CALIFORNIA ICEBERG LETTUCE RESEARCH PROGRAM

April 1, 2007 - March 31, 2008

EPIDEMIOLOGY AND CONTROL OF LETTUCE DROP CAUSED BY *SCLEROTINIA*

Krishna V. Subbarao

Department of Plant Pathology, University of California, Davis

SUMMARY

The four objectives during the current funding cycle were to: 1) compare pre- and post-Endura populations of *Sclerotinia minor* for potential resistance against Endura; 2) re-evaluate lines with 'slow-dying' resistance and screen additional PI lines to identify this type of resistance against *Sclerotinia minor*; 3) continue supporting the breeding program and 4) long-term evaluation of Contans against *Sclerotinia minor*. The pre- and post-Endura populations of *Sclerotinia minor* were compared for potential resistance to Endura. Resistance to Endura was not detected in either population in repeated testing. The recent control failures are perhaps a result of rapid degradation of Endura in fields where the fungicide has been applied repeatedly. Many years ago, we had identified several lines with 'slow-dying' resistance. This evaluation had used a single isolate of *S. minor*. In light of the variation in virulence documented in the populations of *S. minor*, it was important to re-screen the above lines to make sure that 'slow-dying' resistance is stable across the virulence spectrum of the pathogen population. Of the 10 lines that were re-screened, three exhibited 'slow-dying' resistance to a range of *S. minor* isolates. These lines will be screened once again in addition to new germplasm during the coming year. We are continuing our collaborations with Ryan Hayes to develop sources of resistance in lettuce to *S. minor* as well as to *S. sclerotiorum*. Resistance identified thus far in the germplasm is linked to early flowering and transferring this resistance to horticulturally superior lettuce backgrounds is difficult. However, a few families from crosses between low-susceptible lines are being evaluated in the field. We continued with the evaluation of Contans (*Coniothyrium minitans*) for the control of lettuce drop caused by *S. minor* in the Salinas Valley. Two rates of Contans were compared with Endura, two formulations of an experimental Valent product, and an unsprayed control. Soil was sampled from the control, Contans, and Endura treatments after crop emergence to assay for *S. minor* sclerotia. Both Contans treatments had significantly lower numbers of sclerotia in soil relative to the fungicide-sprayed and unsprayed plots despite equal infestation at the beginning of the study. Lettuce drop incidence in the two Contans treatments was identical to that observed in Endura-applied plots. Disease levels in the unsprayed control and the two formulations of V-10135 were twice as high as in either Contans or Endura plots. 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*.

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

PRINCIPAL INVESTIGATOR: **Krishna V. Subbarao**
Department of Plant Pathology
University of California, Davis

COOPERATING PERSONNEL: **BoMing Wu**
Department of Plant Pathology, University of California, Davis

Ryan Hayes
USDA-ARS, Salinas

Steven T. Koike
U. C. Cooperative Extension, Salinas, CA

OBJECTIVES:

1) To compare pre- and post-Endura populations of *Sclerotinia minor* for potential resistance against Endura.
2) Re-evaluate lines with 'slow-dying' resistance and screen additional PI lines to identify this type of resistance against *Sclerotinia minor*.
3) Continue supporting the breeding program.
4) Long-term evaluation of Contans against *Sclerotinia minor*.

PROCEDURES AND RESULTS:

Objective 1: To compare pre- and post-Endura populations of *Sclerotinia minor* for potential resistance against Endura.

Methods. More than 300 isolates of *S. minor* were collected from throughout California between 1999 and 2003. These isolates represented the pre-Endura population as it was registered for use on lettuce only in 2003. Thirty isolates each from five fields in which Endura had been applied since 2003 where control failures had occurred in 2005 were collected to serve as the post-Endura population. Individual cultures were started from sclerotia on potato-dextrose agar (PDA). A 4-mm-diameter agar disk from these cultures of individual isolates was plated on PDA amended with 0, 200, 400, and 600 ppm of Endura with the 600 ppm being the recommended rate for lettuce drop control. All concentrations expressed are based on active ingredient in Endura. Five replicate plates were maintained for each concentration-isolate combination and were incubated at room temperature (22 $\pm$ 2°C). The plates were examined daily and colony diameters of all plates were measured when the growth of *S. minor* in any one plate had reached the edge of the plate. The experiment was conducted twice.

