Provided for non-commercial research and education use. Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Author's personal copy

Biological Control 55 (2010) 92-96



Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon



Efficacy of *Coniothyrium minitans* on lettuce drop caused by *Sclerotinia minor* in desert agroecosystem

P. Chitrampalam ^{a,1}, C.A. Cox ^a, T.A. Turini ^b, B.M. Pryor ^{a,*}

^a Division of Plant Pathology and Microbiology, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA

ARTICLE INFO

Article history: Received 30 January 2010 Accepted 8 July 2010 Available online 13 July 2010

Keywords:
Lettuce drop
Sclerotinia minor
Biological control
Coniothyrium minitans
Paenibacillus polymyxa
Fungicide
Boscolid

ABSTRACT

Field experiments were conducted over 2 years in Yuma County, Arizona and Imperial County, California, to evaluate if increased application rates of a commercial formulation of *Coniothyrium minitans* (Contans) were effective against lettuce drop caused by *Sclerotinia minor*. The efficacy of *C. minitans* at varied application rates were compared to two field isolates of *Paenibacillus polymyxa* and the chemical fungicide Boscolid (Endura). Two applications of manufacture recommended rate of Contans (2.2 kg/ha) did not significantly reduce the incidence of lettuce drop caused by *S. minor*, even though applications at this rate have been shown to be very effective in controlling lettuce drop caused by *Sclerotinia sclerotiorum*. However, two applications of high rates of Contans (6.6, 8.8, or 11 kg/ha), one at planting and one at post-thinning, significantly reduced the incidence of lettuce drop in most trials. Two isolates of *P. polymyxa* each applied at a rate of 9.4 L/ha (10⁹ cfu/ml) were not effective in reducing the incidence of lettuce drop. Two applications of Endura, one at thinning and one at 4 weeks post-thinning, significantly reduced the incidence of lettuce drop in Yuma County, AZ, but not in Imperial County, CA. In summary, successful management of lettuce drop caused by *S. minor* in desert ecosystem could best be achieved with high application rates of *C. minitans*.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Lettuce drop is one of the most common and destructive diseases of lettuce throughout the lettuce producing states of United States and other parts of the world, and causes substantial yield loss every year (Chitrampalam et al., 2008; Melzer and Boland, 1994; Subbarao, 1998; Whipps and Budge, 1990). The disease is caused by two closely related fungi, *Sclerotinia sclerotiorum* (Lib.) de Bary and *Sclerotinia minor* Jagger and both fungi are found in the major lettuce production areas of California and Arizona. *S. minor* is the predominant species in the coastal valley of California, whereas *S. sclerotiorum* is the predominant species in desert lettuce production areas of Yuma County, AZ and Imperial County, CA (Matheron and Porchas, 2004; Subbarao, 1998).

Both fungi produce sclerotia, which function as both survival propagules and primary inoculum in subsequent lettuce crops. *S. minor* infects lettuce primarily through mycelia produced during eruptive germination of sclerotia. However, in addition to mycelial infection, *S. sclerotiorum* may also infect lettuce by ascospores from

apothecia produced by carpogenic germination of sclerotia. Environmental factors such as soil temperature and moisture determine the formation of apothecia and subsequent ascospore production (Subbarao, 1998; Gerlagh et al., 2003; Wu and Subbarao, 2002), but these factors are rarely optimal for the formation of apothecia in desert lettuce production areas (Matheron and Porchas, 2004). Hence, lettuce drop in desert areas is almost always driven by eruptive germination of sclerotia for both *Sclerotinia* species.

Currently, there are no commercial lettuce cultivars with significant resistance to either *Sclerotinia* spp. (Subbarao, 1998). Although, crop rotation of lettuce with broccoli, a non-host, resulted in a significant reduction in number of sclerotia of *S. minor* and subsequent incidence of lettuce drop (Hao et al., 2003), rotation out of lettuce production for any length of time in most areas of Arizona and California is often not justifiable due to the importance of lettuce to the agricultural economy in these two states. In addition, *Sclerotinia* spp. have broad host ranges and extended survival in soil in the absence of hosts (Gerlagh et al., 1999; Subbarao, 1998). Thus, crop rotation is not a suitable management alternative and current management strategies for lettuce drop rely heavily on fungicide applications. However, fungicides currently recommended for lettuce drop, such as dicloran (Botran), iprodione (Rovral), and vinclozolin (Ronilan) have only provided a moderate level

^b University of California Cooperative Extension, Fresno County, CA 93702, USA

^{*} Corresponding author.

