CALIFORNIA ICEBERG LETTUCE RESEARCH PROGRAM

April 1, 2008 - March 31, 2009

EPIDEMIOLOGY AND CONTROL OF LETTUCE DROP CAUSED BY *SCLEROTINIA*

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SUMMARY

The four objectives during the current funding cycle were to: 1) determine the most susceptible stage in the life cycle of *S. minor* to *C. minitans*, and optimize conditions for parasitism of *S. minor* by *C. minitans*; 2) determine the effects of Contans on production and survival of sclerotia of *S. minor*; 3) compare Contans with Endura for controlling lettuce drop caused by *S. minor* in field trials; and 4) continue supporting the breeding program and re-evaluate lines with 'slow-dying' resistance. We conducted laboratory experiments as part of objectives 1 and 2 to determine the most susceptible phase of *S. minor* for attack by *C. minitans.* Three isolates of *S. minor* were challenged at 5, 12 and 22 days after plating with *C. minitans*. The number of viable sclerotia per plate was then counted for each interaction. Fewest sclerotia formed in *S. minor* plates that received *C. minitans* at 5 days in culture. Relative to control (*S. minor* culture with no *C. minitans*), *C. minitans* introduced at 5 and 12 days significantly reduced the number of sclerotia in each plate in all isolates tested. Further studies on the interaction between *C. minitans* and two isolates of *S. minor* revealed that *C. minitans* reduces the number of sclerotia most dramatically in isolates of *S. minor* that also produce abundant sclerotia but the results were less than impressive for isolates that naturally produce fewer sclerotia. Field studies to evaluate different rates of Contans and different treatment times continued. This year, in addition to the ongoing treatments, two new Contans treatments were introduced in plots that were highly infested with *S. minor* sclerotia - a single application of Contans just prior to disking the crop residue and application of Contans at thinning and at harvest. Soil was sampled from all treatments after crop emergence and assayed for *S. minor* sclerotia by wet sieving. Lettuce drop incidence was recorded at weekly intervals until harvest maturity. All Contans treatments had significantly lower numbers of sclerotia in soil relative to the fungicide-sprayed and -unsprayed plots. Lettuce drop incidence in the two Contans treatments applied one week prior to thinning and one week post-thinning was identical to that observed in Endura-applied plots. Since the two new Contans treatments were introduced for the first time in spring 2008, disease levels were not significantly lower than in unsprayed control. However, the Contans treatment applied at thinning and at harvest had the lowest disease in fall 2008. Thus, strategic timing of Contans applications continues to show promise for *S. minor* control. Many years ago, we had identified several lines with 'slow-dying' resistance. Even though these lines ultimately succumb to lettuce drop, there was a significant lag from the time *S. minor* colonized the crown to the time the plants ultimately died. This type of resistance has not been considered previously in a breeding program. We re-evaluated lines previously identified as 'slow-dying' resistant against five isolates of *S. minor*. The results from the original evaluations were analyzed used a new statistical test developed by Ryan Hayes. The differences between the lines were not statistically significant. It is therefore important to focus on the breeding approach Ryan has taken over the past few years that has identified a few lines with heritable resistance. A few families from crosses between low-susceptible lines are being evaluated in the field. The results from this year's trial will be in Ryan Hayes' report.

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PROJECT TITLE: **EPIDEMIOLOGY AND CONTROL OF LETTUCE DROP CAUSED BY *SCLEROTINIA* SPECIES**

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OBJECTIVES:

1. To determine the most susceptible stage in the life cycle of *S. minor* to *C. minitans*, and optimize conditions for parasitism of *S. minor* by *C. minitans*.
2. To determine the effects of Contans on production and survival of sclerotia of *S. minor*.
3. To compare Contans with Endura for controlling lettuce drop caused by *S. minor* in field trials.
4. Continue supporting the breeding program and re-evaluate lines with 'slow-dying' resistance.

PROCEDURES AND RESULTS:

Objectives 1: To determine the most susceptible stage in the life cycle of *S. minor* to *C. minitans*, and optimize conditions for parasitism of *S. minor* by *C. minitans*.

