Combined efficacy assessment of soil solarization and bio-fungicides for management of *Sclerotinia* spp. in lettuce (*Lactuca sativa* L.)

Eficiencia en la combinación de solarización y biofungicidas para el manejo de *Sclerotinia* spp. en lechuga (*Lactuca sativa* L.)

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ABSTRACT

White mould: *Sclerotinia* spp., is the lettuce main disease in the Colombian high Andes. It causes yield losses of up to 70% and is primarily managed with chemical fungicides, while alternative control strategies are poorly used. Soil solarization and a set of chemical and bio-fungicides for *Sclerotinia* management efficacy in lettuce, were determined. Biofungicides included different doses of *Allium sativum* and *Matricaria recutita* extracts and *Trichoderma koningiopsis* suspensions. The fungicide procymidone was also evaluated. Field trials included single or combined treatments application. Non-linear modelling and AUDPC were employed to determine the efficacy of management strategies. Both *S. sclerotiorum* and *S. minor* caused white mould, being *S. minor* the dominant species. Solarization significantly lowered Sclerotia population in the upper soil layers and consequently lowered disease incidence. Highest levels of *Sclerotinia* control were achieved with solarization, particularly in combination with procymidone or *T. koningiopsis* (79.08% and 41.50% disease reduction, respectively). Non-linear modeling of disease epidemic progress curve proved a valuable alternative to AUDPC, which allowed multiple disease development parameters estimation and indirectly the efficacy of management strategies. This work should help the advance of environmentally-sound management of *Sclerotina* spp. in lettuce, under the highly specific growing conditions of the high Andes.

Key words: high Andean tropics, plant extracts, procymidone, soilborne pathogens, *Trichoderma koningiopsis*.

RESUMEN

El moho blanco causado por *Sclerotinia* spp. es la principal enfermedad de la lechuga en la zona altoandina colombiana, causando hasta 70% de pérdidas. El principal control de esta enfermedad ha sido con fungicidas químicos, y poco se han usado otros métodos de control alternativos. En este estudio se determinó el efecto de la solarización y su combinación con biofungicidas y un fungicida químico para el control del hongo *Sclerotinia* spp. en lechuga. Para los biofungicidas se incluyeron diferentes dosis de los extractos de *Allium sativum*, *Matricaria recutita* y una suspensión de *Trichoderma koningiopsis*. El fungicida químico fue procymidona. Se empleó modelación no lineal y ABCPE para evaluar la eficacia de las estrategias de manejo. Se encontró que tanto *S. sclerotiorum* como *S. minor* causan moho blanco, siendo *S. minor* la especie dominante. La población de esclerocios en capas superficiales del suelo disminuyó significativamente con solarización, bajando así incidencia de la enfermedad y demostrando el mejor control, especialmente en combinación con procymidona o *T. koningiopsis* (79,08 y 41,50% de reducción, respectivamente). La modelación no lineal de la curva de progreso epidémico de la enfermedad, es una valiosa alternativa al ABCPE, que permitió estimar múltiples parámetros de desarrollo de la enfermedad e indirectamente la eficacia de estrategias de manejo. El presente trabajo contribuye en la elaboración de estrategias ambientalmente seguras para el control de *Sclerotinia* spp. en lechuga, en las condiciones específicas del trópico alto Andino.

Palabras clave: Trópico alto andino, extractos de plantas, promicidona, patógenos del suelo, *Trichoderma koningiopsis*.

Introduction

Throughout the world, one of the most important diseases of lettuce is white mould, caused by *Sclerotinia minor* Jagger or *S. sclerotiorum* (Lib.) de Bary (Abawi and Grogan, 1979;

Subbarao, 1998). For example, in California (USA), yield losses ascribed to *Sclerotinia* spp. occasionally amount to 60% (Hao and Subbarao, 2005), while in Colombia, yield losses fluctuate between 20 and 70%. Colombian lettuce production is concentrated in the high Andean plateau,

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a region with highly-specific climatic conditions (Pérez, 2003). In recent years, *Sclerotinia* infestations have caused a sharp reduction of the lettuce acreage in various key production zones for this crop.