E-mail address: bmpryor@u.arizona.edu (B.M. Pryor).

¹ Present address: Department of Plant Pathology, University of California, Davis, c/o U.S. Agricultural Research Station, Salinas, CA 93905, USA.

of control of lettuce drop in most situations (Matheron and Matejka, 1989; Matheron and Porchas, 2004; Subbarao, 1998; Chitrampalam et al., 2008). Moreover, *Sclerotinia* spp., have developed resistance to iprodione and vinclozolin under laboratory condition (Hubbard et al., 1997). The heightened concern over fungicide residue on lettuce crops, negative environmental effects from frequent applications of fungicides, and a desire for higher levels of disease control than that provided by currently available fungicides (Matheron and Porchas, 2004), support the development of non-chemical approaches.

Biological control is a viable alternative to chemical strategies and substantial research has been conducted on evaluating biocontrol strategies for the management of Sclerotinia species in many cropping systems (Subbarao, 1998; Budge and Whipps, 1991; Bremer et al., 2000; Partridge et al., 2006b). Trichoderma-based biocontrol products have been tested against Sclerotinia spp. in both greenhouse and field conditions (Chitrampalam et al., 2008; Phillips, 1986; Whipps and Budge, 1990) and have resulted in varying degrees of success from none to significant levels of control (Budge and Whipps, 1991; Chitrampalam et al., 2008; Rabeendran et al., 2006). Coniothyrium minitans and Sporidesmium sclerotivorum have also been used for control of sclerotia-forming fungi in several cropping systems (Adams and Fravel 1990; Subbarao, 1998; Chitrampalam et al., 2008; Bremer et al., 2000). However, to date only C. minitans has been commercially available. Results from the study by Chitrampalam et al. (2008) revealed that two applications of a commercial formulation of C. minitans, Contans, at manufacturer recommended rates (2.2 and 4.4 kg/ha) significantly reduced the incidence of lettuce drop caused by S. sclerotiorum and significantly increased the yield in desert lettuce experiments. However, in the same experiments C. minitans did not provide effective control against S. minor.

The reason for the inefficiency of *C. minitans* against *S. minor* in lettuce is not known, although differences in the biology between these two sister species may contribute. Under both laboratory and field conditions, the number of sclerotia produced by S. minor is far greater than that produced by S. sclerotiorum (Willetts and Wong, 1980). Thus, reported soil sclerotium populations for S. minor are far greater than for S. sclerotiorum in most cropping systems (Abawi and Grogan, 1979; Patterson, 1986). In addition, sclerotia produced by S. minor are very small compared to those of S. sclerotiorum (Willetts and Wong, 1980). As the parasitism of individual sclerotia by C. minitans is mainly dependent upon the detection of sclerotial exudates by the mycoparasite (Partridge et al., 2006b), the quantity of exudates from small sclerotia may not be sufficient to facilitate optimal parasitism. Thus, if both enormous numbers of sclerotia and small size contribute to reduced efficacy of C. minitans against S. minor, then higher application rates of C. minitans than those previously used for effective parasitism of S. sclerotiorum may be required for effective control of this pathogen.

In addition to fungal mycoparasites, several bacterial species have shown promise as biocontrol agents against Sclerotinia spp. Paenibacillus polymyxa, previously known as Bacillus polymyxa, is a common soil bacterium and has broad spectrum of anti-microbial activities. Results from several laboratory studies showed the potential biocontrol effect of P. polymyxa against several soilborne pathogens including Pythium, Phytophthora, Fusarium, Rhizopus, Aspergillus, and Sclerotinia spp. (Yang et al., 2004; Oedjijono et al., 1993). In 2005, several isolates of *P. polymyxa* were recovered from sclerotia of S. sclerotiorum collected from lettuce production fields in AZ. Preliminary results on the effect of these isolates on S. minor and other soilborne fungi in vitro indicated that they were more efficient in inhibiting mycelial growth of Sclerotinia than commercial strains of Bacillus subtilis used as the active agent in several commercially available bacterial-based biocontrol products (unpublished results). This suggested that these strains may have additional utility in biocontrol strategies for lettuce drop management programs in the southwest deserts.

The long term objective of our research is to develop biocontrol strategies for management of lettuce drop caused by both *Sclerotinia* spp. in desert agroecosystems of Arizona and California. The specific objective of this study was to evaluate if increased applications rates of commercially formulated *C. minitans* increased efficacy against lettuce drop caused by *S. minor*. An additional objective was to evaluate the efficacy of native *P. polymyxa* strains against *S. minor* under field conditions.

