CALIFORNIA ICEBERG LETTUCE RESEARCH PROGRAM

April 1, 2009 - March 31, 2010

EPIDEMIOLOGY AND CONTROL OF LETTUCE DROP CAUSED BY *SCLEROTINIA*

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SUMMARY

Over the past three years, we have been evaluating the biocontrol product Contans (*Coniothyrium minitans*) against lettuce drop caused by *Sclerotinia minor* in the Salinas Valley. Field experiments were continued again this year to evaluate both the rates of Contans as well as the efficacy of timing and frequency of application of the product on lettuce. In addition, laboratory experiments to evaluate the hypothesis that the most susceptible phase of *S. minor* is mycelial for attack by *C. minitans* were expanded to a range of isolates. Isolates of *S. minor* were challenged in culture at purely mycelial, at few immature sclerotial and at fully mature sclerotial phases. Four-wk-old cultures of these treatments were harvested for sclerotia, air-dried, and weighed. The sclerotia were also plated on medium to determine recovery of *C. minitans*. Fewest sclerotia formed in *S. minor* plates that received *C. minitans* at mycelial stage. Reductions in the sclerotia were in the treatment that received *C. minitans* after a majority of sclerotia had formed were not significantly different from the control treatment that received no *C. minitans*. *C. minitans* was recovered from nearly all sclerotia from the treatment that received *C. minitans* at the mycelial stage. While our hypothesis that the introduction of *C. minitans* to mycelial cultures of *S. minor* results in reductions of sclerotial numbers was proven right, there were significant isolate differences. Field studies to evaluate different rates of Contans and different treatment times continued in 2009. Last year, in addition to the established treatments with Contans, we introduced two new treatments with Contans in plots that were highly infested with *S. minor* sclerotia. The two new treatments were a single application of Contans just prior to disking the crop residue after harvest; and application of Contans at thinning and at post-harvest. Lettuce drop incidence was recorded at weekly intervals until harvest maturity. Lettuce drop incidence during both spring and fall was low and yet there were treatment differences. All Contans treatments had significantly lower numbers of sclerotia than Endura and unsprayed control treatments. Lettuce drop incidence in all Contans treatments except the one applied only at harvest was slightly higher but statistically not significant from that observed in Endura-applied plots. A single application of Contans at harvest had disease levels intermediate between the other Contans treatments and unsprayed control. 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*. The ideal approach to lowering lettuce drop infections appears to be to employ Contans to lower the number of sclerotia in soil and Endura to prevent infection within a cropping season. Resistance to *S. minor* in lettuce germplasm was analyzed used a new statistical test developed by Ryan Hayes. It is therefore important to focus on the breeding approach Ryan has taken over the past few years that has identified a few lines such as Eruption 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 effects of different rates and application times of *C. minitans* on sclerotial population of *S. minor* in the soil and on incidence of lettuce drop over multiple crop seasons.
2. Efficacy of *Coniothyrium minitans* against different growth phases and different MCGs of *S. minor*.
3. Continue supporting the breeding program and re-evaluate lines with 'slow-dying' resistance.

PROCEDURES AND RESULTS:

Objectives 1: To determine the effects of different rates and application times of *C. minitans* on sclerotial population of *S. minor* in the soil and on incidence of lettuce drop over multiple crop seasons.

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 plots 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 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), 4) Contans sprayed only once on the residue prior to disking; 5) Contans sprayed twice – once at post-thinning and a second time on the residue prior to disking; and 6) infested but unsprayed

control. 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 prevent plot interactions. Data were collected only from 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 was 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, 2008, and 2009. 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 cropping 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 another Contans treatment application was made 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: The results from all years are summarized in this report. Analysis of variance demonstrated that overall year, seasons and treatment, as well as interactions among treatment × year and year × season significantly affected the incidence of lettuce drop (Table 1). Separate analysis for each crop showed that effect of treatment was not significant in fall 2006 when all plots were uniformly infested with sclerotial inoculum and the lettuce drop incidence was uniformly high. The differences among treatment were significant for all other crops from spring 2007 to fall 2009 (Tables 3 and 4). Overall incidence of lettuce drop decreased over years, but slightly increased from spring crop to fall crop within a year. Specifically, drop incidence was significantly lower in plots treated with Endura than in control plots for all crops except during fall 2006 and spring 2008 (Table 3). Drop incidence in plots treated with Contans was mostly comparable to incidence in plots treated with Endura and significantly lower than the incidence in control plots, with exception of fall 2006 and spring 2008, as well as for the treatment of 2.2 kg/ha in fall 2008 and spring 2009 (Figs. 1 and 2). Similarly, the analysis of variance on sclerotial populations showed significant effect of year, season and treatment, as well as interactions of treatment × year and year × season (Table 2). Overall the sclerotium population increased from spring crop to fall crop within a year, especially in 2007, and decreased from fall crop to the subsequent spring crop, especially from fall 2007 (when numbers of sclerotia in soil were very high) to spring 2008. The sclerotium population showed significant differences among treatments from spring 2007. Sclerotium population was significantly lower in plots