The symptoms in both species are: watery soft rot with development of white cottony mycelia on different parts of lettuce plants (Abawi and Grogan, 1979; Bolton *et al.,* 2006). At given times during the disease cycle, survival structures such as sclerotia or apothecia are produced (Abawi and Grogan, 1979; Clarkson *et al.*, 2004). Sclerotia can remain viable in the soil for up to 10 years (Adams and Ayers, 1979), which complicates management of *Sclerotinia* spp. A key prerequisite for successful *Sclerotinia* sp. management is the sound appreciation of disease development under specific (e.g., climatic, crop management) conditions.

Currently, *Sclerotinia* is most commonly managed using the fungicide procymidone (Patrício *et al.,* 2006; Wilson *et al.*, 2005). A sole reliance on chemical fungicides can lead to *Sclerotinia* resistance development, while also impacting biodiversity and interfering with key ecosystem services (Sorensen and Stewart, 2000). Additionally, *Sclerotinia* management is not based on a sound knowledge of causal agent or appreciation of extent of infestation. For the particular case of *Sclerotinia*, a single pest management strategy has not yielded satisfactory control and integrated tactics (combining physical, cultural, chemical or biological control) are urgently needed (Subbarao, 1998).

Recent research has spurred the development of alternatives for chemical fungicides. Soil disinfection through solarization has proven an effective strategy for control of pathogens, such as *Sclerotinia* (Phillips, 1990; Katan, 2000; Ferraz *et al.,* 2003). Although solarization combined with applications of procymidone yields substantial reductions of *S. minor* incidence (Patrício *et al.,* 2006), efficacy of such practice depends on local climatic conditions. Biological control of *Sclerotinia* has also been investigated, with various antagonistic fungi affecting white mould sclerotia both under *in vitro* as in field conditions (Jones and Stewart, 2000; Cheng *et al.,* 2003; Rabeendran *et al.,* 2006). Although successful biological control of *Sclerotinia* is mainly restricted to greenhouse production systems, Ávila and Gutiérrez (1991) report effective control of *S. sclerotiorum* with *Trichoderma harzianum* under field conditions in Colombia. Also, *Trichoderma koningiopsis* (Th003) Oudemans, has shown potential for *Sclerotinia* control (Cotes *et al.*, 2007). Lastly, extracts from *Allium sativum* and *Matricaria recutita* have shown promise for management of fungi like *Botrytis* and *Colletotrichum*

under *in vitro* conditions (Bianchi *et al.,* 1997). However, the efficacy of several of these management alternatives remains to be investigated under the particular growing conditions of the Colombian high Andes.

Non-linear models are commonly used for the evaluation of disease development and quantification of host pathogen and environmental effects on fungal epizootics (Bowers and Kinkel, 1997). Such models allow the analysis of disease development and can help assess the efficacy of disease management strategies (Campbell and Madden, 1990). Non-linear models could therefore serve to determine the efficacy of management alternatives for *Sclerotina* sp. in lettuce under specific environmental (i.e., climatic) conditions.

At present, Colombian lettuce growers mainly rely on chemical fungicides and largely lack workable alternatives for *Sclerotinia* disease management. The aim of this article was assessment of the effects of soil solarization and application of chemical or bio-fungicides on *Sclerotinia* spp. in lettuce in the Colombian high Andes. The specific objectives of this work are to evaluate the effect of two plant extracts and *T. koningiopsis* suspensions in a lettuce crop under open-field conditions; and to contrast the use of non-linear modelling with that of area under disease progress curve (AUDPC).

Materials and methods

Study area: Research was carried out in the municipality of Cota (4°49'05" N and 74°07'20" W) (Cundinamarca, Colombia). Specific climatic conditions of this locality are: annual mean temperature: 13.7°C, annual rainfall: 700 mm, and altitude: 2,547 m. Experiments were conducted in fields planted (700 m^2) with lettuce (*Lactuca sativa* var. Coolguard®), 11 plants/m^2, between January and May 2006. Lettuce fields with high *Sclerotinia* spp. incidence were previously selected in November 2005.

Sample collection and disease identification. A total of 20 diseased plants were randomly collected in a selected field for experimentation and taken to the Phytopathology laboratory at the "Centro de Investigaciones y Asesorías Agroindustriales" (CIAA) (Chia, Cundinamarca) to confirm identity of pathogens. Fungal samples were isolated in potato dextrose agar (PDA) and pathogens were identified based on macro and microscopic characteristics. For *Sclerotinia* identification, we used identification keys developed by Kohn (1979). In the laboratory (22.5±0.5 °C, 92±5% RH), we described morphological and developmental characteristics of the collected fungal species.

