.1	0.0	15.4	7.7	0.0	53.8
Untreated	Earliglow	9	66.7	0.0	0.0	0.0	0.0	33.3
Azoxystrobin	Earliglow	16	25.0	0.0	25.0	0.0	0.0	50.0
Azoxystrobin + phosphites	Earliglow	8	62.5	0.0	0.0	0.0	0.0	37.5
2009								
Untreated	Northeaster	32	37.5	12.5	12.5	21.9	3.1	12.5
Azoxystrobin	Northeaster	27	7.4	0.0	0.0	7.4	0.0	85.2
Azoxystrobin + phosphites	Northeaster	31	6.5	6.5	0.0	0.0	0.0	87.1
Untreated	Earliglow	33	39.4	0.0	27.3	18.2	0.0	15.2
Azoxystrobin	Earliglow	27	7.4	0.0	29.6	18.5	3.7	40.7
Azoxystrobin + phosphites	Earliglow	23	4.3	8.7	8.7	0.0	13.0	65.2

^a Treatments: azoxystrobin (Abound) applied by chemigation; phosphites (Phostrol: mono- and dibasic sodium, potassium and ammonium phosphites) applied as foliar spray.

^b Fungal isolation frequency calculated based on total number of fungi isolated from 25 root pieces per treatment in 2008 and 50 root pieces per treatment in 2009.

^c Other category includes: *Acremonium*, *Alternaria*, *Botrytis*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Epicoccum*, *Gliocladium*, *Humicola*, *Mucor*, *Nectria*, *Penicillium*, *Phoma*, *Phomopsis*, *Sphaerodes*, *Streptomyces*, and *Tetracladium* spp.

Due to the biology of *Rhizoctonia*, resistance may not develop quickly; however, mildly resistant isolates have been reported in *Rhizoctonia solani* AG3 on potatoes (Djébal et al., 2014). One or two well-timed applications per season may provide the measure of control necessary without unduly exposing the pathogen to selection pressure. In addition, alternating with fungicides like potassium phosphite may provide control while managing fungicide resistance development. Further research is needed to confirm the efficacy of post-plant fungicide application through chemigation in on-farm trials. Interestingly, a new fungicide, fluopyram (Indemnify, Bayer Crop Science, Research Triangle Park, North Carolina, USA), in the succinate dehydrogenase inhibitor (SDHI) class has been registered for control of both nematodes and fungal diseases in turfgrass and should be evaluated for control of black root rot for inclusion in a post-plant disease management program to increase the longevity and maintain productivity of perennial strawberry fields.

Acknowledgements

The authors gratefully acknowledge funding from the Michigan State Horticultural Society and the North American Strawberry Growers Association. The authors would also like to thank Phillip De Lange and Gary Bardenhagen for graciously allowing use of their farms for the field experiments. We would also like to thank Fred Warner from the MSU Diagnostic Clinic for providing nematode counts, Jerri Gillett and Laura Miles for critical reading of the manuscript, and Christine Bates for technical assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.cropro.2017.10.012>.