treated with Contans in all crops except fall 2006 when the field was first artificially infested and in fall 2008 when no significant difference was observed in drop incidence in the preceding spring crop (Table 4). Sclerotium population in plots treated with Endura was comparable to the high levels in control plots and statistically not significantly different (Table 4). There was a positive correlation between the amount of sclerotial inoculum assayed in each plot during a crop season and the percent disease incidence observed at the end of the crop season (Fig. 3). The analysis of variance on the sclerotial residue showed significant effects of treatment, year and interaction involving these two factors. Similarly, there was a positive correlation between final incidence in a crop and the sclerotial levels observed in the subsequent crop, the relationship could be fitted to two different linear model for spring and fall crops separately (Fig. 4). Both the disease progress and sclerotial density data from 2009 seasons were similar to results obtained during previous years.

TABLE 1. Analysis of variance for the fixed effect of year, cropping season, treatment and interactions on the final lettuce drop incidence

Effect	NumDF	DenDF	*F* value	Pr > *F*
Year	3	9	423.51	<.0001
Season	1	9	22.92	0.001
Year*Season	2	9	9.43	0.0062
Treatment	5	87	17.29	<.0001
Year*Treatment	11	87	6.38	<.0001
Season*Treatment	5	87	1.74	0.1331
Year*Season*Treatment	8	87	1.78	0.0918

NumDF = Numerator degrees of freedom; DenDF = Denominator degrees of freedom.

TABLE 2. Analysis of variance for the fixed effect of year, cropping season, treatment and their subsequent interactions on sclerotial population in the soil

Effect	NumDF	DenDf	F value	Pr. >F
Year	2	6	174.13	<.0001
Season	1	9	38.07	0.0002
Year*season	2	9	50.56	<.0001
treatment	5	78	16.76	<.0001
year*treatment	8	78	5.96	<.0001
season*treatment	5	78	0.24	0.9411
year*season*treatment	8	78	0.42	0.9032

NumDF = Numerator degrees of freedom; DenDF = Denominator degrees of freedom.

TABLE 3. The long term effect of different application rates and application times of Contans and Endura on the sclerotial population of *S. minor* from 2006-2009

Treatments	Sclerotia population (No. of sclerotia/100cc of soil)*						
	2006	2007		2008		2009	
		Spring	Fall	Spring	Fall	Spring	Fall
Contans 2.2kg/ha[w]	a15.0	b4.9b	a23.1bc	b4.4b	b2.0c	b1.3b	b1.8d
Contans 4.4kg/ha [w]	ab15.0	b6.3b	a20.5c	b4.8b	b4.0bc	b1.6b	b2.1dc
Contans 2.2kg/ha [x]				a5.3b	a5.1ab	a3.4b	a1.8d
Contans 2.2kg/ha[y]				a3.9b	a5.7ab	a3.5b	a3.0bc
Endura [z]	bc15.0	b22.3a	a40.1a	cd8.9a	cd6.7a	cd7.3a	d3.4ab
Unsprayed control	bc15.0	b19.8a	a39.2ab	cd7.6a	cd7.1a	cd7.2a	d4.2a

The letters on the right side of each column indicates the ANOVA between the treatments for each season and it should be read top to bottom. The letters on the left side of the each column indicates the ANOVA between the season for each treatment and it should be read left to right. Columns with different letters are significantly different according to Holm-Sidak method ($P<0.05$) based on the F test in analysis of variance.
*Sclerotia population was enumerated for each treatments after crop emergence, [w] Treatment consisted of three sprays, one at one week prior, one at after thinning and one at before disking the first crop. [x] Treatment consisted of one spray at harvest. [y] Treatment consisted of two sprays, one at post thinning and one at harvest. [z]Fungicide Endura applied twice, one at thinning and one at two weeks post thinning.