Germination of sclerotia was determined by plating 25 sclerotia of each isolate on two Petri dishes containing media amended with 0, 200, 400, and 600 ppm of Endura. Sclerotia were not considered to have germinated unless the developing colony grew at least 1-cm-diameter on the medium. Germinated sclerotia were counted weekly for 5 weeks, and the experiment was conducted twice.

Results. All isolates tested regardless of whether they were from the pre- or post-Endura population were highly sensitive to Endura as isolates from neither population grew on any medium containing Endura. Colonies developed only on medium with no Endura. Similarly, germination of sclerotia occurred only on medium that lacked Endura. These results suggest that the control failures observed in the five fields that had used Endura since its registration on lettuce were not caused by the development of resistance in the pathogen population. However, degradation of boscalid (active ingredient in Endura) by other microorganisms in soil has not been investigated. In all likelihood, continuous use of Endura in these fields perhaps selected for microbes that rapidly degrade this fungicide and this in turn led to the breakdown of the fungicide soon after its application similar to the well documented soil microbial degradation of dicarboximide class of fungicides such as Rovral and Ronilan.

Objective 2: Re-evaluate lines with 'slow-dying' resistance and screen additional PI lines to identify this type of resistance against *Sclerotinia minor*.

There are difficulties inherent in the screening of any plant species for resistance to Sclerotinia diseases. Complete resistance to necrotrophic pathogens such as *Sclerotinia* is rare. Also, the onset of disease may be erratic, and, after initial infection, disease may progress very rapidly, so that small differences between individual lines may not be discernible if rating is done only at the end of an experiment. To measure low levels of resistance, some researchers have used inoculation methods that limit the time of contact between the host and pathogen, or they have measured relative lesion sizes under greenhouse or laboratory conditions. Low to moderate levels of genetic resistance in peanut were quantified in the laboratory by measuring the rate of lesion elongation on stems inoculated with cultures of *S. minor*. In alfalfa, it has been demonstrated that low levels of measurable resistance to *Sclerotinia* can be accumulated and increased so that later generations are more resistant than the parents.

Lettuce is not suited to methods used for measuring low levels of resistance in other crops. Lettuce plants have very short crowns, with a rosette of leaves occurring very close to the soil, which makes this kind of measurement difficult. Thus, alternative methods are needed for measuring low levels of resistance in lettuce and for determining the optimal timing of these measurements. Previously developed greenhouse assay has included the evaluation of resistance in lettuce against *S. sclerotiorum* using colonized agar blocks as inoculum, and based ratings on the number of days from inoculation until plant death and percent of survivors 15 days after inoculation. During greenhouse screening of lettuce lines for *S. minor* resistance, we observed that the time after inoculation until onset of symptoms was variable, and that after first symptoms were observed, some lines took considerably longer than others to collapse. Using this parameter that we termed as 'slow-dying' we had previously evaluated 177 lettuce germplasm lines for resistance to lettuce drop and had identified 10 lines that exhibited differential levels of 'slow-dying' resistance. Slow dying measurements may provide a means of identifying lettuce lines with low but measurable levels of resistance that would be useful in a lettuce drop resistance breeding program. When these evaluations were made, we had employed a single isolate of *S. minor* to determine resistance. Subsequent evaluation of the virulence among *S. minor* isolates had shown that isolates also exhibit differential virulence. It was therefore important to re-test the 10 lines that exhibited differential 'slow-dying' resistance against isolates exhibiting differential virulence to determine that the resistance is effective against a range of isolates.

Methods: Four-week old seedlings of 10 lettuce cultivars and Plant Introductions previously identified as possessing 'slow-dying' resistance were inoculated with five isolates of *Sclerotinia minor* that possessed differential virulence. For each isolate, three rye seeds infested with the mycelium was placed at the base of each plant and rated for the slow dying interval or interval from infection until plant death. Each line was tested twice using ten plants per test. Lines were ranked according to the differences of the mean value of the line.