2. Materials and methods

Field experiments were conducted at the University of California Desert Agricultural Research and Extension Center, Holtville, CA, in 2006-2007 and 2007-2008, and at the University of Arizona Yuma Agricultural Center, Yuma, AZ in 2007-2008 and 2008-2009 to evaluate different application rates of commercially available C. minitans (ContansTM) along with P. polymyxa strains against lettuce drop caused by S. minor. Experiment sites were free from both lettuce cultivation and Sclerotinia disease in the previous 2-3 years. This experiment utilized a randomized complete block design with three replicate blocks. Sclerotia of S. minor produced in the laboratory according to methods described by Matheron and Porchas (2004) were used as disease inoculum for all trials. Inoculum was evenly broadcast by hand across the top of each bed immediately before planting and was lightly incorporated into the top centimeter of soil during seeding. In California experiments, two levels of sclerotia inoculum (1.45 and 14.5 g of sclerotia/40 m bed) and in Arizona experiments, three levels of sclerotia inoculum (3.69, 7.25 and 14.5 g/40 m bed) were used to test the efficacy of different treatments under different pathogen pressures. Uninfested control treatments consisted of no inoculum and infested control treatments consisted of no treatment. Crisphead (cv. Winterhaven) lettuce, known to be very susceptible to lettuce drop, was used as the host. Lettuce was planted on beds with 102 cm between bed centers and two rows of lettuce spaced 30 cm apart per bed. Each treatment plot consisted of four 10 m beds. Within each plot, all beds received pathogen inoculum and/or biocontrol or chemical applications, but only the center two beds were evaluated to fully separate the effect of each treatment. Application rates tested for Contans (C. minitans; Prophyta, Germany) in California experiments were 2.2 and 11 kg/ha. As the results from our previous study conducted at Arizona (Chitrampalam et al., 2008) and the results from the first year (2006–2007) of the CA experiment of the current study revealed that the Contans at 2.2 kg/ha did not provide any significant control against S. minor, the recommended rate of Contans (2.2 kg/ha) was excluded in Arizona experiment which was conducted a year later. In Arizona, application rates were 4.4, 6.6, 8.8, and 11 kg/ha. Each rate of Contans was properly suspended in 1.9 L of water in a sprayer and applied evenly across the planting bed surface. Contans was applied twice, once immediately after planting and once after thinning at approximately 4 weeks post-emergence.

Paenibacillus polymyxa inoculum was prepared by collecting the log phase culture grown in L media (tryptone 10 g, yeast extract 5 g, NaCl 5 g and p-glucose 1 g/L of water) at 25 °C. The raw bacterial culture in L media was mixed with 1.9 L of water in a sprayer at the rate of 9.4 l/ha with concentration of 10⁹ cfu/ml and applied evenly across the planting bed surface three times (immediately after planting, at thinning, and at one month post-thinning). As P. polymyxa-095 did not show any promise of control of lettuce drop in 2006–2007 of California experiment, it was excluded from Arizona experiment which was conducted a year later. A chemical treatment consisting of two applications of Endura (a.i. = Boscolid)

at 0.77 kg/ha at thinning and at one month post-thinning, was included for comparison as a chemical standard recommended in desert lettuce production.

Sprinkler irrigation was used for the duration of all experiments. All other cultural practices standard for desert lettuce production in Arizona were applied for each trial including pre-plant applications of the herbicide Kerb (a.i. = pronamide; Dow Agro-Sciences, Indianapolis, IN), pre-plant and supplemental fertilization, and manual thinning and weeding as needed. At plant maturity and harvest, the numbers of asymptomatic healthy, lettuce plants from the center two beds were recorded and the incidence of lettuce drop was calculated based upon the number of asymptomatic healthy heads in uninfested control plots.

2.1. Statistical analysis

Disease incidence (DI) for each plot was calculated by subtracting the number of asymptomatic heads at harvest from the number of heads in the non-inoculated control plots, then dividing by the number of heads in the non-inoculated control plots. Analysis of variance was performed on values of DI for each treatment using Sigmastat software package (Systat Software Inc., San Jose, CA, USA). Treatments on plots with varying density of sclerotial inoculum were analyzed separately. A χ^2 test for homogeneity of variance for data from each year in each location was carried out to test whether the 2 years of data from Yuma and from Holtville experiments could be combined as single data sets.