TABLE 4. The long term effect of different application rates and application times of Contans and Endura on lettuce drop caused by *S. minor* from 2006-2009

Treatments	Lettuce drop incidence at the end of the crop season (%)						
	2006	2007		2008		2009	
		Spring	Fall	Spring	Fall	Spring	Fall
Contans 2.2kg/ha [w]	a88.4a	c19.6b	b32.4b	c19.7bc	bc29.0dc	d5.0b	d5.0b
Contans 4.4kg/ha [w]	a84.5ab	bc21.3b	bc27.4b	cd17.5c	b33.0bc	d5.0b	d5.3b
Contans 2.2kg/ha [x]				b30.2a	a42.0ab	c8.3a	c6.1b
Contans 2.2kg/ha [y]				a25.7ab	a20.0d	b5.0b	b4.1b
Endura	a78.2ab	bc25.7b	b32.0b	cd16.2c	bc26.0dc	d4.0b	d3.4b
Unsprayed control	a85.0ab	bc50.1a	b58.4a	d24.2abc	c45.0a	e10.0a	e8.4a

The letters on the right side of each column indicates the ANOVA between the treatments for each season and it should be read top to bottom. The letters on the left side of the each column indicates the ANOVA between the season for each treatment and it should be read left to right. Columns with different letters are significantly different according to Holm-Sidak method ($P<0.05$) based on the *F* test in analysis of variance. , [w] Treatment consisted of three sprays, one at one week prior, one at after thinning and one at before disking the first crop. [x] Treatment consisted of one spray at harvest. [y] Treatment consisted of two sprays, one at post thinning and one at harvest. [z]Fungicide Endura applied twice, one at thinning and one at two weeks post thinning.

Objective 2: Efficacy of *Coniothyrium minitans* against different growth phases and different mycelial compatibility groups (MCGs) of *S. minor*.

Methods. Laboratory experiments were conducted to determine the most susceptible growth phase in *S. minor* to *C. minitans* parasitism and to test the effect of *C. minitans* on different mycelial compatibility groups (MCG) of *S. minor*. Five *S. minor* isolates were randomly chosen each from MCGs I, II and III and one isolate from MCG IV as only few isolates were available in this group. One of the five isolates from MCG I produced sclerotia inconsistently and was therefore removed from the analysis. The three growth phases of *S. minor* tested for the susceptibility to *C. minitans* parasitism were: (i) mycelial phase before initiation/formation of sclerotia (4^{th} day of incubation); (ii) early sclerotial formation phase when sclerotia were still brown (6^{th} day of incubation); (iii) sclerotial maturation phase, (15^{th} day of incubation). A mycelial plug of each isolate obtained from 4-day-old cultures on PDA was placed at the center of PDA plates and incubated at 20 C for either 4, 6 or 15 days depending on the treatments to which they were assigned. Conidial suspensions of *C. minitans* prepared from a 14-day-old culture on PDA were uniformly distributed into each *S. minor* plate (@ 2 X 10^7 conidia/ml/ plate) either on 4, 6 or 15^{th} day. Plates distributed with 1 ml of sterilized water served as a negative control. Each treatment had three replicates and the experiment was conducted twice. These plates were incubated at 20 C for 14 days. Two sets of observations were taken from each treatment; (i) sclerotial mortality: Five sclerotia from each plate were randomly picked and surface sterilized with 70% ethyl alcohol for 1 minute and rinsed with sterilized water twice. These sclerotia were placed individually on the PDA agar plug (1-cm diameter) amended with streptomycin sulfate and incubated at 20 C for a week. Observations on the number of sclerotia germinated with and without *C. minitans* infection and dead sclerotia were taken; (ii) sclerotial dry weight: Sclerotia from each plate were collected separately, air dried for about 36 hrs in hood and weighted.