Results: As expected, there were significant virulence differences among the isolates tested. Three isolates were significantly more virulent than the other two isolates tested (Table 1). Similarly, of the 10 lines tested, three lines exhibited 'slow-dying' resistance against the five isolates tested (Table 2). The other six lines were not significantly different from the susceptible line. Thus, additional germplasm evaluations should identify more lines that possess this type of resistance. We intend to do this and also evaluate breeding material from Ryan Hayes for this type of resistance over the next few years.

Table 1. Effects of *Sclerotinia minor* isolates on slow-dying interval

Isolate	Replications[a]	Slow-dying interval[b]
Bm005	24	5.38a
Bm107	24	5.03a
Bm004	24	4.24b
Bm010	24	4.12b
Bm160	24	3.76b

[a]: Each replication consists of ten plants.
[b]: Means followed by the same letter are not different according to LSD.

Table 2. Average slow-dying intervals calculated from the response of lettuce germplasm lines and cultivars to 5 isolates of *Sclerotinia minor*

PI-lines	Replications[a]	Slow-dying interval[b]
PI-250020	2	10.71a
PI-273589	2	5.86b
PI-207490	2	4.84c
BRG[c]	2	4.25cd
PI-177415	2	3.93cd
Salinas	2	3.81cd
PI-171669	2	3.80cd
PI-178924	2	3.77cd
PI-173710	2	3.64cd
PI-177419	2	3.52d
PI-171665	2	3.38d
PI-226641	2	3.35d

[a]: Each replication consisted of ten plants.
[b]: Means followed by the same letter are not different according to LSD.
[c]: BRG= Batavia Reine des Glaces

Objective 3. Continue supporting the breeding program.

After realizing the differential virulence exhibited by *S. minor* isolates in 2006, field evaluation of the breeding material began incorporating sclerotia from a mixture of isolates with varying virulence. This was also continued in 2007 and the results from the field evaluation of breeding material using this technique will be in the report by Ryan Hayes.

Objective 4. Long-term evaluation of Contans against *Sclerotinia minor*.

Considerable research has been conducted on evaluating biological control strategies for the management of Sclerotinia diseases in varied cropping systems although most studies revealed only moderate effectiveness. Most contemporary research involves the use of mycoparasitic fungi *Trichoderma spp*., *Sporidesmium sclerotivorum*, and *Coniothyrium minitans*. However, in field trials conducted in Salinas, CA, and in Yuma, AZ, neither *Trichoderma spp.* nor *Sporidesmium sclerotivorum* was efficacious. In contrast, *C. minitans* available as a commercial formulation Contans, consistently reduced lettuce drop incidence caused by *S. sclerotiorum* by over 96% in the Imperial Valley, CA and Yuma, AZ under a very high disease pressure (70% disease incidence in controls). In Yuma during 2001-2003 consistently better control of lettuce drop caused by *S. sclerotiorum* was achieved using Contans than using 20 chemicals tested, including Rovral and Ronilan (Pryor, personal communication). These studies clearly demonstrated the potential of Contans for control of lettuce drop caused by *S. sclerotiorum* and has become a standard lettuce drop management strategy in winter lettuce production systems in California.

In previous tests, Contans was not effective against lettuce drop caused by *S. minor* in either the Salinas Valley or the Imperial Valley trials. Since *C. minitans* is an effective parasite of sclerotia of both *S. sclerotiorum* and *S. minor*, it is unclear why Contans was ineffective against *S. minor* in these trials. Whether enough Contans was applied in these studies, or whether applications were made at appropriate times has remained unanswered. It is critical to investigate whether an application of Contans on infected plants prior to plowing the residue alone will increase the parasitic destruction of *S. minor* sclerotia by *C. minitans* and reduce the number of sclerotia formed since the mycelial phase of *S. minor* is generally considered most susceptible to environmental stresses and attacks by other organisms. Our ultimate goals are to develop environmentally-friendly strategies for the management of lettuce drop using *C. minitans* in combination with rotation and/or irrigation management, to reduce the dependence of the lettuce industry on a few chemical fungicides that will be continually reviewed for registration. The goal of this project is to develop an effective method to control lettuce drop caused by *S. minor* using *C. minitans*. Specifically, the objectives of the proposed project include:

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 fungicide Endura for controlling lettuce drop caused by *S. minor* in field trials.

    a. To determine the effects of two rates of Contans and three application times on the densities of total and viable sclerotia of *S. minor* in the soil compared with Endura.
    b. To determine the effects of two rates of Contans and three application times on incidence of lettuce drop over multiple crops compared with Endura.
    c. To determine the effects of two rates of Contans and three application times on the colonization of *S. minor* sclerotia by *C. minitans* compared with Endura.

At this time, we have results only for the main objective 3.

Methods: Field trials were conducted to determine the effects of Contans on lettuce drop caused by *S. minor* at the USDA Station in Salinas 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 post-thinning, 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. In addition, two formulations of an experimental fungicide from Valent Corportation were also tested in 2007. 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 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. 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 whole crop seasons to promote uniform seedling emergence, and 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 sprayed in the corresponding plot. 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. Ten 500-mL soil samples were collected from top 0-10 cm soil layer from the middle four beds in each plot at lettuce seedling emergence and at the end of each cropping season immediately after disking and conventional tillage. Sclerotia of *S. minor* were retrieved using the wet sieving method. Total number of sclerotia per 100 cc soil, percentage of viable sclerotia, and percentage of sclerotia infested with *C. minitans* was determined for all plots. Effects of treatments on lettuce drop incidence, total sclerotia, viable sclerotia and sclerotia infested by *C. minitans* were determined statistically using the PROC mixed procedures in SAS (Release 8.0, SAS Institute Inc., Cary, NC, USA).

Results: Neither formulations of the experimental fungicide was effective against lettuce drop caused by *S. minor* in either season during 2007 (Figs. 1 and 2). These two treatments will be discontinued and in their place, we will begin testing two other application methods of Contans from 2008. 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 2007 crops relative to unsprayed treatments, and the disease level was comparable to two applications of Endura (at thinning and two weeks post-thinning) (Figs. 1 and 2). Considering that uniform high disease incidence (about 80%) on the first crop in 2006 fall (Fig. 3) was established across all plots via seeding sclerotia along seed lines at the beginning of the study, these results are highly significant. While the lower levels of lettuce drop in Contans treatments were associated with significantly lower densities of sclerotia in the soil, the lower levels of lettuce drop despite the high inoculum in the Endura treatment might be attributable to the prevention of infection by *S. minor* (Fig. 4). This study suggested that *C. minitans* can also be used for management of lettuce drop caused by *S. minor* although the rate of Contans applications tested in these studies may be uneconomical. It is desirable to test whether a single application of Contans at an optimal stage will accomplish the goal of reducing both the soilborne sclerotia and the lettuce drop incidence relative to the Endura treatment. If indeed it does, then it would be even more economical than the Endura treatment, and can be used as an environmentally-friendly biopesticide in integrated management of lettuce drop. This will lead to further reductions in the use of Endura, and avoids/or delays the potential disease control failures from extensive applications of Endura. These treatments are being tested in 2008.