Methods. *C. minitans* was isolated from the commercially available Contans, purified and maintained on PDA plates for use in the laboratory experiments. This experiment included 36 treatments (3 isolates, three media, three inoculation times), each with 3 replications. Three isolates of *S. minor* (Bm004, Bm005, Bm010 collected from lettuce in the Salinas Valley) maintained on PDA were plated on to PDA plates, autoclaved rye seeds in flasks, and autoclaved 1-cm potato cubes in flasks, at 12 plates/flasks for each isolate. At early stages of mycelial extension before sclerotia were produced (5 days post-plating), at early stages of sclerotium formation before many sclerotia turned black (12 days post-plating), and at one week before most sclerotia matured (22 days post-plating), conidial suspension (at a concentration of about 2 x 10^7 conidia/mL) of *C.*

minitans was prepared from a 14-day-old culture on PDA plates and one ml suspension was uniformly distributed into each of the 3 plates/flasks of the *S. minor* culture. One week after maturation of sclerotia, they were harvested, dried and weighed on a per flask basis. This experiment was conducted twice. In a separate study, two isolates that inherently produced different quantities of sclerotia were further studied to determine if the efficacy of *C. minitans* is uniform across isolates that produce differential numbers of sclerotia. In this study, *C. minitans* was introduced into 5-day-old and 8-day-old cultures of the two isolates. Cultures that did not receive *C. minitans* served as controls. Cultures were incubated for three weeks, and one week after maturation of most sclerotia, they were harvested, dried and weighed on a per flask basis. This experiment was also conducted twice.

Results: *C. minitans* introduced into 5-day-old *S. minor* cultures when the fungus growth was purely mycelial significantly reduced the number of sclerotia produced by each isolate tested (Fig. 1). The suppression of total sclerotia produced progressively decreased when *C. minitans* was introduced to *S. minor* cultures at 12 days when a few sclerotia had formed and at 22 days when mature sclerotia produced was maximal (Fig. 1). The number of sclerotia produced in flasks that received *C. minitans* at 22 days was nearly the same as the cultures that did not receive *C. minitans* (Fig. 1) in contrast to the significant reductions observed in cultures that received *C. minitans* earlier.

Isolates that naturally produced greater numbers of sclerotia in culture were more susceptible to attack by *C. minitans* than isolates that produced fewer sclerotia. This was best illustrated by the significant reductions in number of sclerotia produced by isolate Bm004 when *C. minitans* was introduced into either 5-day-old or 8-day-old *S. minor* cultures relative to cultures that did not receive *C. minitans* (Fig. 2). In contrast, isolate Bm010 produced as many sclerotia in association with *C. minitans* introduced into 5-day-old cultures as in cultures without *C. minitans* (Fig. 2). Even though there were reductions in the number of sclerotia produced when *C. minitans* was introduced into cultures into 8-day-old cultures relative to the control, the reductions were not as dramatic as with Bm004 (Fig. 2).

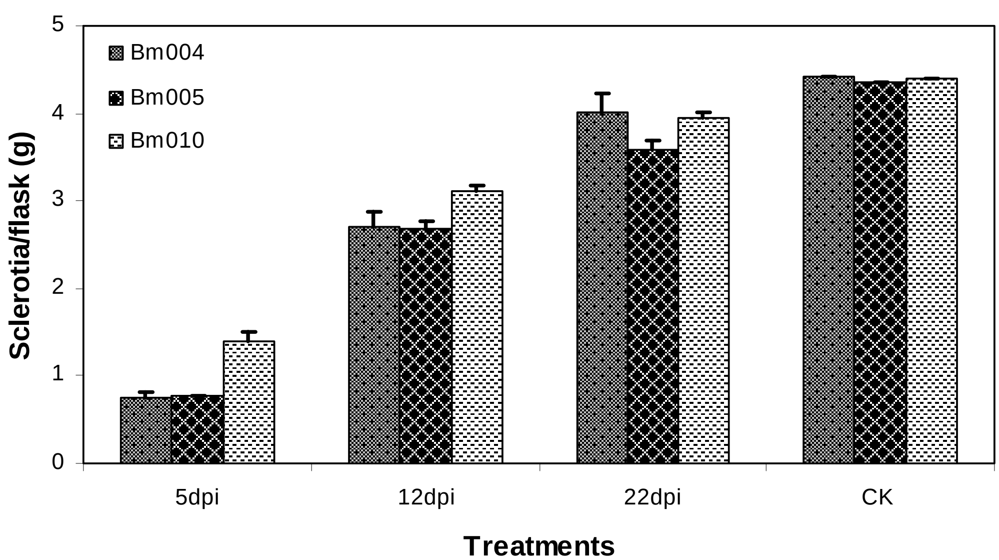

Fig. 1. Sclerotia per flask (g) produced by three isolates of *Sclerotinia minor* after inoculations with the biocontrol agent *Coniothyrium minitans* into 5-, 12- and 22-day-old cultures. The *S, minor*

cultures were either mycelial, with few immature sclerotia, or fully mature sclerotia when *C. minitans* was introduced. Sclerotia per flask was quantified from 4-wk-old cultures when most sclerotia were mature.