In addition, soil samples of selected field were taken and Sclerotia found were characterized morphologically (e.g. number of sclerotia produced) and their infectivity was quantified through Koch´s postulates trials (Agrios, 2005). In these trials, we used viable sclerotia obtained from soil samples and reproduced on PDA in the laboratory. Next, lettuce plants were inoculated with agar discs containing isolates of the collected fungi. A total of 20 replicates were conducted per fungal species.

Field experimental design: In January 2006, a former lettuce field (700 m^2) was superficially tilled, and 42.0 x 1.5 m beds were established. The field experiment was set up as a randomized split-plot design with five replications. The main treatment was solarization, while subplots consisted of seven different treatments: plant extracts of garlic (*Allium sativum* L.) and chamomile (*Matricaria recutita* L.) at two concentrations (1 and 25 g L^{-1}), *T. koningiopsis* WG (1 g L^{-1}), procymidone (1 g L^{-1}) and an absolute control (without applications). For the application of treatments was used a water volume of 300 L. Applications in each treatment were conducted on the basis of each plant. Each bed was divided into two equal 21 m^2 plots, excluding the area of the edges, which were assigned to the main treatment (i.e., with or without solarization); each subplot had an area of 3 m^2. For the solarization treatment, a 152 µm thick transparent plastic cover was placed during four weeks on each main plot, while no-solarization beds were left bare. Throughout the experiment, we recorded climatic data with a climate station (Micrometros®) and monitored soil temperature profiles up to 5 cm deep with T-type (coppec-constantan) thermocouples coupled to a remote datalogger (EJ-2E, Escort DLS, Auckland, New Zealand) probes at 10 min intervals. Daily average soil temperature was calculated for subset data at major radiation hours (10 a.m. and 3 p.m.) and used to determine the solarization effect on soil temperature. After four weeks, plastic cover was removed from the solarization plots. Furthermore, we took seven soil samples up to 25 cm of depth (100 g) per main plot and recorded the number of sclerotia per soil sample through sieving (2 mm, 1 mm and 250 µm) and visual counts. Previous verifications of necessary assumptions over data, a Student´s t-test were used to determine statistical differences in temperature and number of viable sclerotia between treatments: solarization and non-solarization.

Biofungicides: Garlic and chamomile extracts have shown to inhibit growth of *S. clerotiurum* mycelia under *in vitro* conditions (Espinosa and Niño, 2008). Both plant extracts were elaborated using a "Purines" method. "Purines" are obtained through anaerobic fermentation of selected plant tissues. For "garlic-purin" were used cloves of garlic, while for "chamomile-purin" were used flowers and leaves (Jiménez *et al.,* 2007). *T. koningiopsis* (Th003) is a biopesticide prototype developed by the biological control lab of the Bioindustry and Biotechnology Centre of the Colombian Corporation for Agricultural Research-Corpoica, the dose used was 1x10^6 spores/mL. Prior to experimental use, we determined the colony forming units (cfu) per cc of the fungal suspension.

Chemical product: The chemical fungicide treatment was procymidone application, a product that is commonly used by local farmers (Jiménez *et al.,* 2007). Within each of the experimental plots, lettuce seedlings were planted with four open leaves at 0.3 x 0.3 m distance. Plant extracts and fungal suspensions were applied on a weekly basis following planting, while procymidone was applied every two weeks until 30 days prior to harvest. Prior to planting, the fungal suspension was also applied at a weekly basis on lettuce seedlings and upon planting. At planting, we incorporated 1 g of *T. koningiopsis* WG.

Data collection and analysis: In each treatment, all plants were visually checked and were recorded the number of diseased plants at a weekly basis. Temporal patterns of *Sclerotinia* incidence were adjusted to different mathematical models (Campbell and Madden, 1990; Xu, 2006), obtaining the best fit with the logistic model presented in equation (1).

$$y_i = \frac{\alpha}{1 + e^{-K(dat-\gamma)}} \qquad (1)$$

Where y_i is the number of diseased plants during the ith observation, α the maximum disease incidence (horizontal asymptote), e the natural log basis, K the slope in the inflection point, dat the number of days after planting and γ the time (in *dat*) at which the curve inflection point occurs. For each treatment, α, K and γ were calculated. To determine the logistic curves goodness of fit to the recorded data, the root mean square error was calculated (RMSE). For *Sclerotinia* disease development within each treatment, the area under the disease progress curve (AUDPC) was determined using the trapezoidal integration method (Campbell and Madden, 1990; Xu, 2006) as indicated in equation (2).

$$AUDPC = \sum_{i=1}^{n} \left(\frac{y_i + y_{i+1}}{2} \right)(t_{i+1} - t_i) \qquad (2)$$

Where, y_i is the number of diseased plants at the ith observation, t_i is days after planting, and n the total number of observations.