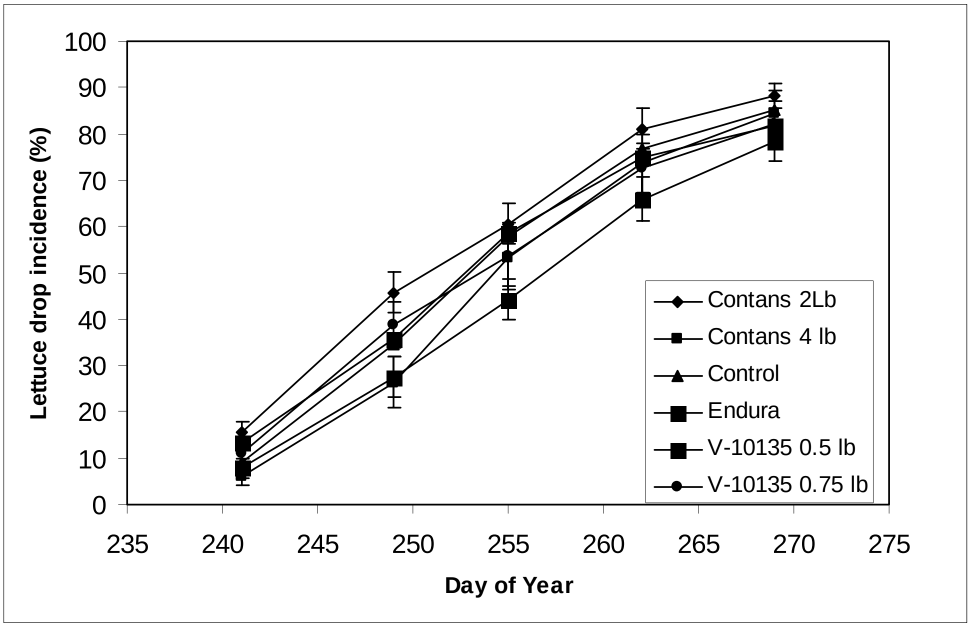

Fig. 1. Lettuce drop progress curves for the different treatments in fall 2006 after the plots were infested with laboratory-produced *Sclerotinia minor* sclerotia.

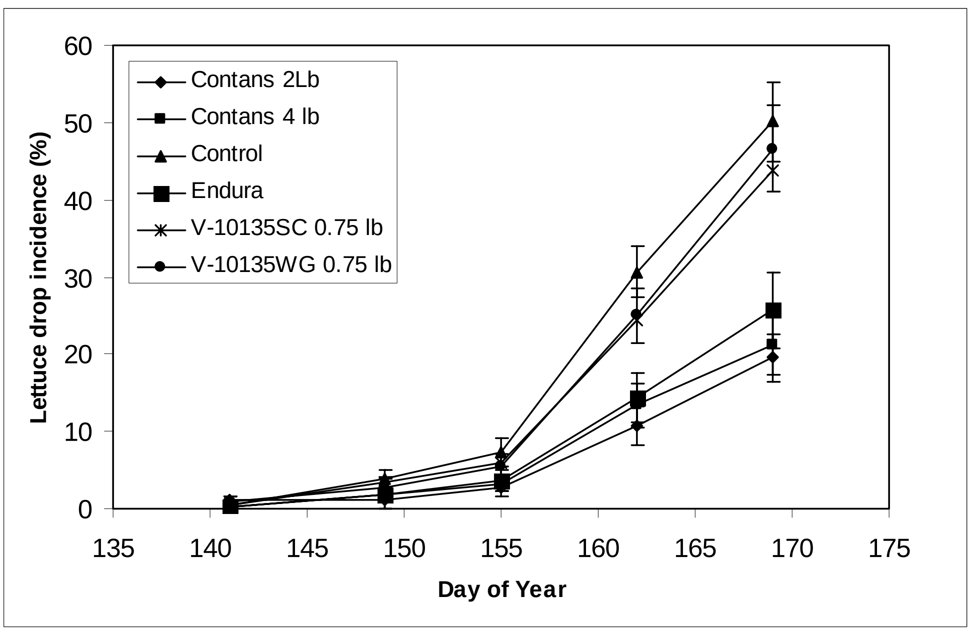

Fig. 2. Lettuce drop progress curves in the various treatments during the 1997 spring season. Notice that the two Contans treatments had identical final disease incidence and was not significantly different from the Endura treatment.