Results. Analysis of variance using mixed model revealed that *Coniothyrium minitans* treatment significantly reduced ($P < 0.0001$) sclerotial production at all three growth phases of *S. minor* tested in all four MCG of *S. minor*. However, effects of MCG, the time of inoculation of *C. minitans* and all interactions ($P < 0.0001$) of MCG, inoculation time and treatment were all statistically significant (Fig. 5). MCG1, which was the most predominant MCG during the previous field survey tended to be most sensitive to *C. minitans* at early mycelia phase (Fig. 5) while isolates from all MCG showed decreased sensitivity to *C. minitans* as they got close to maturity in terms of dry weight of total sclerotia. Similarly, results of analysis of variance on sclerotial mortality data also revealed that significant differences among both MCGs and time of inoculation of *C. minitans* ($P < 0.0001$). Unlike the dry weight of sclerotia and no significant interaction between the time of inoculation and MCG ($P = 0.5791$) was found. Percent sclerotial mortality decreased with the increase of age of *S. minor* and inoculation of *C. minitans* at mycelial phase of *S. minor* resulted in significantly higher percent of sclerotial mortality than that at sclerotial formation and maturation phase. The results also revealed that *C. minitans* had significantly different effects on isolates belonging to different MCGs. The percent sclerotial mortality for isolates in MCG IV was significantly higher than that in other MCGs at all three phases tested. The percent sclerotial mortality in MCG I after *C. minitans* treatment was also significantly higher than that in MCG II and MCG III in all phase, but comparable (in mycelial

phase) or lower than that in MCG IV (in other two phases). MCG II & III was the least sensitive to treatment with *C. minitans*.

Objective 3: Continue supporting the breeding program and re-evaluate lines with 'slow-dying' resistance.

Assessing resistance in field experiments can be complicated by fast bolting or small stature lettuce lines that may escape, rather than resist the pathogens. Therefore, methods to select resistant lines from morphologically variable populations are needed. We used *S. sclerotiorum* and *S. minor*-infested field experiments, regression analysis, field experiments with artificially high plant densities, and *S. sclerotiorum* ascospore inoculations to identify lettuce lines with resistance to both pathogens. Three replicated experiments in *S. sclerotiorum*-infested fields were conducted in Yuma, Ariz. and three replicated experiments in a *S. minor*-infested field were conducted in Salinas, Calif. using diverse populations of iceberg, romaine, leaf, butterhead, Latin, oilseed lettuce and wild relatives of lettuce, and genetic variation for the incidence of lettuce drop from mycelial infections was identified. In two *S. minor* field experiments, a quadratic regression model was developed that related rapid bolting with reduced lettuce drop. Regression residuals were calculated, and eight cultivars or PIs had negative residuals in two independent field experiments, indicating higher resistance than predicted by their rate of bolting. Eruption, a small statured Latin cultivar, had significantly lower disease levels than susceptible cultivars in experiments with high plant densities, indicating that its small size did not facilitate disease escape. Ascospore inoculations confirmed resistance in 'Eruption' and *L. virosa* SAL012, while the oilseed lettuce PI251246 may have partial resistance to infection. These lines will likely be useful for the development of *Sclerotinia spp*. resistant lettuce cultivars.