Statistical differences for α, K, γ and the AUDPC between the various treatments were determined, using a factorial Anova, followed by a Tukey HSD post-hoc analysis. Statistics were carried out using statistical software package R (R Development Core Team, 2006).

Results

The following results with respect to white mould are reported at first under the high Andes conditions. Laboratory observations indicate that two different species of *Sclerotinia* were present: *S. minor* and *S. sclerotiorum*. Sclerotia forming patterns differed between both species: either in concentric circles (*S. minor*) or random (*S. sclerotiorum*). In laboratory, cultures of both species, the presence of *Fusarium* spp. colonies that limited or inhibited *Sclerotinia* spp. development was observed. In Koch´s postulate trials, 85% and 75% of plants inoculated with *S. minor* and *S. sclerotiorum* sclerotia respectively, showed typical disease symptoms (Subbarao, 1998; Ekins *et al.*, 2005), identical to the ones observed in the field.

Effect on soil temperature: Temperatures in solarization plots (45.3±10.8°C) were significantly higher than in plots without solarization (29.3±3.6°C) (Student´s t-test, $|t|$=79.16, df= 4734, P<0.0001) (Fig. 1). Upon establishment of the lettuce crop, the number of sclerotia in solarization plots was significantly lower than in non-solarization plots (Student´s t-test, $|t|$=3.90, df=23, P=0.0018). In plots without solarization, we recorded 3.91±0.57 via-ble sclerotia/100 g of soil, while solarization plots had 0.80±0.31 viable sclerotia/100 g of soil.

Effect on disease development: Use of functions fitted to disease curve progress for assessment management practices is one of key the foci of the study. In general, logistic curves fit well to the recorded data (Tab. 1). Solarization treatments had a significant effect on maximum *Sclerotinia* incidence (α), while biofungicides significantly affected both α and K (Tab. 2). The absence of significance for the interaction between solarization and product (Tab. 2) indicates that the effects are independent; furthermore, Fig. 2 suggest that the combination of solarization and product is different to the completely untreated (non-solarization) control. Also, time until maximum disease rate (γ) did not differ between treatments. Although disease symptoms generally appeared after approx. 20 d, K was reached 40-50 d after planting. Maximum disease rate (between 40 and 50 d) was reached independent of treatment (Fig. 2). The solarization treatment significantly affected disease incidence (α), compared to non-solarization plots (Tab. 3). Maximum disease incidence (α) was significantly lower in the procymidone treatment than in plots where *T. koningiopsis* or plant extracts were applied. Also, significant differences were found for α between "*T. koningiopsis*" and "plant extracts" treatments. The procymidone treatment was also typified by the highest rate of *Sclerotinia* development (K). Identical results were obtained using an interpretation of the AUDPC (Tab. 2).
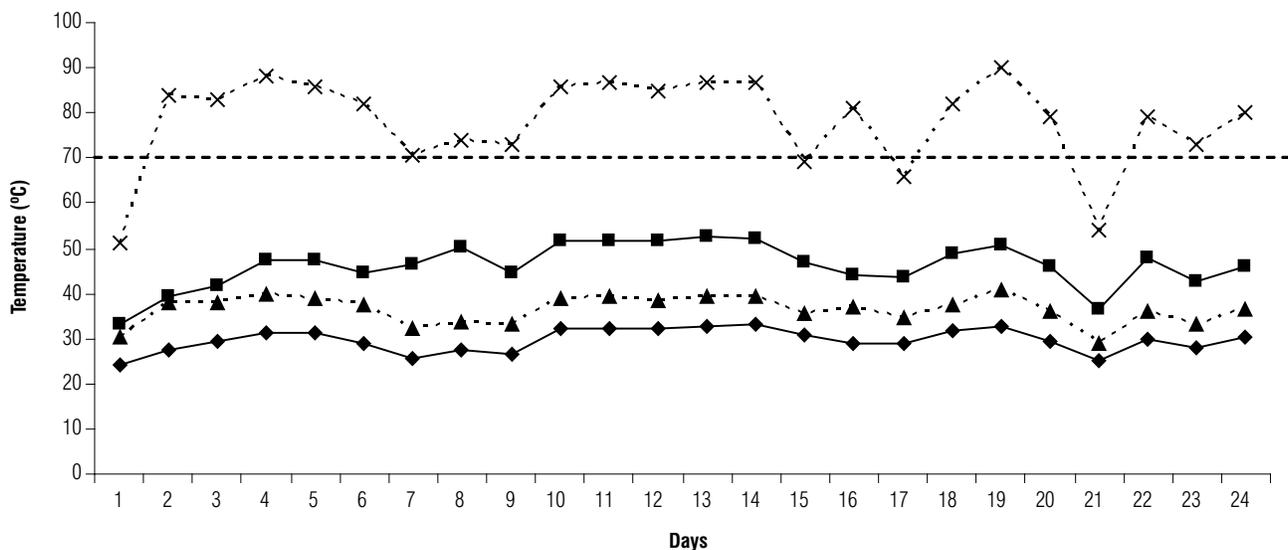

