Arizona Iceberg Lettuce Research Council
Lettuce Research Proposal Final Report, 2007-2008

Part I. IDENTIFICATION

A. Title: Development of an effective biocontrol strategy for the management of lettuce drop disease caused by the plant pathogenic fungus *Sclerotinia minor*

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C. Location of research: The University of Arizona, Tucson, AZ, and Yuma Agricultural Research Station, Yuma, AZ.

Significance, need, and benefit to lettuce industry.

Arizona and California account for over 95% of the US lettuce production with approximately 73% of production devoted to crisphead (iceberg) lettuce, 15% to romaine lettuce, and 12% to leaf lettuce, both green and red. Disease management is a principle activity for growers of all types of lettuce as viral, bacterial, and most importantly, fungal diseases can seriously impact quality and yield. Among the important fungal diseases affecting lettuce, lettuce drop, caused by *Sclerotinia* spp., is one of the most common and destructive diseases resulting in yearly losses in all lettuce-production regions for all types of lettuce. Approximately 60-70% of the lettuce acreage is treated with fungicides specifically for lettuce drop, and even with the use of these chemicals losses from this disease can range from 5 to 50%. Thus, improved management strategies for lettuce drop, including development of non-chemical treatments, will have direct benefits to lettuce growers in both Arizona and California.

Brief summary of previous research relevant to proposal

Lettuce drop is caused by two closely related fungi, *Sclerotinia minor* and *S. sclerotiorum*. Both fungi are present in the lettuce-growing areas of Arizona but *S. sclerotiorum* is the predominant species. However, and most importantly, the occurrence of *S. minor* in the desert production areas has been increasing in recent years. This confounds current disease management strategies as the two fungi do not respond similarly to control tactics.

Both fungi produce hard, durable structures known as sclerotia, which function as survival structures and also as disease inoculum in subsequent lettuce crops. Although both fungi cause similar disease symptoms in all lettuce types, their ecology is different in several respects. Whereas population densities of *S. sclerotiorum* sclerotia may be in the range of 5-20 sclerotia/100 g soil on the high end, *S. minor* may produce over 200 sclerotia in the same volume of soil (36). This presents significant challenges to controlling *S. minor* in lettuce fields, and this challenge has been demonstrated in our previous studies, which are detailed below.

Considerable research has been devoted to evaluating biological control strategies for the management of *Sclerotinia* diseases in different cropping systems, although, most studies reveal only moderate success. However, Studies conducted by Pryor from 2001-2003, and Pryor and Subbarao from 2003-2005 revealed that Contans was capable of reducing the incidence of lettuce drop due to *S. sclerotiorum* by over 95%, which was far superior to any chemical product tested. Interestingly, *C. minitans* at label rates had only a limited effect against *S. minor* which an increasingly common species in the desert regions. Most recently, a very interesting result from field trials conducted by our lab in 2006-2007 revealed that two applications at higher-than-label rates of Contans (10 lb/acre) reduced the incidence of lettuce drop disease caused by *S. minor* by 85%, even under very high disease pressure. This level of disease suppression has not been achieved previously with any other biocontrol agents against *S. minor* and at the disease pressure tested.

Another promising biocontrol agent that might be used in conjunction with *C. minitans* is *Paenibacillus polymyxa*, previously known as *Bacillus polymyxa*, a common soil bacterium with a broad spectrum of antimicrobial activities. Results from field trials in 2006-2007 revealed that three applications of *P. polymyxa*-011 significantly prevented the lettuce drop pathogen caused by *S. minor* and was the next best biocontrol treatment to high rates of Contans (10 lb/acre). And both the high rate of Contans and Paenibacillus performed better than Endura.

Long-range objectives, including estimated time frame to achieve objectives.

The long-range objective of this study is to develop an effective biocontrol strategy for control of *Sclerotinia minor* in lettuce. This would clearly compliment our success in controlling S. sclerotiorum and would complete our ultimate objective of several years of providing effective and economical biocontrol options for managing lettuce drop in Arizona, regardless of the causal agent.

Objectives for 2007-2008 proposed research.

Specific objectives are:

1. Optimize the application rate of Contans that results in the highest level of suppression of *S. minor* at the lowest application rate. Continue studies on the efficacy of *Paenibacillus polymyxa* and evaluate the complimentary activities of this bacterium with Contans and other chemical fungicides to successfully manage *Sclerotinia minor* in lettuce under field conditions.
2. Determine inhibition spectrum of *Paenibacillus polymyxa* against other soilborne pathogens of lettuce such as *Fusarium oxysporum lactucae, Botrytis cinarea*, *Rhizoctonia solani*, *Phoma exigua*, and *Verticillium dahliae*, and test the compatibility of *P. polymyxa* with chemical fungicide used against lettuce drop pathogens in in vitro.