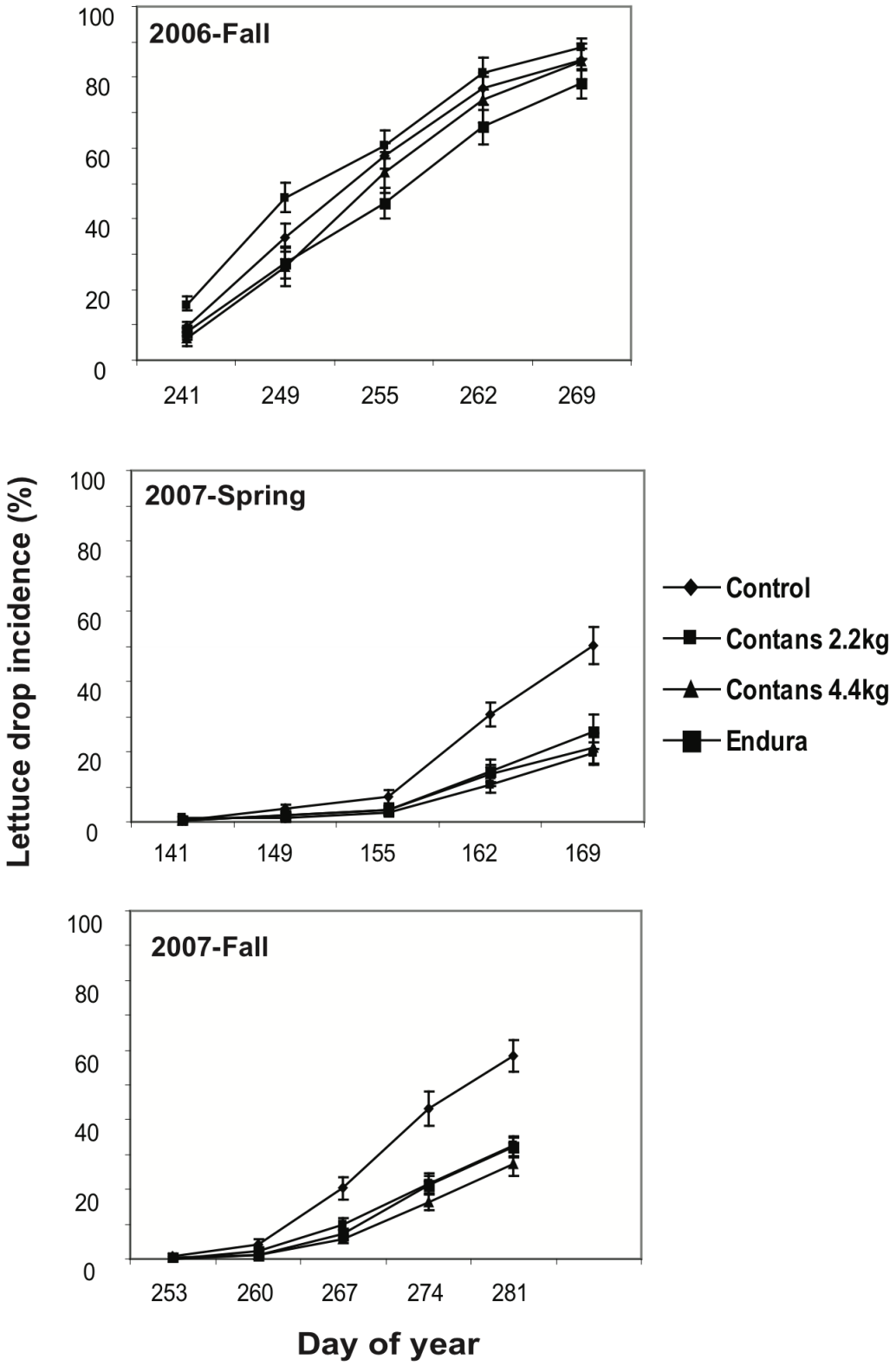

Figure 1. Progression of lettuce drop in plots infested with *Sclerotinia minor* and treated either with different rates of Contans or Endura from 2006 to 2007.