FIGURE 1. Temperature fluctuation throughout the duration of the solarization experiment (i.e., 25 days) in field plots with solarization and without solarization. The various lines represent average temperature in plots with solarization (—■—), average temperature in plots without solarization (—◆—), maximum temperature in plots with solarization (- ✕ -) and maximum temperature in plots without solarization (- ▲ -).

TABLE 1. Assessment of goodness of fit using root mean square error (RMSE), between logistic curves and the recorded data, for each of the different treatments included in the field study.

Treatments	Calculated Root Mean Square Error (RMSE)	
	Solarization	Non-solarization
Procimydone 1 g L⁻¹	0.99	1.60
T. koningiopsis WG Th003	0.81	1.93
Garlic 1 g L⁻¹	2.11	1.87
Garlic 25 g L⁻¹	1.26	1.58
Chamomile 1 g L⁻¹	4.08	2.33
Chamomile 25 g L⁻¹	0.39	0.89
Control	2.07	1.08

Discussion

Experimental evidence showed that white mould in lettuce in the Colombian high Andes is caused by both *S. minor* and *S. sclerotiorum*. Although solarization reduced the number of viable sclerotia, procymidone and *T. koningiopsis* were most effective in lowering white mould disease incidence. Except for use of *T. koningiopsis*, the combined application of solarization and biofungicides yielded a greater level of *Sclerotinia* spp. control. However, Hoyos-Carvajal *et al.* (2008) suggest that mycoparasitic capacity of *Trichoderma* species varies according to pathogen. Therefore, the results of this study would be restricted to the dominant species,

TABLE 2. Effects of solarization and biofungicides on *Sclerotinia* spp. disease development, using logistic parameters (α, γ and K) and AUDPC.

Parameter	Source	DF	MS	F	P>F
Disease incidence (α)	Solarization	1	1752	11.83	<0.05*
	Product	6	2355.1	20.79	<0.05*
	Product x Solarization	6	179.4	1.70	0.163
Time[1] of maximum rate of disease development (γ)	Solarization	1	66.25	4.14	0.112
	Product	6	39.83	1.98	0.107
	Product x Solarization	6	30.52	1.32	0.286
Maximum rate of disease development (K)	Solarization	1	0.1317	0.19	0.684
	Product	6	5.4221	6.50	<0.05*
	Product x Solarization	6	0.8416	1.72	0.159
AUDPC	Solarization	1	1313145	14.59	<0.05*
	Product	6	1131384	12.57	<0.05*
	Product x Solarization	6	134499.3	1.49	0.222

[1] Time in days after planting.
* Values followed by an asterisk are statistically significant (Anova, $P \leq 0.05$).

TABLE 3. Effect of different treatments on selected *Sclerotinia* spp. disease development parameters (α, γ and K values) and AUDPC values. Treatments included: solarization, application of procymidone, *Trichoderma koningiopsis* suspension, garlic and chamomile extracts. The effect of plant extracts on disease development was determined for two different doses (1 g L⁻¹ and 25 g L⁻¹).