3. Results

3.1. California trials

The χ^2 test for homogeneity of variance revealed that the data between the 2 years were significantly different (P = 0.001) and so the data sets were analyzed separately. In 2006-2007, the incidence of lettuce drop in control plots with low and high inoculum levels were 5% and 56%, respectively. In the experiment with low sclerotial inoculum, neither the application of biocontrol products nor chemical application resulted in a difference in lettuce drop incidence relative to the control plots (Table 1). However, two applications of the high rate of Contans and two application of Endura resulted in a lower incidence of disease (1% and 2%, respectively), but were not significantly different (P = 0.941 and 0.979, respectively) compared to infested control plots. In plots with high level of sclerotia inoculum, only two applications of the high rate of Contans resulted in significantly lower incidence of disease (16%) compared to infested control plots (56%). The incidence of disease in plots treated with either P. polymyxa-011, Contans (recom-

Table 1Effect of different biocontrol and chemical products on lettuce drop incidence in plots infested with different level of sclerotial inoculum of *Sclerotinia minor* in Holtville, CA.

Treatments	Percent disease incidence			
	2006–2007		2007-2008	
	I ^a	IIp	I ^a	IIp
Untreated infested control	5.2a	55.9a	1.2a	22.9a
P. polymyxa-011	7.9a	30.7ab	0.0a	23.5a
P. polymyxa-095	4.6a	45.3a	0.0a	27.2a
Contans 2.2 kg/ha	2.5a	39.5ab	2.2a	11.3a
Contans 11 kg/ha	1.0a	16.1b	0.0a	11.6a
Endura	2.0a	34.1ab	0.0a	11.0a

Column with different letters are significantly different according to Tukey's test (P < 0.05)

mended rate), or Endura was neither significantly different from that of the control plots nor from the high Contans application (Table 1).

In 2007–2008, the incidence of lettuce drop in control plots with low and high sclerotia inoculum was 1% and 23%, respectively, which were considerably lower than that of the 2006–2007 experiment. With low inoculum level, neither the application of biocontrol products nor the application of Endura resulted in a significant difference in lettuce drop incidence relative to the control plots (Table 1). At the high inoculum level, plots treated with either low or high rates of Contans (2.2 or 11 kg/ha) or Endura resulted in lower disease incidence (11%, 12%, and 11%, respectively) compared to control plots, but these differences were not statistically significant (P = 0.413, 0.438, and 0.388, respectively). In addition, there was no significant difference in the disease levels between the low and high rate of Contans at either low (P = 0.963) or high $S.\ minor$ inoculum levels (P = 0.388) (Table 1).

3.2. Arizona trials

The χ^2 test for homogeneity of variance revealed that the data between the 2 years were not significantly different (P = 0.9887) and the data sets were combined and analyzed as one data set. The disease incidence in the control plots containing low (3.69 g sclerotia/40 m bed), medium (7.25 g sclerotia/40 m bed), and high (14.5 g of sclerotia/40 m bed) level of inoculum were 20%, 38%, and 53%, respectively (Table 2). In plots containing low level of S minor inoculum, two applications of high rate of Contans (6.6 and 11 kg/ha) and two applications of Contans (4.4 kg/ha) combined with one application of Endura resulted in a significantly less incidence of disease (5%, 2%, and 3%, respectively) compared to the infested control plots. The incidence of disease from other treatments including the fungicide Endura were not significantly different from either control plots or from the treatments that significantly reduced the incidence of disease.

In plots containing medium level of sclerotia inoculum (7.25 g of sclerotia/40 m bed), the plots treated with either 6.6, 8.8, or 11 kg/ha Contans resulted in significantly lower incidence of disease than the infested control plots (Table 2). There was no significant difference in lettuce drop suppression among the Contans treatments. Treatment with two applications of Endura alone and two applications of Contans (4.4 kg/ha) combined with one application of Endura also significantly decreased the incidence of disease compared to the infested control. The incidence of disease in plots treated with *P. polymyxa*-011 combined with Endura was

Table 2Effect of different biocontrol and chemical products on lettuce drop incidence in plots infested with different level of sclerotial inoculum of *Sclerotinia minor* in Yuma, AZ in combined 2007–2008 and 2008–2009 trials.