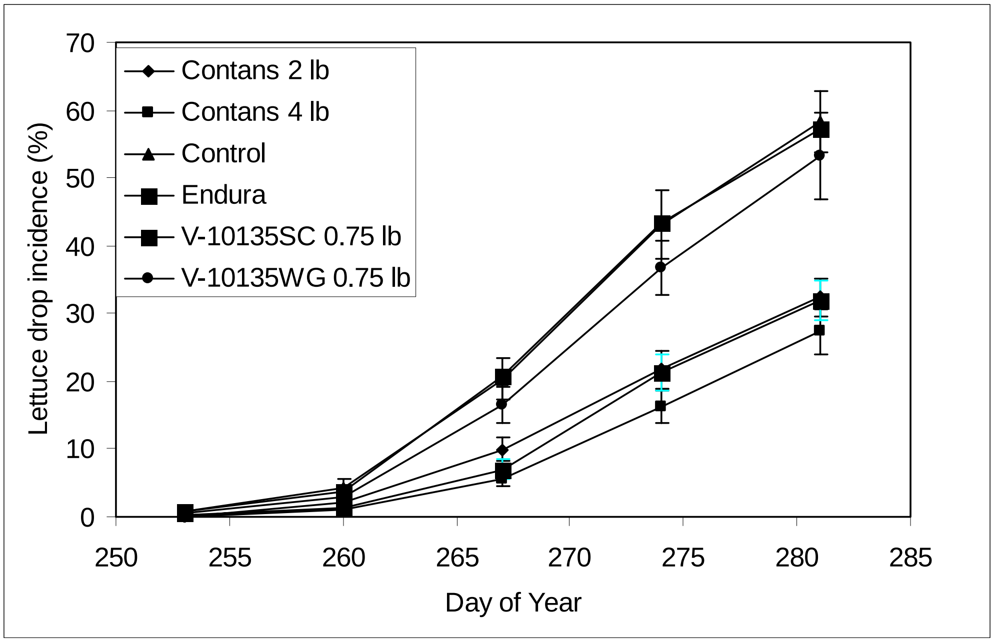

Fig. 3. Lettuce drop progress curves in the various treatments during the 1997 fall season. Notice that the two Contans treatments had identical final disease incidence and was not significantly different from the Endura treatment.

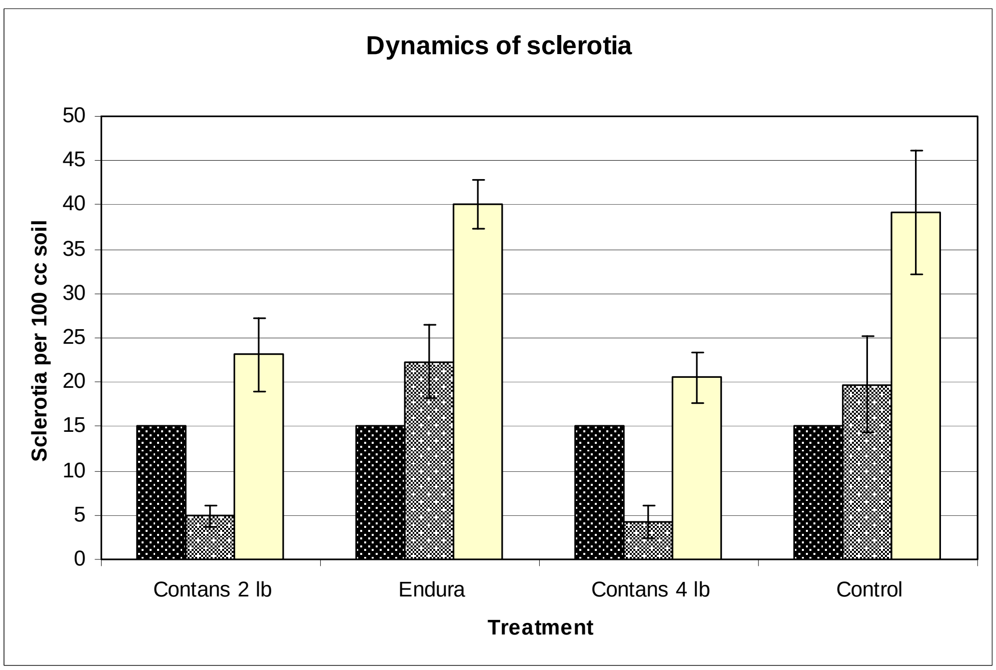

Fig. 4. Number of sclerotia in 100 cc soil from the four treatments at soil infestation in 2006 fall (first bar in each treatment), at planting in 2007 spring (middle bar), and 2007 fall (last bar). Notice the significant increases in both Endura and Control treatments relative to the two Contans treatments.