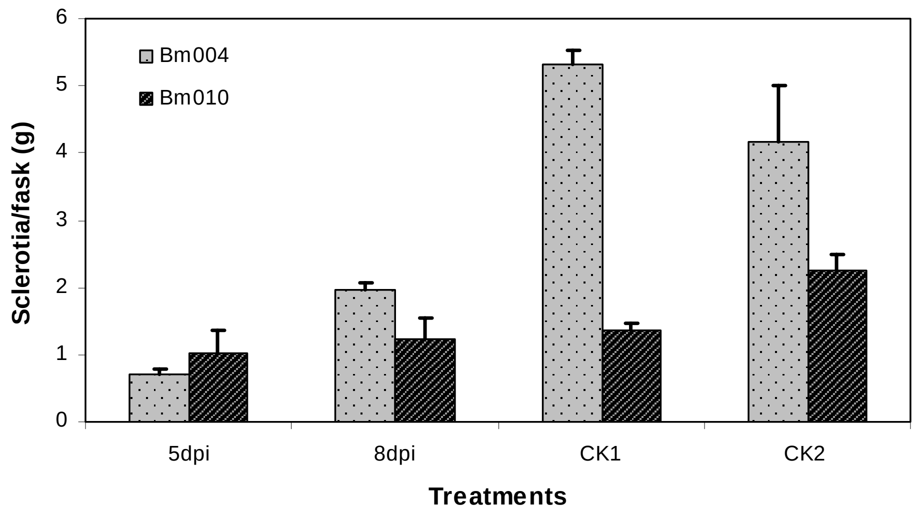

Fig. 2. Sclerotia per flask (g) produced by *Sclerotinia minor* that naturally produced high numbers of sclerotia (Sm004) or low numbers of sclerotia (Sm010) in the presence or absence (CK1 and CK2) of *Coniothyrium minitans* introduced into either 5-day-old or 8-day-old cultures of *S. minor*. Sclerotia per flask was quantified from 4-wk-old cultures when most sclerotia were mature.

Objective 2: To determine the effects of Contans on production and survival of sclerotia of *S. minor*.

Methods. These studies are currently ongoing and the results will be presented in the next report. However, methods being employed are described below. Lettuce cultivar "Salinas" was sown into 20-oz foam cups, and one seedling per cup was maintained after thinning at the 3rd leaf stage. At the 10th leaf stage, lettuce plants will be inoculated by placing a *S.-minor*-infested rye seed next to the crown. On 2, 5, and 14 days post-inoculation, when some, many or even all plants have developed drop symptoms, Contans will be sprayed onto lettuce plants at two 2 and 4 mL of a 1/1000 Contans WG aqueous solution. Immediately after the last spray (at 80% disease incidence) lettuce and/or debris will be incorporated into soil. Half of them will be watered while others will be maintained dry, sclerotia will be retrieved 4 weeks later by wet sieving. The numbers of viable sclerotia and sclerotia infested by *C. minitans* will be determined using the methods described above. The experiment includes 12 treatments (3 spray time × 2 spray rate × 2 moisture levels) each with 4 replications (cups) arranged with a complete random design, and the experiment will be replicated three different times. The reduction in the number of sclerotia per infected lettuce plant would be derived from these studies and compared among different treatments.

Objective 3. Long-term field evaluation of Contans against *Sclerotinia minor*.

Methods: Field experiments were again conducted to determine the effects of Contans on lettuce drop caused by *S. minor* at the USDA Station in Salinas on plants originally established in 2007. Treatments were arranged in a randomized complete block design with four replications.