Treatments	Disease development parameter						AUDPC value	
	Disease incidence (α)		Time[1] of maximum rate of disease development (γ)		Maximum rate of disease development (K)			
	Solarization	Non-solarization	Solarization	Non-solarization	Solarization	Non-solarization	Solarization	Non-solarization
Procimydone 1 g L⁻¹	14.13 a	15.11 a	49.63 a	41.18 a	2.86 a	1.60 a	197.50 a	307.50 a
T. koningiopsis Th003	39.51 b	33.56 b	40.59 b	40.80 a	0.19 b	0.90 a	653.50 a	667.50 ab
Garlic 1 g L⁻¹	43.54 b	57.73 c	45.35 ab	41.90 a	0.27 b	0.37 a	966.50 b	1,287.50 bc
Garlic 25 g L⁻¹	44.87 b	59.14 c	43.71 ab	40.56 a	0.26 b	0.26 a	841.00 b	1,290.00 c
Chamomile 1 g L⁻¹	56.70 b	62.59 c	41.35 ab	42.36 a	0.28 b	0.22 a	1,237.50 b	1,277.50 bc
Chamomile 25 g L⁻¹	51.50 b	62.75 c	46.78 ab	43.25 a	0.20 b	0.17 a	882.50 b	1,272.50 bc
Control	43.92 b	67.54 c	42.39 ab	46.13 a	0.28 b	0.23 a	867.50 b	1,467.50 c

[1] Time in days after planting.
* Values within the same column followed by the same letter are not significantly different (Tukey's HSD test, $P \leq 0.05$).
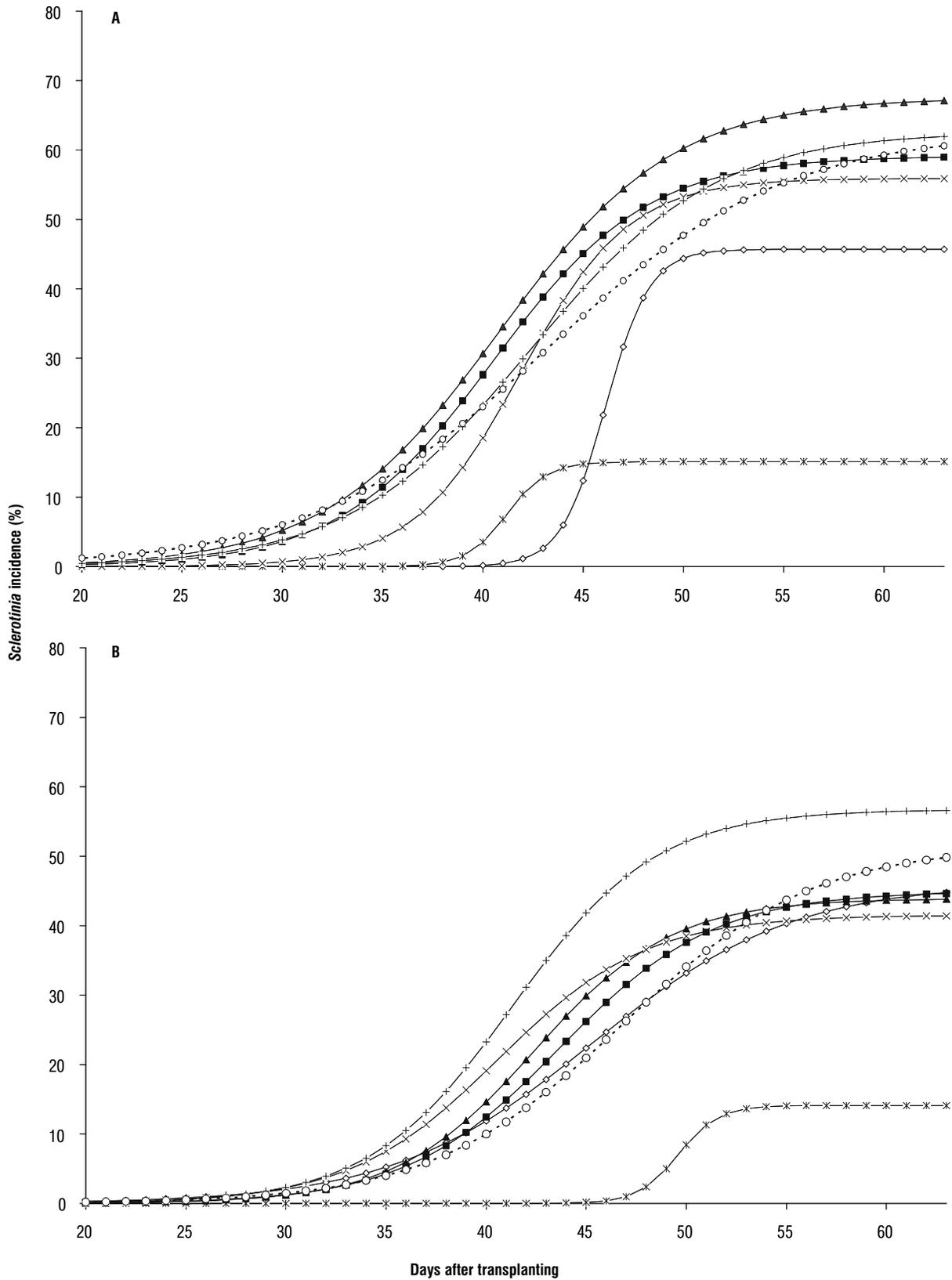
FIGURE 2. Disease curve progress for each one of treatments: *T. koningiopsis* 1 g L^{-1} (–◇–), procimydone 1 g L^{-1} (-✳-), garlic 1 g L^{-1} (–✕–), garlic 25 g L^{-1} (–■–), chamomile 1 g L^{-1} (-+-), chamomile 25 g L^{-1} (⋯○⋯) and control plot (–▲–); A, without solarization; B, with solarization.