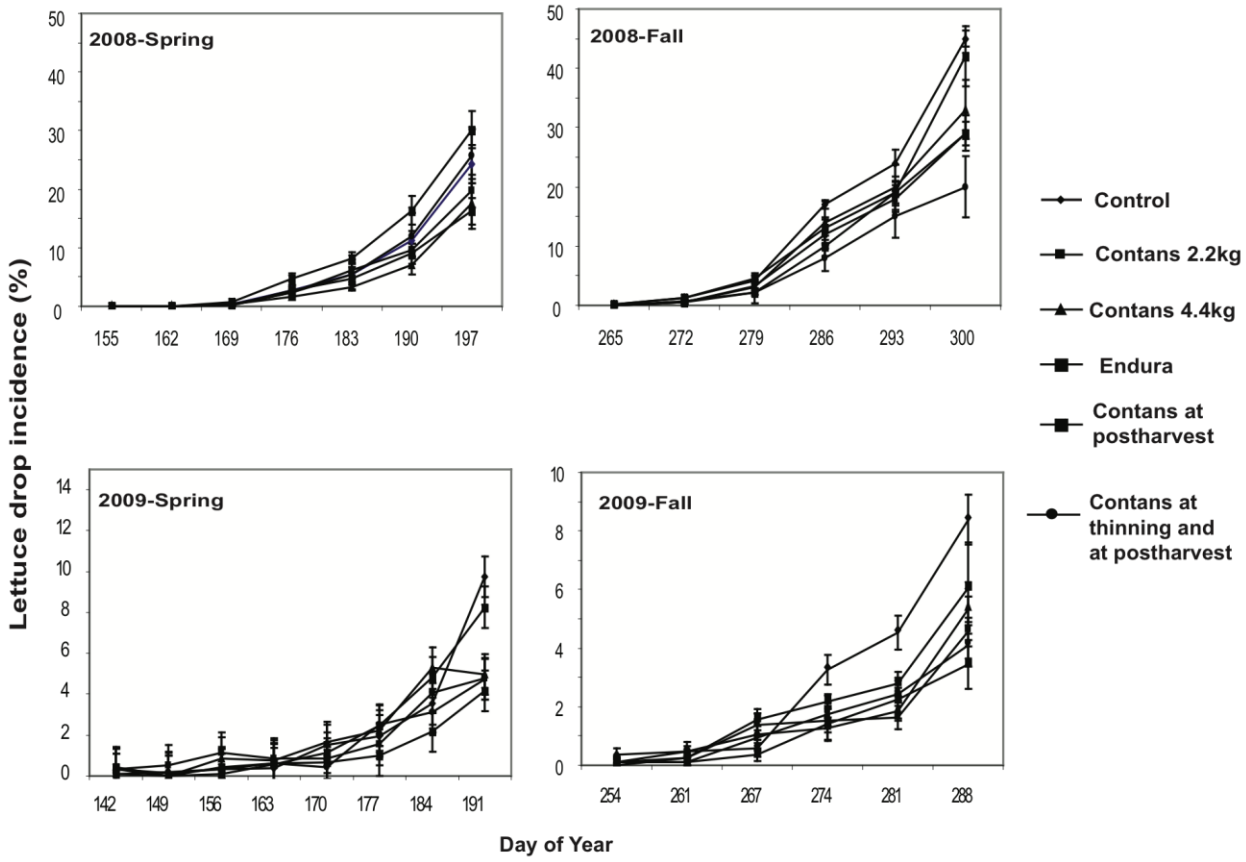

Figure 2. Progression of lettuce drop in plots infested with *Sclerotinia minor* and treated either with different rates of Contans at different times or with fungicide Endura from 2008 to 2009.