Treatments included: 1) Endura (0.5 lb a.i./A) applied at post-thinning and again one week after the first spray, 2) Contans 2 lb/A applied three times (one wk prior to and one wk following thinning, and finally on the residue prior to disking under), 3) Contans 4 lb/A applied three times (one wk prior to and one wk following thinning, and finally on the residue prior to disking under), and 4) infested but unsprayed control. We introduced two additional treatments with Contans this year in plots that were previously used to study the efficacy of two formulations of an experimental fungicide from Valent Corportation. These additional treatments were: Contans sprayed only once on the residue prior to disking and Contans sprayed twice – once at post-thinning and a second time on the residue prior to disking. Plots were 8 beds (1-m between bed centers) wide and 8 m long and were separated by 2 m of bare soil between blocks to avoid plot interactions. Data were collected only on the middle 4 beds of each plot to avoid interactions from proximal plots. The experimental field was artificially infested with sclerotia of *S. minor* once in 2006 fall by distributing laboratory produced sclerotia with a planter along the seed lines prior to planting the first lettuce crop. Lettuce was planted in 2006 and nearly 80% of the plants regardless of the treatment developed lettuce drop and significantly augmented the number of sclerotia in soil. In the two Contans treatments, Contans at the corresponding rates were applied on the residue before disking in October 2006. Crisphead lettuce cultivar 'Salinas' was planted in April and July for the summer and fall seasons in 2007 and 2008. Fertilization of the experimental site was done using 'best management practices', i.e., <160 Kg N ha^{-1}, with banded, split applications. The plants were irrigated with sprinklers throughout the crop seasons to promote uniform seedling emergence, and to provide moisture required for parasitism of *S. minor* by *C. minitans*. Corresponding Contans treatments were initiated on the plots prior to thinning, the crop was thinned one week later and Endura and the new treatment with Contans was sprayed in the corresponding plots. One week after thinning, a second application of the Contans treatments was made on the corresponding plots. Two weeks after thinning, Endura was sprayed a second time on the corresponding plot and lettuce drop caused by *S. minor* was monitored weekly in all plots. Following harvest, Contans at the appropriate rates was applied on all Contans plots. Ten 500-mL soil samples were collected from top 0-10 cm soil layer from the middle four beds in each plot at seedling emergence and sclerotia of *S. minor* were retrieved using the wet sieving method. Total number of sclerotia per 100 cc soil was determined for all plots. The plots were monitored for lettuce drop incidence weekly until harvest and expressed as the percentage of the total plants present in the middle four rows of each plot. Effects of treatments on lettuce drop incidence and total sclerotia were determined statistically using the PROC mixed procedures in SAS (Release 8.0, SAS Institute Inc., Cary, NC, USA) and disease progress curves were generated from the incidence data.

Results: Contans applied three times, one week prior to and after thinning, as well as before disking the first crop, reduced the lettuce drop caused by *S. minor* by nearly 50% on both spring and fall 2008 crops relative to the unsprayed control, and the disease level was comparable to two applications of Endura (at thinning and two weeks post-thinning) (Figs. 3 and 4). The two new Contans treatments were not effective in the spring season (Fig. 3). However, the Contans treatment applied at post-thinning and at harvest provided the best control of lettuce drop during the fall (Fig. 4). The reduction in lettuce drop observed with this treatment during fall was far greater than that observed with Endura. However, we only have data from one season for this treatment and need to evaluate its performance during 2009 before recommending its adaptation. The other new Contans treatment that was applied only at harvest did not provide any control during 2008 but its efficacy could only be tested during the fall as the treatment was not applied at harvest of the 2007 fall crop. The two rates of Contans applied three times within a season had the least number of sclerotia in the soil (Figs. 5 and 6). While the lower levels of lettuce drop in Contans treatments were correlated with significantly lower levels of sclerotia, the lower levels of lettuce drop despite the presence of higher inoculum in the Endura treatment was attributable to the prevention of infection by *S. minor*. According to the manufacturer's projections, it currently costs about $60 per acre per application to apply Contans (2 lb rate) and is nearly the same as Endura. Efforts are currently underway to optimize the application of Contans to further reduce application costs.