Results

Objective 1: Optimize the application rate of Contans and determine the compatibility of *Paenibacillus polymyxa* isolates with chemical fungicide to successfully manage *Sclerotinia minor* in lettuce under field conditions

In order to optimize the application rate of Contans and determine the compatibility of *P. polymyxa* isolates with chemical fungicide to successfully manage the lettuce drop disease caused by *Sclerotinia minor* under field conditions, trial was conducted at Yuma Agricultural Research Station, Yuma, AZ. Trial was set up in a RCBD with 3 blocks. Each treatment plot consisted of 4 beds (42”), 25’ in length with a 10’ buffer between different plots within a row. Prior to planting, sclerotia of *S. minor* was prepared and broadcasted on the tops of each bed at high and low inoculum levels. Differential inoculum rates were applied (3.63, 7.25, and 14.5 g sclerotia/100 ft bed) to allow the examination of Contans and *P. polymyxa* performance under both high and low disease pressure. Planting following sclerotium distribution allowed the inoculum to be lightly incorporated into the upper inch of soil. The experiment was planted with head lettuce ‘Winterhaven’ using standard planting equipment and seed densities. All plots were managed using standard agricultural practices for lettuce. Fertilization and herbicide application, cultivation, and thinning were conducted as in commercial operations.

All treatments are listed in **Table1**. Contans was prepared as per manufacturer’s recommendation and applied two times: one at planting and one at immediately after thinning. For *Paenibacillus polymyxa*, a cell suspension of bacteria were prepared from the log phase culture at a concentration of 10^9cfu/ml in L media and applied at three times. Sprinkler irrigation and standard practices for lettuce in the Imperial Valley were used for the duration of the trial. At plant maturity, the number of healthy, symptomless, lettuce plants were recorded from the center two rows of each plot. These data were analyzed statistically by ANOVA.

Results are presented in **Fig. 1.** Results revealed significant increases of disease in control plots as disease inoculum levels increased from 3.63 g to 14.5 g (21% disease and 50% disease, respectively). At low inoculum rates, there were no significant differences between 6, 8, or 10 lbs Contans/acre, and all rates controlled disease nearly 100%. At medium and high inoculum levels, there were no significant differences in disease incidence between 6, 8, and 10 lbs/acre of Contans, resulting in disease incidence of 19% and 27%, respectively. There were no statistically significant differences between disease incidence in the biocontrol plots and the plots in which the fungicide Endura was used, although at the highest inoculum level, two applications of Endure or one application of Contans followed by one application of Endure resulted in the lowest level of disease. At 4 lbs/acre of Contans results were no different than the untreated controls. Thus, results reveal that 6 lbs/acres Contans was just as effective as 10 lbs/acre, and dramatically more effective than 4 lbs/acre. These findings are very promising and suggest that much lower rates of the biocontrol product can be used than revealed in previous studies for control of *S. minor* and these rates were as effective as standard chemical fungicides. Further work in the optimization of application (e.g. the application through irrigation systems) may further increase the efficacy of the biocontrol product leading to a further reduction in application rates toward a level that is more cost effective.

Results from the application of the bacterium *P. polymyxa* were not as promising as in previous studies. At all disease inoculum levels, treatments using *P. polymyxa* resulted in no significant increase in disease suppression over the control plots. In combining *P. polymyxa* with the conventional fungicide Endura, significant disease reduction was achieved at the low and medium inoculum levels, but not at the high inoculum levels. Reasons for these results that were substantially different from previous studies are not know. However, this inconsistency in performance is typical of many commercially available biocontrol products with the notable exception of Contans, which has performed consistently in our studies for the last 7 years.

Objective 2: Determine inhibition spectrum of *Paenibacillus polymyxa* against other soilborne pathogens of lettuce such as *Rhizoctonia solani*, *Phoma exigua*, and *Fusarium*

***oxysporum* and test the compatibility of *P. polymyxa* with chemical fungicides used against lettuce drop pathogens in *in vitro* experiments.**

Inhibition studies were performed on both broth culture and cell free extracts (supernatant) of *P. polymyxa*. Cell free extracts were prepared by the method of Swadling et al. Inhibition tests were performed on PDA plates. A 5 mm mycelial plug obtained from actively growing test fungus was placed at the center of a Petri plate containing PDA, and subsequently two 50 μl spots of *P. polymyxa* broth culture or cell-free extract obtained from stationary culture were placed on two opposing edges of the plate. Plates were incubated for 3-5 days at 25, 28 and 30 C. The zone of inhibition was measured as the final distance between the edge of bacterial and fungal colonies and statistically analyzed. All tests were replicated 5 times and the experiments were repeated twice.

Results are presented in **Table 2.** Results of inhibition study using cell-free supernatant were not as successful as those using bacterial suspensions. Several other methods were also tried to extract the cell free supernatant and test the inhibitory activity, but the results were generally the same: no inhibition. At this point, the exact reason for the lack of inhibitory activity of cell free supernatant of *P. polymyxa* is not known. However, it is speculated that *P. polymyxa* isolates may require the presence of a target fungus to produce inhibitory substance. Experiment to test this hypothesis is currently in progress.