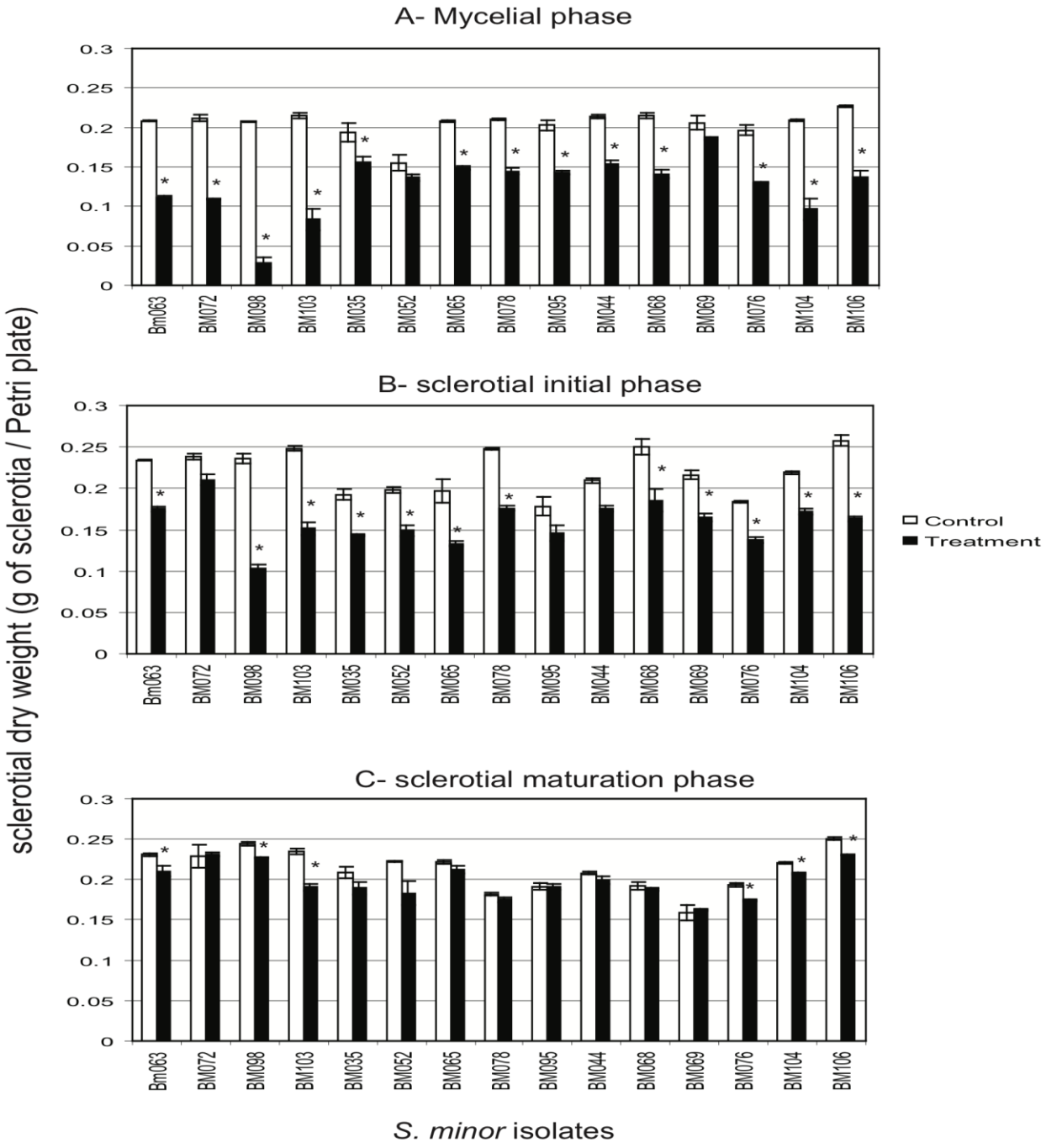

Figure 3. Efficacy of *Coniothyrium minitans* against different phases of growth in culture of several isolates of *Sclerotinia minor* belonging to different mycelial compatibility groups (MCGs) in reducing sclerotial production. **MGC I :** BM063, BM072, BM098, BM103; **MCG II :** BM035, BM052, BM065, BM078, BM095; **MCG III :** BM044, BM068, BM069, BM076, BM104; **MCG IV :** BM106