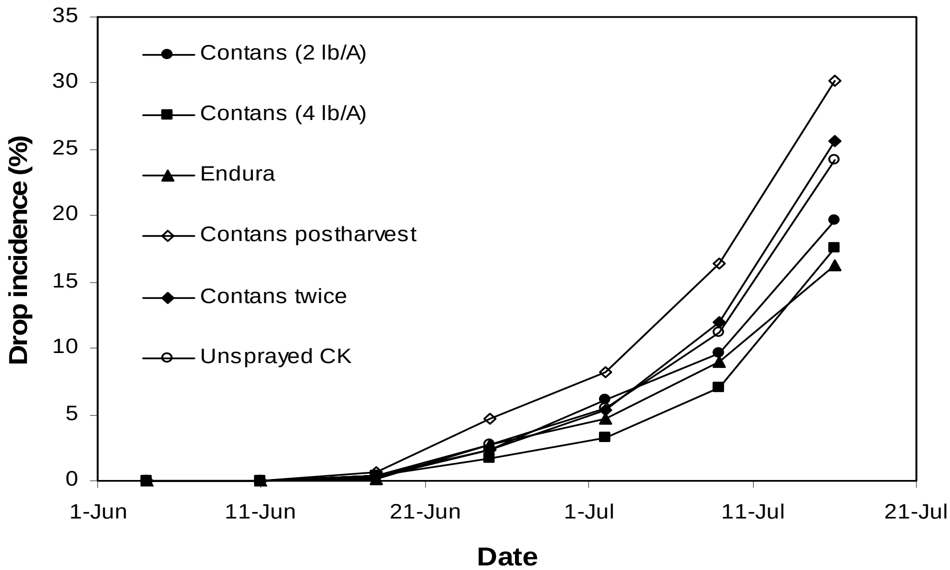

Fig. 3. Lettuce drop progress curves for the different treatments in spring 2008. Contans (2 lb/A and 4 lb/A) was applied three times during the season; Endura was applied twice; Contans (2 lb/A) was applied at thinning and at harvest; and the Contans (2 lb/A) was applied only once at postharvest. Unsprayed control did not receive any treatment.

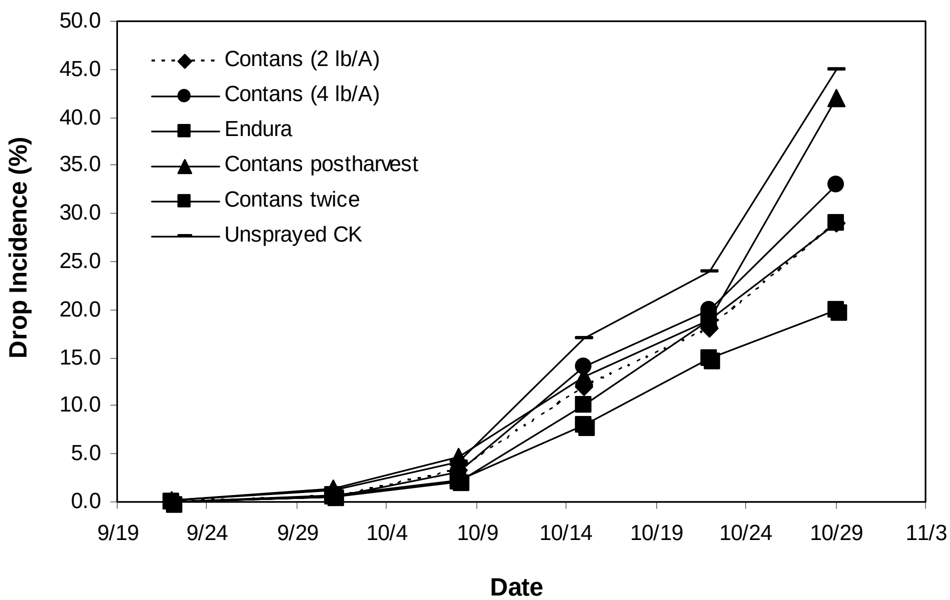

Fig. 4. Lettuce drop progress curves for the different treatments in fall 2008. Contans (2 lb/A and 4 lb/A) was applied three times during the season; Endura was applied twice; Contans (2 lb/A) was applied at thinning and at harvest; and the Contans (2 lb/A) was applied only once at postharvest. Unsprayed control did not receive any treatment.