S. minor and vary in areas where the dominant species is *S. sclerotiorum*.

Results reported in this study only emanate from experiments conducted during one cropping cycle, but with all treatments repeated five times. Given the difficulty in locating farmer-owned fields with high *Sclerotinia* incidence and setting up trials with farmers' consent, we were unable to repeat the experiment for another cycle. Nevertheless, the results were deeming indicative of the different disease management strategies effect, under the specific environmental conditions of the study region.

Although *S. sclerotiorum* has been reported from the Colombian high Andes (Avila and Gutierrez, 1991; Arias *et al*., 2007), this is the first record of *S. minor* in this region. The work showed that white mould in lettuce is caused by both species, but that *S. minor* was the dominant species in most lettuce fields. Upon presence of both *Sclerotinia* spp. in a given site, one species commonly causes the majority of disease symptoms (Subbarao, 1998). Such results could indicate that in the study region, *S. minor* likely is the main cause of white mould. Also, as *S. minor* produces more sclerotia than *S. sclerotiorum*, the former species may more likely cause fungal epidemics. Characterization of the *Sclerotinia* species complex associated with white mould in lettuce has broader implications for management of this disease.

Our work also hinted the occurrence of (natural) biological control of *Sclerotinia* in lettuce fields, with a potential role of naturally-occurring *Fusarium* sp. colonies. In the laboratory, these colonies inhibited *Sclerotinia* mycelia growth, indicating that *Sclerotinia* populations could be naturally regulated by other fungi (Adams and Ayers, 1979; Rodriguez *et al.,* 2006). Such interactions may eventually be manipulated to improve *Sclerotinia* biological control in field conditions. However, additional research is needed to determine the specific interactions between *Fusarium* spp. and both *Sclerotinia* species. Additionally, it is necessary identify the species of *Fusarium* and to quantify their potential as biological control agents.

The effectiveness of solarization greatly depends on overall weather conditions, and more specifically on ambient temperature or solar radiation. In general, solarization treatments are effective when temperatures surpass 70ºC for >30 min (Stapleton *et al.,* 2000; Flint and Gouveia, 2001). During the field trials conducted in the present study, temperatures in solarization plots exceeded 70°C for at least 4 h daily throughout the experiment, and this practice

therefore successfully disinfected soil. The observed reduction of white mould incidence following solarization, is in agreement with reports of its effect on pestiferous fungi in general (Stapleton *et al.* 2000; Ferraz *et al.* 2003) and specifically on lettuce plants infected by *S. minor* (Patricio *et al.,* 2006). Also, solarization may easily be adopted in the region, as plastics are abundantly available as waste materials from the local floriculture sector. As experiments were conducted during January and May the efficacy of solarization as a disease management practice will need to be validated during the remainder of the year.