In order to test the sensitivity of *P. polymyxa* to the fungicide Endura, *P. polymyxa* was grown in shake cultures (L media) containing different concentration of fungicide (0, 100, 300, 500, 700 and 900 p.p.m) at 30 C. The culture was continuously be shaken for 26-28 hrs at 110 rpm. Optical density of liquid cultures was taken at every three hour intervals to measure the rate of bacterial growth. In addition, viable bacterial population was estimated by preparing series of dilution from the stationary culture and plating them on LB medium.

Compatibility of *Paenibacillus polymyxa* was tested with different concentrations of Endura. Results revealed complete inhibition of growth at 500 ppm Endure for both *P. polymyxa* strains. In addition, results revealed high sensitivity at both 100 and 300 ppm Endura. Although Endura is a fungicide, its activity against *Paenibacillus* is expected as it mode of action is to disrupt succinate dehydrogenase, the only enzyme that participates in both the mitochondrial and bacterial electron transport chain. As such, Endura would be expected to show activity against other bacterial-based biocontrol products as well as normal soil microflora.

Appendices:

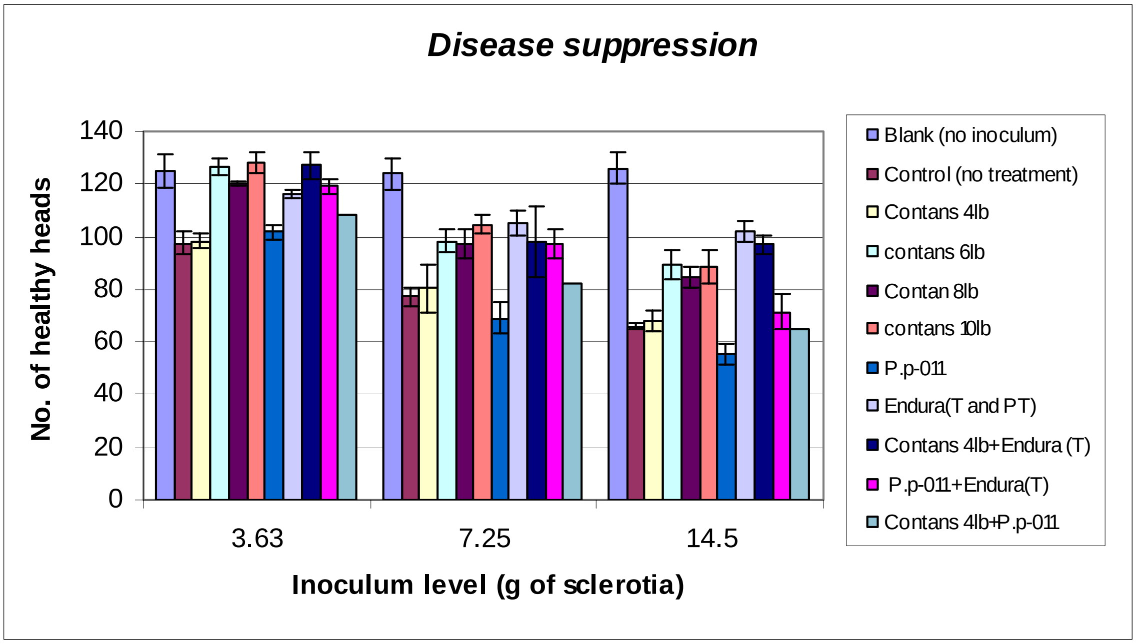
Table 1. Lettuce trial 2007-2008- Yuma*: Sclerotinia minor*
Treatments:
Contans-4 lb
1. Sprayed on to the bed immediately after planting
Contans-6 lb
2. Sprayed on to the bed immediately after planting
Contans-8lb
3. Sprayed on to the bed immediately after planting
Contans-10lb
4. Sprayed on to the bed immediately after planting
Other treatments:
5. Paenibacillus polymyxa-095-immediately after planting, thinning, and post thinning
6. Chemical treatment-Boscolid thinning and post thinning
7. 1 + 6
8. 5 + 6
9. 1 + 5
10. Control, no treatment
11. Blank, no inoculum and no treatment

Table 2: Inhibition of soilborne fungi by *P. polymyxa* in *in-vitro* experiments.

Fungi	Width of inhibition zone (mm)*	
	P. polymyxa-011	*P. polymyxa*-095
S. sclerotiorum	7.8	5.8
S. minor	9.4	7.0
Botrytis sp.	10.0	9.2
Fusarium lactucae	15.0	10.6
Phoma. sp	21.2	21.0
Rhizoctonia sp	5.0	4.4
Macrophomina sp.	12.0	11.0
Coniothyrium minitans	20.2	11.0

*Average of five replications. Values for no inhibition would be 0.0.

Fig. 1.


Disease suppression
No. of healthy heads
140
120
100
80
60
40
20
0
3.63
7.25
14.5
Inoculum level (g of sclerotia)
Blank (no inoculum)
Control (no treatment)
Contans 4lb
contans 6lb
Contan 8lb
contans 10lb
P.p-011
Endura(T and PT)
Contans 4lb+Endura (T)
P.p-011+Endura(T)
Contans 4lb+P.p-011