Treatments	Percent disease incidence Sclerotial inoculum level (g of sclerotia/ 40 m bed)		
	3.69	7.25	14.5
1. Untreated infested control	19.5a	38.4a	52.6a
2. Contans 4.4 kg/ha	15.1ab	29.7ab	37.3ab
3. Contans 6.6 kg/ha	5.3b	17.5b	35.3b
4. Contans 8.8 kg/ha	8.9ab	22.5b	34.0b
5. Contans 11 kg/ha	2.3b	22.3b	25.9b
6. Paenibacillus polymyxa-011	14.8ab	39.7a	45.9ab
7. Endura	8.4ab	22.0b	22.0b
8. Combined treatment 2 and 7	3.2b	22.3b	27.8b
9. Combined treatment 2 and 6	9.9ab	28.1ab	47.4ab
10. Combined treatment 6 and 7	8.8ab	25.7ab	33.1b

Column with different letters are significantly different according to Tukey's test (P < 0.05).

^a Plots contained low level of sclerotial inoculum (1.45 g of sclerotia/40 m bed).

^b Plots contained high level of sclerotial inoculum (14.5 g of sclerotia/40 m bed).

neither significantly different from that of control plots (P = 0.222) nor from that of plots that yielded significantly lower disease incidence (P = 1.000) (Table 2).

In plots containing high level of sclerotial inoculum, two applications of the fungicide Endura yielded the lowest incidence of disease (22%) followed by the highest rate of Contans (26%) and Contans combined with Endura (28%), and all were significantly different from the control (53%). Plots treated either with 6.6 and 8.8 kg/ha of Contans were also significantly different from the control (35% and 34%, respectively). There was no significant difference between any of the Contans treatments. The incidence of disease from plots treated with *P. polymyxa*-011 combined with one application of Endura (33%) was also significantly lower than that of the control. Treatment of *P. polymyxa* alone or combination with Contans did not significantly reduce the incidence of disease compared to the control (Table 2).

4. Discussion

This study demonstrated that lettuce drop caused by S. minor could be successfully managed with the commercial biocontrol product Contans (C. minitans) in desert agroecosystem. However, the rate of application of Contans required to manage S. minor in the field was much higher than that previously reported to manage S. sclerotiorum (Chitrampalam et al., 2008). Only the increased rates of Contans effectively suppressed S. minor at both locations when it was applied twice during the crop cycle: once at planting and once at thinning. Although the same type of sclerotial inoculum was used in 2006-2007 and 2007-2008 in California experiment, the disease incidence induced by S. minor in 2007-2008 was three times lower than that of in 2006-2007. As the weather factors observed in 2007-2008 at Imperial County, were similar to that observed in 2006-2007 (Anonymous, 2008b), and the sclerotia inoculum used at Imperial County, were also from the same sclerotia source that induced substantial disease in Yuma experiment in 2007–2008, the effect of weather factor and efficiency of sclerotia inoculum for low disease incidence was discounted. The difference was perhaps more likely due to poor field management in 2007-2008 (untimely irrigation, ineffective weeding, etc.) which may have hindered both S. minor and C. minitans growth resulting in low disease incidence and suboptimal disease suppression even with high rates of Contans.

The results from this study were consistent with the result from Chitrampalam et al. (2008) and reiterated that the recommended rates of Contans (2.2 and 4.4 kg/ha) was insufficient to control lettuce drop caused by S. minor. The reason for the required higher level of Contans required to suppress S. minor could be due to a combination of (1) poor survivability or infectivity of C. minitans in desert ecosystems at the time of planting, (2) the reduced amount of exudates released from small size sclerotia that stimulate C. minitans parasitism, and/or (3) a much greater number of sclerotia produced by S. minor compared to S. sclerotiorum. Results from previous study showed that mycoparasitic activity of C. minitans was as high as 98% at temperatures ranging from 14 to 22 °C and the activity decreased dramatically to 9% at temperatures >28 °C (McQuilken et al., 1997). The average maximum air temperature in lettuce production areas in both Yuma and Imperial County during lettuce planting and at the time of first application of biocontrol was 26-27 °C (Anonymous, 2008a,b), which is comparatively higher than the temperature favorable for C. minitans growth and sclerotial parasitism (Partridge et al., 2006a). Thus, the higher temperature prevalent in lettuce production field at the time of application of biocontrol product might reduce survival and subsequent establishment of C. minitans population in the soil.