Although solarization has considerable potential for soil disinfection and *Sclerotinia* control, its effect on beneficial soil-inhabiting organisms remains controversial. Increased periods of solarization generally allow toxic substances to accumulate and may have sub-lethal effects on beneficial organisms (Flint and Gouveia, 2001). On the other hand, certain antagonistic organisms are heat-resistant and may even be activated by solarization practices (Ferraz *et al.,* 2003; Stevens *et al.,* 2003; Porras *et al.,* 2007). Considering the potential importance of *Sclerotinia* biological control (i.e., through action of *Fusarium* spp.), solarization treatments may require continuous monitoring and fine-tuning. More specifically, long-term effects of elevated temperatures on beneficial fungi need to be investigated and their impact on *Fusarium* inoculum density and viability waits to be determined.

The high efficacy of procymidone treatment reported in this study corresponds with previous research (Wilson *et al.,* 2005; Patricio *et al.,* 2006; Arias *et al*., 2007; Cotes *et al*., 2007). However, the independent effect when using procymidone in solarized plots is somewhat surprising. For example, Patricio *et al.* (2006) reported increased control of *S. minor* when combining solarization with procymidone treatments. Contrarily, Arias *et al.* (2007) reported vastly lower levels of *S. sclerotiorum* control using the same experimental set-up in the high Andes region. This discrepancy between both studies could be attributed to the fact that Arias *et al.* (2007) carried out their research during a different time of the year (with dissimilar climatic conditions) and in plots with low *Sclerotinia* infestation. Results in this study are similar to those of Arias *et al.* (2007), with respect to low efficacy of combined use of solarization and procymidone.

The level of *Sclerotinia* spp. control by *T. koningiopsis* Th003 was lower than that described by Escande *et al.* (2002) for *T. koningiopsis* strains TK1 and TK2 and for *T. harzianum* (Avila and Gutierrez, 1991; Jones and Stewart, 2000). Such

differences could be attributed to high *Sclerotinia* spp. infestation levels in the experimental plots or the particular formulation of the fungal suspension, considering it was a prototype. Nevertheless, this work showed significant bio-control action of *T. koningiopsis* Th003 against *Sclerotinia* spp., with this bio-pesticide eventually constituting a viable alternative to procymidone. The description of *Sclerotinia* spp. control with *T. koningiopsis* is one of few reports of *Sclerotinia* biological control under field conditions (Alabouvette *et al.,* 2006). Along this line, although little is known about *Trichoderma* spp. behaviour in the field, results hint at a reduced activity of *T. koningiopsis* in solarization plots. At the end of the experiment, *T. koningiopsis* cfus were lower (6.91 cfu/mL) in solarization plots than in non-solarization plots (8.57 cfu/mL), which may indicate that solarization eventually compromises the efficacy or viability of *T. koningiopsis*. Secondary succession of microorganisms, after solarization, may affect the establishment of *T. koningiopsis*.

Although garlic and chamomile extracts effectively inhibited *Sclerotinia* growth in the laboratory, no control was reported under field conditions. This lack of *Sclerotinia* control can be ascribed to two factors: firstly, certain environmental factors could cause a quick degradation of the active ingredients and impede the action of plant extracts under field conditions. Secondly, the artisan preparation of plant extracts may allow contamination with micro-organisms, which may cause degradation of active ingredients. Additional research is needed to determine the exact causal factors of this lack of control of plant extracts.

Modelling approaches are routinely used for evaluating disease development and determining the effect of various factors (e.g., disease management) on fungal epidemics. AUDPC values are valuable descriptors of disease development, and have been widely used in plant pathology (Campbell and Madden, 1990; Van Maanen and Xu, 2003; Xu, 2006). However, results indicated that a more useful alternative to AUDPC analyses is the adjustment of the epidemic progress curve to a theoretical model, which allowed estimation of multiple parameters of disease development over time. Computation of these parameters then provides the basis for informed pest management decision making, such as the targeting of fungicidal sprays. Field results indicated that, in order to be effective, control measures should be taken prior to γ, i.e. during the first 40 d after planting.

This research is of crucial importance for the formulation of disease management protocols against *Sclerotinia* in Colombia and eventually throughout the Andes. Solarization and application of biofungicides (i.e., *T. koningiopsis*) have proven to be viable alternatives to chemical control of white mould in lettuce, while being safe, low-cost and environmentally-sound technologies which can easily be adopted by local small-scale vegetable farmers.

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