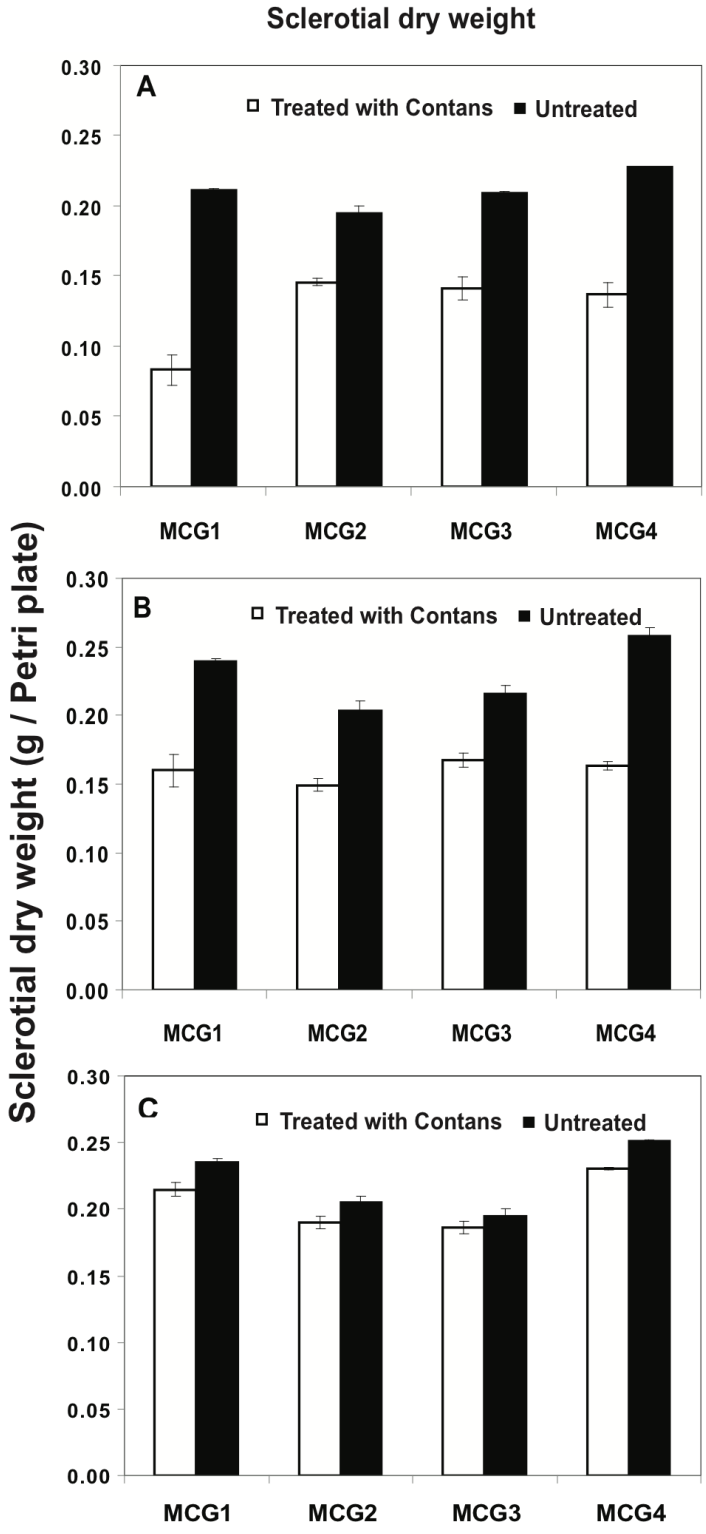

Figure 4. Efficacy of *Coniothyrium minitans* against different phases of growth of different MCGs of *Sclerotinia minor* in reducing sclerotial production. *C. minitans* inoculated at **A:** mycelial phase, **B**: early sclerotial formation phase, **C:** maturation phase

24 – 48 hr
24

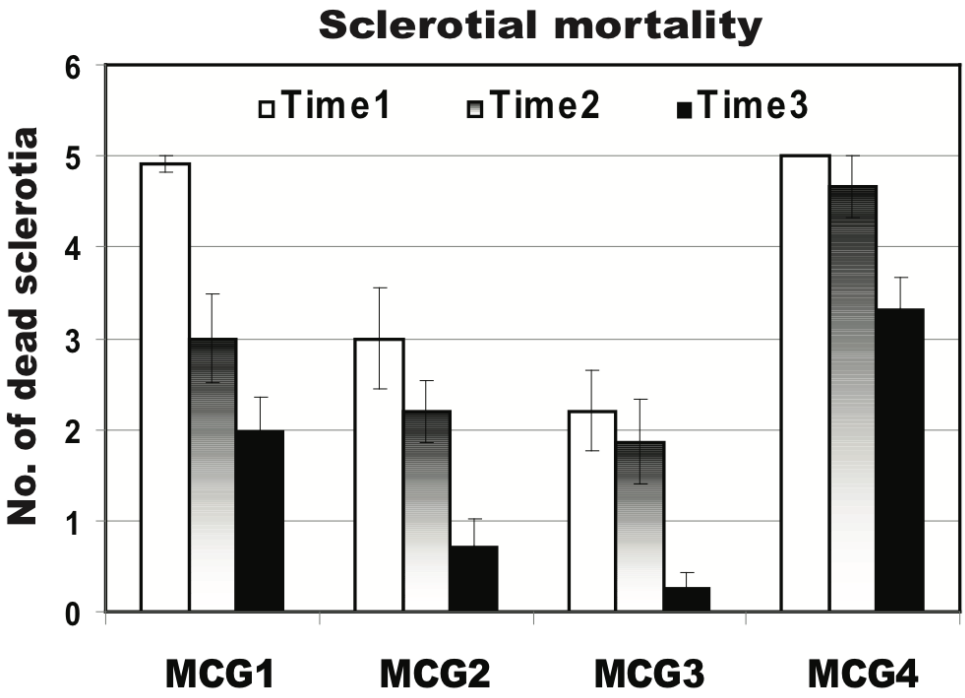

Figure 5. Efficacy of *Coniothyrium minitans* against different phases of growth of different MCGs of *Sclerotinia minor* in causing sclerotial mortality. *C. minitans* inoculated at Time 1: mycelial phase, Time 2: early sclerotial formation phase and Time 3: maturation phase