Additionally, C. minitans cannot grow saprophytically in soil and requires the attraction to and parasitism of sclerotia in soil for optimal survival. Attraction to and parasitism of sclerotia in soil is mainly dependant upon stimulants exuded from sclerotia, and to some extent soil microfauna (Grenadine and Marciano, 1999; Partridge et al., 2006b; Williams and Whipps, 1995). As S. minor produces small size sclerotia, the amount of stimulants exuded from these sclerotia are likely less than that from sclerotia of S. sclerotiorum, which are much larger and likely release more exudates. Thus, the potential of C. minitans for effective attraction to and parasitism of sclerotia of S. sclerotiorum via exudates may be higher at the recommended rates of Contans (2.2-4.4 kg/ha), even considering exposure to high temperatures and low C. minitans survivability. However, under the same condition, the potential of *C. minitans* for effective attraction to and parasitism of sclerotia of S. minor may be greatly reduced. In addition, the direct contact between sclerotia and C. minitans spores is very critical for infection (Budge and Whipps, 1991: Partridge et al., 2006b). Therefore, the increase in application rate of C. minitans (6.6–11 kg/acre) could possibly increase the quantity of surviving C. minitans inoculum and maximize the chance of contact between C. minitans spores and the smaller S. minor sclerotia, subsequently increasing the chance of effective parasitism. Furthermore, as the temperature at the time of planting in the lettuce production areas of Yuma and Imperial are not optimal for *C. minitans* survival, whereas the temperature at the time of thinning is more favorable, one application of a high rate of Contans at the time of thinning may alone be sufficient to successfully manage lettuce drop caused by S. minor. However, further studies would be needed to support these hypotheses. Previous studies have shown that C. minitans can successfully parasitize the sclerotia of S. minor and subsequently reduced the incidence of disease in lettuce and peanut under very specific conditions (Isnaini and Keane, 2007; Rabeendran et al., 2006), thus, the potential for enhancing Contans performance in specific applications or application window is quite possible.

Recently, Endura has been recommended for the control of lettuce drop caused by S. minor and results from a previous study revealed that it significantly improved lettuce drop control (Matheron and Porchas, 2004). In this study, Endura served as a standard fungicide at both locations, however, the fungicide significantly suppressed the lettuce drop in only one location. Reasons for the differential performance of Endura with different location are not known. However, it has been suggested in the previous study that the half life of any active ingredients in the field depends on the interaction of several factors, of which hydrolytic and photochemical breakdown, microbial conversion and decomposition, and movement of the active ingredients are considered as the major factors. Slade et al. (1992) also found that the efficacy of the fungicides against Sclerotium cepivorum in onion were significantly lower in non-sterile soil than that in sterilized soil and the reduced efficacy was related to the presence of microbes in the non-sterile soil. Moreover, microbial degradation of fungicides is often exacerbated by high soil pH >6.5 (Subbarao, 1998). As the pH (>7) and soil types (silty clay loam) are relatively similar at both experimental locations (Anonymous, 2010a,b), the reason for the differential performance of Endura at these two locations could possibly be due to the difference in the soil microbial diversity and population.

Paenibacillus polymyxa is a common soil bacterium and enhances plant growth either indirectly by secreting diffusible metabolites, fixing atmospheric nitrogen, living as an endophyte, and, most importantly, antagonizing deleterious plant pathogens by producing antifungal and antibacterial metabolites (Dijksterhuis et al., 1999; Choong-Min et al., 2005). Although both isolates of *P. polymyxa* successfully inhibited the growth of both *Sclerotinia* spp. *in vitro* (data not shown), they did not control *S. minor* in field

conditions. Reasons for the ineffectiveness of *P. polymyxa* against *S.* minor in field condition are not known. As both isolates of P. polymyxa grow well at 30 °C, the higher temperature prevalent in Yuma and CA at the time of lettuce production was likely not the reason for the ineffectiveness. In previous studies on bean, a strain of P. polymyxa isolated from the senescent blossoms of bean was shown to inhibit both mycelial and ascospore growth of S. sclerotiorum in vitro, but was ineffective in controlling white mold under field conditions. It was presumed that this ineffectiveness was due to its inability to replace native epiphytic bacterial population and flourish as an epiphyte (Yuen et al., 1991) and, thus, suggested that resident microbial communities play a critical role in the efficacy of this potential biocontrol agent. In this study, the soil microbial communities at the experimental plots were not characterized and the survival of P. polymyxa after the application were not determined, thus, the impact of native soil microbial community on P. polymyxa survival and disease suppression cannot be discounted. Formulation is also very critical for the success of any biocontrol agent in the field, and protects the antagonist from desiccation and increases their stability and viability in the field (Zidack and Quimby, 2002). In this study, P. polymyxa was applied as an aqueous log phase culture as opposed to a more exacting commercial formulation, which could also be one of the possible reasons for its failure in the field.