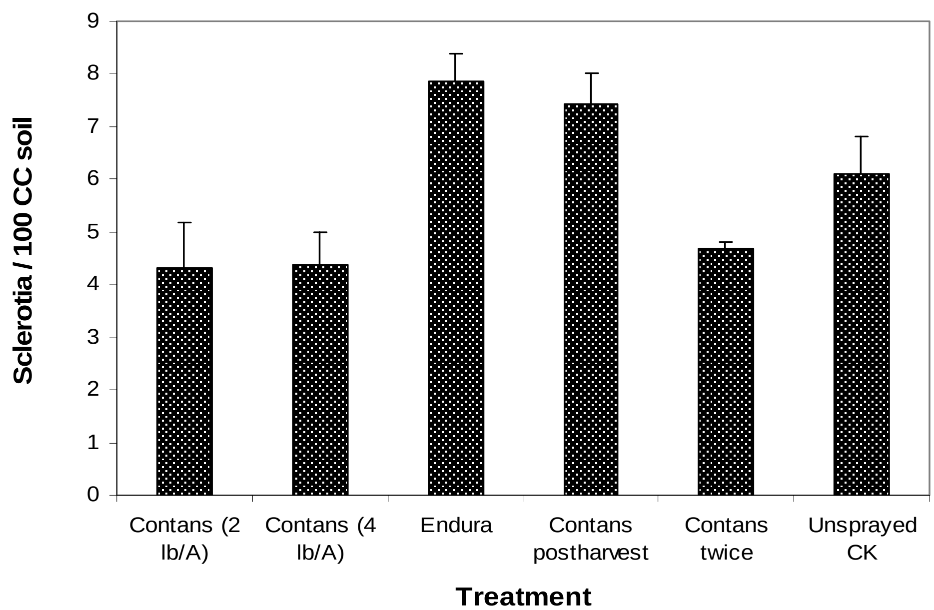

Fig. 5. Number of sclerotia in 100 cc soil from the different treatments in 2008 spring season. Notice the significant reductions in the number of sclerotia in 3 of the 4 Contans treatments.

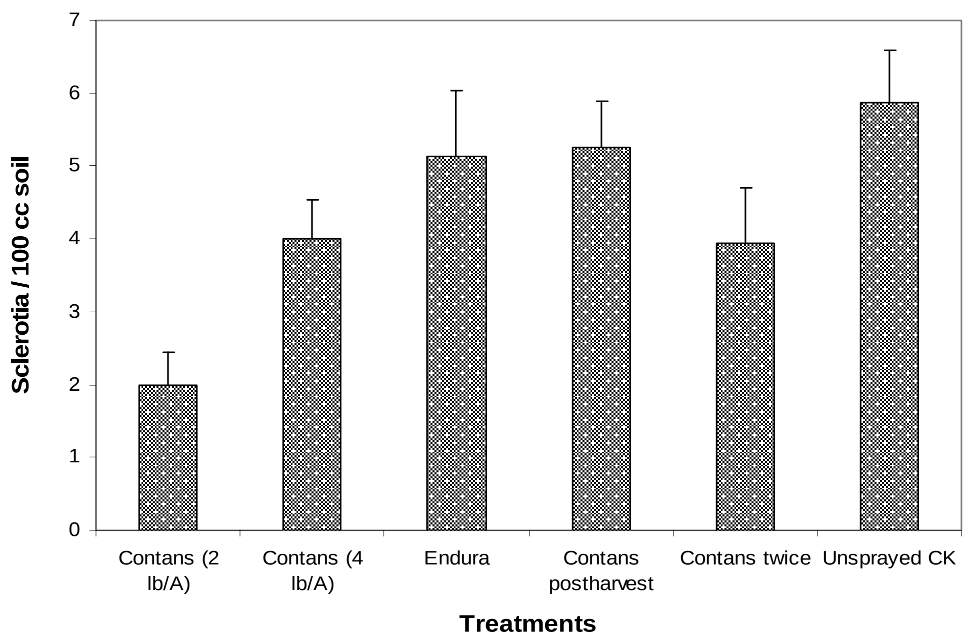

Fig. 6. Number of sclerotia in 100 cc soil from the different treatments in 2008 fall season. Notice the significant reductions in the number of sclerotia in 3 of the 4 Contans treatments.

Objective 4: Continue supporting the breeding program and re-evaluate lines with 'slow-dying' resistance.

The slow-dying resistance data that had been identified after evaluating 177 lettuce germplasm lines was tested with the new statistical program that Ryan Hayes developed. The differences between the lines were not statistically significant. It is therefore important to focus on the breeding approach Ryan has taken over the past few years that has identified a few lines with heritable resistance. A few families from crosses between low-susceptible lines are being evaluated in the field. The results from this year's trial will be in Ryan Hayes' report. We have been providing inoculum for field infestation of the plot in which Ryan Hayes evaluates resistance against *S. minor*. The sclerotia of isolates Sm001, Sm004, Sm005, and Sm010 produced on autoclaved potato pieces are dried and then mixed in equal proportions before seeding them into the field plots. Production of nearly 4 Kg of sclerotia takes about 4 months and we have been supplying this inoculum to the breeding program for over 4 years and will continue to facilitate this in the future.