References

- Adetutu, E.M., Ball, A.S., Osborn, A.M., 2008. Azoxystrobin and soil interactions: degradation and impact on soil bacterial and fungal communities. *J. Appl. Microbiol.* 105, 1777–1790.
- Ajwa, H.A., Klose, S., Nelson, S.D., Minuto, A., Gullino, M.L., Lamberti, F., Lopez-Aranda, J.M., 2003. Alternatives to methyl bromide in strawberry production in the United States of America and the Mediterranean region. *Phytopathol. Mediterr.* 42, 220–244.
- Anke, T., 1995. The antifungal strobilurins and their possible ecological role. *Can. J. Bot.* 73, 940–945.
- Barnett, H.L., Hunter, B.B., 1998. *Illustrated Genera of Imperfect Fungi*, fourth ed. APS Press, St. Paul, MN, USA.
- Barron, G.L., 1968. *The Genera of Hyphomycetes from Soil*. Williams and Wilkins, Baltimore, MD, USA.
- Batchelor, T., 1998. Assessment of Alternatives to Methyl Bromide. United Nations Environment Programme (UNEP) Methyl Bromide Technical Options Committee (MBTOC). Nairobi, Kenya.
- Bird, G.W., 1971. Influence of incubation solution on the rate of recovery of *Pratylenchus brachyurus* from cotton roots. *J. Nematol.* 3, 378–385.
- Botha, A., Denman, S., Lamprecht, S.C., Mazzola, M., Crous, P.W., 2003. Characterisation and pathogenicity of *Rhizoctonia* isolates associated with black root rot of strawberries in the Western Cape Province, South Africa. *Australas. Pl. Pathol.* 32, 195–201.
- Cal, A.D., Martinez-Treceno, A., Lopez-Aranda, J.M., Melgarejo, P., 2004. Chemical alternatives to methyl bromide in Spanish strawberry nurseries. *Plant Dis.* 88, 210–214.
- D'Ercole, N., Nipoti, P., Manzali, D., 1988. Research on the root rot complex of strawberry plants. *Acta Hortic.* 265, 497–506.
- Djébal, N., Elkahoui, S., Taamalli, W., Hessini, K., Tarhouni, B., Mrabet, M., 2014. Tunisian *Rhizoctonia solani* AG3 strains affect potato shoot macronutrients content, infect faba bean plants and show in vitro resistance to azoxystrobin. *Australas. Plant Pathol.* 43, 347–358.
- Domsch, K.H., Gams, W., Anderson, T.H., 1980. *Compendium of Soil Fungi*, second ed. Academic Press, New York, NY, USA.
- Ellis, M.A., Funt, R.C., Wright, S., Demchak, K., Wahle, E., Doohan, D., Welty, C., Williams, R.N., Brown, M., 2006. *Midwest Strawberry Production Guide*. Bulletin 926. Ohio State University, Columbus, OH, USA.
- FRAC, 2017. *Mode of Action of Fungicides—FRAC Classification on Mode of Action 2016*. Fungicide Resistance Action Committee. <http://www.frac.info/>.
- Funt, R.C., Ellis, M.A., Welty, C., 1997. *Midwest Small Fruit Pest Management Handbook*. Ohio State University Extension Bulletin 861.
- Glass, B.W., 2008. *Alternatives to Methyl Bromide for Controlling the Black Root Rot Complex of Strawberry*. MS. Thesis. Michigan State University, East Lansing, MI, USA.
- Hancock, J.F., 1999. *Strawberries*. CABI Publishing, Wallingford, UK.
- Hildebrand, A.A., 1934. Recent observations on strawberry root rot in the Niagara Peninsula. *Can. J. Res.* 11, 18–31.
- Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for extracting nematodes from the soil. *Plant Dis. Rep.* 48, 692.
- LaMondia, J.A., 2004. Field performance of twenty-one strawberry cultivars in a black root rot-infested site. *J. Am. Pomol. Soc.* 58, 226–232.
- Liu, Y., Khan, M.F.R., 2016. Penthhiopyrad applied in close proximity to *Rhizoctonia solani* provided effective disease control in sugar beet. *Crop Prot.* 85, 33–37.
- Louws, F.J., Driver, J.G., Leandro, L., 2004. Evaluation of Fungicide Pre-plant Dip and Spray Applications to Manage Phytophthora Crown Rot on Strawberry Plugs. Fungicide and Nematicide Tests 60, SMR041. APS Press, East Lansing, MI, USA.
- Martin, F.N., 2000. *Rhizoctonia* spp. recovered from strawberry roots in central coastal California. *Phytopathology* 90, 345–353.
- Martin, F.N., Bull, C.T., 2002. Biological approaches for control of root pathogens of strawberry. *Phytopathology* 92, 1356–1362.
- Martin, F.N., Hancock, J.F., 1983. Chemical factors in soils suppressive to *Pythium ultimum*. In: Parker, C.A., Rovira, A.D., Moore, K.J., Wong, P.T.W. (Eds.), *Ecology and Management of Soilborne Plant Pathogens*. APS Press, St. Paul, MN, USA, pp. 113–116.
- Martin, S.B., Lucas, L.T., 1984. Characterization and pathogenicity of *Rhizoctonia* spp. and binucleate *Rhizoctonia*-like fungi from turfgrasses in North Carolina. *Phytopathology* 74, 170–175.
- Miles, T.D., Woodhall, J., Miles, L.A., Wharton, P.S., 2013. First report of a binucleate *Rhizoctonia* (AG-A) from potato stems infecting potatoes and sugar beets in the Pacific Northwest. *Plant Dis.* 97, 1657.
- Njoroge, S.M.C., Kabir, Z., Martin, F.N., Koike, S.T., Subbarao, K.V., 2009. Comparison of crop rotation for verticillium wilt management and effect on *Pythium* species in conventional and organic strawberry production. *Plant Dis.* 93, 519–527.
- Particka, C.A., Hancock, J.F., 2005. Field evaluation of strawberry genotypes for tolerance to black root rot on fumigated and nonfumigated soil. *J. Am. Soc. Hortic. Sci.* 130, 688–693.
- Perry, S., Ramsdell, D., 1994. Strawberry Diseases in Michigan. *Ag. Facts Extension Bulletin E-1728*. Michigan State University, East Lansing, MI, USA.
- Porras, M., Barrau, C., Arroyo, F.T., Santos, B., Blanco, C., Romero, F., 2007. Reduction of *Phytophthora cactorum* in strawberry fields by *Trichoderma* spp. and soil solarization. *Plant Dis.* 91, 142–146.
- Rebollar-Alviter, A., Madden, L.V., Ellis, M.A., 2005. Efficacy of azoxystrobin, pyraclostrobin, potassium phosphite, and mefenoxam for control of strawberry leather rot caused by *Phytophthora cactorum*. *Plant Health Prog* 2005-0107-01-RS.
- Roskopf, E.N., Chellemi, D.O., Kokalis-Burelle, N., Church, G.T., 2005. Alternatives to methyl bromide: A Florida Perspective. Online. APSnet Features <http://dx.doi.org/10.1094/APSnetFeature/2005-0605>.
- Sharon, M., Freeman, S., Kuninaga, S., Sneh, B., 2007. Genetic diversity, anastomosis groups and virulence of *Rhizoctonia* spp. from strawberry. *Eur. J. Plant Pathol.* 117, 247–265.
- Shennan, C., Muramoto, J., Lamers, J., Mazzola, M., Roskopf, E.N., Kokalis-Burelle, N., Momma, N., Butler, D.M., Kobara, Y., 2014. Anaerobic soil disinfestation for soilborne disease control in strawberry and vegetable species: current knowledge and future directions. *Acta Hortic.* 1044, 165–175.
- Strong, F.C., Strong, M.C., 1927. Investigations on the black root of strawberries. *Phytopathology* 21, 1041–1059.
- Subbarao, K.V., Kabir, Z., Martin, F.N., Koike, S.T., 2007. Management of soilborne diseases in strawberry using vegetable rotations. *Plant Dis.* 91, 964–972.
- Wilhelm, S., 1987. Black root rot. In: Maas, J.L. (Ed.), *Compendium of Strawberry Diseases*, second ed. APS Press, St. Paul, MN, USA, pp. 53.
- Wing, K.B., Pritts, M.P., Wilcox, W.F., 1994. Strawberry black root rot: a review. *Adv. Strawb. Res.* 13, 11–19.