In summary, results from this study clearly demonstrated that the management of lettuce drop caused by *S. minor* is possible using the biocontrol agent *C. minitans*, but requires different strategies than the management of lettuce drop caused by *S. sclerotiorum*. Additionally, the fungicide Endura was not consistently effective against *S. minor*. Future studies will focus more on the environmental conditions that contribute to optimal survival of *C. minitans* inoculum following application, and on the factors that contribute to optimal parasitism of sclerotia of *Sclerotinia* species in soil in desert agroecosystems.

Acknowledgments

This work was supported in part by the University of Arizona, College of Agriculture and Life Sciences, and the Arizona Department of Agriculture Grant SCBGPO7-17.

References

- Abawi, G.S., Grogan, R.G., 1979. Epidemiology of diseases caused by *Sclerotinia* species. Phytopathology 69, 899–904.
- Adams, P.B., Fravel, D.R., 1990. Economical biological control of *Sclerotinia* lettuce drops by *Sporidesmium sclerotivorum*. Phytopathology 80, 1120–1124.
- Anonymous, 2008a. Arizona Meteorological Network, University of Arizona, Tucson, AZ.
- Anonymous, 2008b. California Weather Data: Report, University of California, Integrated Pest Management Program.
- Anonymous, 2010a. Natural Resources Conservation Service, United States of Department of Agriculture.
- Anonymous, 2010b. Yuma Agricultural Center, University of Arizona, Tucson, AZ. Bremer, E., Huang, H.C., Selinger, L.J., Davis, J.S., 2000. Competence of *Coniothyrium minitans* in preventing infection of bean leaves by *Sclerotinia sclerotiorum*. Plant Pathology Bulletin 9, 69–74.
- Budge, S.P., Whipps, J.M., 1991. Glasshouse trials of *Coniothyrium minitans* and *Trichoderma* species for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. Plant Pathology 40, 59–66.
- Chitrampalam, P., Figuli, P.J., Matheron, M.E., Subbarao, K.V., Pryor, B.M., 2008. Biocontrol of lettuce drop caused by *Sclerotinia sclerotiorum* and *S. minor* in Desert Agroecosystems. Plant Disease 92, 1625–1634.
- Choong-Min, R., Chia-Hui, H., Locy, R.D., Kloepper, J.W., 2005. Study of mechanisms for plant growth promotion elicited by rhizobacteria in *Arabidopsis thaliana*. Plant and Soil 268, 285–292.

