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METHODS OF INOCULATION OF SUNFLOWER HEADS WITH *Sclerotinia sclerotiorum*

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SUMMARY

Sclerotinia head rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a major disease in sunflower (*Helianthus annuus* L.). Development of hybrids with adequate genetic resistance is necessary to reduce yield losses. The objective of this study was to find an effective technique of inoculating sunflower heads with *Sclerotinia sclerotiorum* for screening and identifying resistant and susceptible genotypes.

A factorial experiment was conducted to evaluate the effectiveness of different inoculation procedures. The factors were bag color, water treatment, inoculum type, and rate of inoculation.

Based on the results of this study, the inoculation procedure recommended to sunflower researchers who wish to screen lines or hybrids for Sclerotinia head rot resistance is spraying the heads at the beginning of flowering with 5 cm^3 of a suspension of ascospores containing 5000 ascospores per milliliter and covering the heads with brown paper bags immediately after inoculation. Application of additional water is not needed. Measurements of infection could begin as early as 35 days after inoculation.

Key words: ***Sclerotinia sclerotiorum*, head rot, sunflower, disease resistance**

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is one of the most important pathogens of sunflower (*Helianthus annuus* L.). It is encountered in all sunflower-growing regions of the world (Gulya *et al.*, 1997). The fungus can attack several plant parts and cause stalk rot/wilt or head rot. Sclerotinia head rot is considered a major disease in Europe, Argentina, and the United States. Severe attacks can cause losses up to 100% (Sackston, 1992).

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The available chemical controls are difficult to perform or ineffective (Péres and Regnault, 1985). Thus, development of hybrids with adequate genetic resistance is necessary. Although complete resistance in cultivated sunflower has not been reported, significant differences in the level of susceptibility have been identified in diverse germplasm (Leclerq, 1973). Methods for screening germplasm are needed to detect those differences.

The degree of infection is affected by the presence of sclerotia in the soil and weather conditions, so it varies between years and regions. Artificial infections using ascospores and mycelium have been demonstrated useful for screening sunflower lines and hybrids (Tourvieille and Vear, 1984; Vear and Guillaumin, 1977).

The objective of this study was to find an effective technique of inoculating sunflower heads with *Sclerotinia sclerotiorum* for screening and identifying resistant and susceptible genotypes.

MATERIALS AND METHODS

The sunflower hybrid HA 403 × RHA 373 was chosen for treatment because this hybrid was known to be susceptible to Sclerotinia head rot. Both lines are USDA-ARS germplasm releases.

The experiment consisted of 24 treatments arranged in a 2 × 2 × 2 × 3 factorial designed. The factors were bag color, water treatment, inoculum type, and rate of inoculation. Rates of inoculation were nested within inoculum types. The bag colors were white and brown. The water treatments were additional water and no additional water. The inoculum types were suspensions of ascospores and ground mycelia/wheat. The rates of inoculation for the ascospore treatment were 1000, 5000, and 15000 ascospores per milliliter of suspension. The rates of inoculation for the ground mycelia/wheat treatment were 1, 5, and 15 cm^3 of ground mycelia/wheat. All the factors were considered as fixed.

The experimental design was a randomized complete block design with three replicates. Each plot consisted of a single 6-m row with a spacing of 75 cm between rows. Plants were thinned by hand to a spacing of approximately 30 cm between plants. Ten heads were treated per plot.

The experiment was planted at Fargo, ND, USA, on May 25, 2000. Heads were treated at the beginning of flowering, R5.1 stage (Schneiter and Miller, 1981). The inoculation of heads with ascospores was done by spraying florets with 5 cm^3 of a suspension with 1000, 5000, or 15000 ascospores per milliliter of distilled water. The concentration of ascospores was measured with a hemocytometer. The inoculation of heads with ground mycelia/wheat was done by scattering 1, 5, or 15 cm^3 of a ground mycelia/wheat mixture on the floral surface after the surface was sprayed with 5 cm^3 of water. Immediately after inoculation with both types of inoculum, the heads were covered with either white or brown paper bags. In the additional-water treatments, 5 cm^3 of water were applied to the heads each day of the two days following inoculation. All heads were inoculated on the same day (August 10).

Five treatments with no inoculation were included as controls. Four control treatments had either white or brown paper bags, and either additional water appli-

cation or no additional water application. The remaining control treatment had no bags and no additional water application. The controls did not show any infection and were not included in the analyses.

The heads were uncovered 28 days after inoculation and the symptoms of Sclerotinia head rot were measured on each head using a scale ranging from 0 to 5 (0, no symptoms; 1, less than 12.5% of the head infected; 2, between 12.5 and 25% of the head infected; 3, between 25 and 50% of the head infected; 4, between 50 and 90% of the head infected; 5, more than 90% of the head infected). A "severity index" was calculated by averaging the scores of the heads inoculated in each plot. The same measurements were also taken 35 and 42 days after inoculation.