- Dijksterhuis, J., Sanders, M., Gorris, L.G.M., Smid, E.J., 1999. Antibiosis plays a role in the context of direct interaction during antagonism of *Paenibacillus polymyxa* towards *Fusarium oxysporum*. Journal of Applied Microbiology 86, 13–21.
- Gerlagh, M., Goosen-van de Geijn, H.M., Fokkema, N.J., Vereijken, P.F.G., 1999. Longterm biosanitation by application of *Coniothyrium minitans* on *Sclerotinia sclerotiorum*-infected crops. Phytopathology 89, 141–147.
- Gerlagh, M., Goossen-van de Geijn, H.M., Hoogland, A.E., Vereijken, P.F.G., 2003. Quantitative aspects of infection of Sclerotinia sclerotiorum sclerotia by Coniothyrium minitans-timing of application, concentration and quality of conidial suspension of the mycoparasite. European Journal of Plant Pathology 109 489–502
- Grenadine, A., Marciano, P., 1999. Interaction between *Sclerotinia sclerotiorum* and *Coniothyrium minitans* strains with different aggressiveness. Phytoparasitica 27, 1–6
- Hao, J.J., Subbarao, K.V., Koike, S.T., 2003. Effects of broccoli rotation on lettuce drop caused by *Sclerotinia minor* and on the population density of sclerotia in soil. Plant Disease 87, 159–166.
- Hubbard, J.C., Subbarao, K.V., Koike, S.T., 1997. Development and significance of Dicarboximide resistance in *Sclerotinia minor* isolates from commercial lettuce fields in California. Plant Disease 81, 148–153.
- Isnaini, M., Keane, P.J., 2007. Biocontrol and epidemiology of lettuce drop caused by *Sclerotinia minor* at Bacchus march, Victoria. Australasian Plant Pathology 36, 295–304.
- Matheron, M.E., Matejka, J.C., 1989. Evaluation of new fungicides for the control of leaf drop of lettuce. Journal of the Rio Grande Valley Horticultural Society 42, 71–74.
- Matheron, M.E., Porchas, M., 2004. Activity of Boscolid, Fenhexamid, Fluazinam, Fludioxonil, and Vinclozolin on growth of *Sclerotinia minor* and *S. sclerotiorum* and development of lettuce drop. Plant Disease 88, 665–668.
- McQuilken, M.P., Budge, S.P., Whipps, J.M., 1997. Biological control of *Sclerotinia sclerotiorum* by film-coating *Coniothyrium minitans* on to sunflower seed and sclerotia. Plant Pathology 46, 919–929.
- Melzer, M.G., Boland, G.J., 1994. Epidemiology of lettuce drop caused by *Sclerotinia minor*. Canadian Journal of Plant Pathology 16, 170–176.
- Oedjijono, M., Line, A., Dragar, C., 1993. Isolation of bacteria antagonistic to range of plant pathogenic fungi. Soil Biology Biochemistry 25, 247–250.
- Partridge, D.E., Sutton, T.B., Jordan, D.L., 2006a. Effect of environmental factors and pesticides on mycoparasitism of *Sclerotinia minor* by *Coniothyrium minitans*. Plant Disease 90, 1407–1412.
- Partridge, D.E., Sutton, T.B., Jordan, D.L., Curtis, V.L., Bailey, J.E., 2006b. Management of Sclerotinia blight of peanut with the biological control agent *Coniothyrium minitans*. Plant Disease 90, 957–963.
- Patterson, C.L., 1986. The comparative biology, epidemiology, and control of lettuce drop caused by Sclerotinia minor and S. sclerotiorum, and the genetic analysis of vegetative and sexual compatibility in S. minor. Ph.D. Thesis, University of California, Davis.
- Phillips, A.J.L., 1986. Factors affecting the parasitic activity of Gliocladium virens on the sclerotia of Sclerotinia sclerotiorum and a note on its host range. Journal of Phytopathology 116, 212–220.
- Rabeendran, N., Jones, E.E., Moot, D.J., Stewart, A., 2006. Biocontrol of Sclerotinia lettuce drop by Coniothyrium minitans and Trichoderma hamatum. Biological Control 39, 352–362.
- Slade, E.A., Fullerton, R.A., Stewart, A., Young, H., 1992. Degradation of the dicorboximide fungicides iprodione, vinclozolin, and procymidione in Patumahoe clay loam soil, New Zealand. Pesticide Science 35, 95–100.
- Subbarao, K.V., 1998. Progress toward integrated management of lettuce drop. Plant Disease 82, 1068–1078.
- Whipps, J.M., Budge, S.P., 1990. Screening for sclerotial mycoparasites of Sclerotinia sclerotiorum. Mycological Research 94, 607–661.
- Willetts, H.J., Wong, J.A.L., 1980. The biology of *Sclerotinia sclerotiorum*, *S. trifoliorum*, and *S. minor* with emphasis on specific nomenclature. Botanical Review 46, 101–165.
- Williams, R.H., Whipps, J.M., 1995. Growth and transmission of *Coniothyrium minitans* in soil. Bulletin of the International Organization for Biological Control 18, 39–43.
- Wu, B.M., Subbarao, K.V., 2002. Sclerotial survival of *S. minor* and *S. sclerotiorum* in California. Phytopathology 92, S88 (Abstr.).
- Yang, J., Kharbanda, P.D., Mirza, M., 2004. Evaluation of *Paenibacillus polymyxa* PKB1 for biocontrol of *Pythium* disease of cucumber in a hydroponic system. In: Proceedings of the XXVI IHC-Managing Soil-Borne Pathogens. Acta Horticulturae 635, ISHS 2004.
- Yuen, G.Y., Godoy, G., Steadman, J.R., Kerr, E.D., Craig, M.L., 1991. Epiphytic colonization of dry edible bean by bacteria antagonistic to *Sclerotinia* sclerotiorum and potential for biological control of white mold disease. Biological Control 10, 293–301.
- Zidack, N.K., Quimby Jr., P.C., 2002. Formulation of bacteria for biological weed control using the stabilize method. Biocontrol Science and Technology 12, 67–74.