Analyses of variance were performed using PROC GLM (SAS Institute, 1990). An *F*-protected LSD (P=0.05) was calculated for each significant source of variation.

RESULTS AND DISCUSSION

The experimental mean of severity index of *Sclerotinia sclerotiorum* infection was 2.1, 3.1, and 3.5, and the coefficient of determination was 0.67, 0.85, and 0.86 for the first (28 days), second (35 days), and third (42 days) dates of measurement after inoculum application, respectively.The coefficient of determination was higher for the second date than for the first date, indicating that a greater proportion of the variation was explained by the model when measurements were taken on the second date. Although the coefficient of determination was somewhat higher for the third date than for the second date, the disease symptoms were more evident on the second date due to less natural senescence confounding the measurement of severity index. Therefore, measurements 35 days after inoculation are recommended.

Table 1: Analyses of variance for severity index of sunflower heads 28, 35, and 42 days after inoculation with *Sclerotinia sclerotiorum*

Source of variation	df	Mean square		
		28 d	35 d	42 d
Replicate	2	0.383	0.369	0.072
Bag color (B)	1	5.969**	5.899**	5.654**
Water treatment (W)	1	0.407	0.134	0.328
B × W	1	0.606	0.449	0.207
Inoculum type (I)	1	21.725**	57.141**	55.095**
B × I	1	1.026	0.006	0.696
W × I	1	0.053	0.689	0.997
B × W × I	1	0.021	0.134	0.023
Rate (I) [R(I)]	4	2.495**	1.439**	1.212**
B × R(I)	4	0.277	0.143	0.204
W × R(I)	4	0.629	0.266	0.251
B × W × R(I)	4	0.541	0.218	0.263
Error	46	0.510	0.289	0.262

** Significant at the 0.01 level of probability.

The analyses of variance for the three dates of measurement showed no significant interactions among the factors (Table 1). Sclerotinia infection on heads with different bag colors, inoculum types, and rates within inoculum types were significant for the three dates.

The severity index was significantly higher for brown bags than for white bags (Table 2). The purpose of the bags was to keep the level of moisture sufficiently high to permit infection. It was hypothesized that white bags would reflect sunlight more than brown bags, keeping the moisture content higher. However, the brown bags appeared to have equal amount of moisture on the heads when compared with the white bags. It is possible that the brown bags created a warmer environment, promoting faster and higher infection rates.

Table 2: Comparisons among severity index means of sunflower heads for the bag colors, types of inoculum, and rates within inoculum types

Treatment	Days after inoculation		
	28 d	35 d	42 d
White bag	1.8	2.8	3.2
Brown bag	2.4	3.4	3.8
LSD (0.05)	0.3	0.3	0.2
Mycelia	1.6	2.2	2.6
Ascospores	2.7	4.0	4.4
LSD (0.05)	0.3	0.3	0.2
1000 ascospores cm^{-3}	2.2	3.8	4.2
5000 ascospores cm^{-3}	2.8	4.1	4.4
15,000 ascospores cm^{-3}	3.0	4.1	4.4
LSD (0.05)	0.6	0.4	0.4
$1cm^3$ mycelia/wheat	1.0	1.7	2.1
5 cm^3 mycelia/wheat	1.9	2.5	2.7
15 cm^3 mycelia/wheat	1.8	2.5	2.9
LSD (0.05)	0.6	0.4	0.4

The severity index was significantly higher for the ascospore inoculation than for the mycelium inoculation (Table 2). Therefore, inoculations using a suspension of ascospores were more effective than those using the ground mycelia/wheat mixture.

On the first date of measurement, the severity index was significantly higher for the medium (5000 ascospores cm^{-3}) and high (15,000 ascospores cm^{-3}) ascospore rates than for the low rate (1000 ascospores cm^{-3}) (Table 2). However, the severity index for the three rates was not significantly different on the second and third dates of measurement. The significance of rate within inoculum types was mainly due to differences among mycelium rates.

The additional water treatments were not significant for any of the dates of measurement (Table 1). A continuous wet period of 42 h after contamination is necessary for successful infection (Lamarque, 1983). However, the additional water

applied in each of the two days following the inoculation did not increase the infection. Therefore, the moisture content under the bags was sufficiently maintained by the initial inoculation procedure.

CONCLUSIONS

Based on the results of this study, the inoculation procedure recommended to sunflower researchers who wish to screen lines or hybrids for Sclerotinia head rot resistance is spraying the heads at the R5.1 stage with 5 cm^3 of a suspension of ascospores containing 5000 ascospores per milliliter of suspension and covering the heads with brown paper bags immediately after inoculation. Application of additional water is not needed. Measurements of infection could begin as early as 35 days after inoculation. This inoculation procedure is quite similar to the successful ascospore test reported by Tourvieille and Vear (1984).

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MÉTODOS DE INOCULACIÓN DEL CAPÍTULO DE GIRASOL CON *Sclerotinia sclerotiorum*

RESUMEN

La podredumbre húmeda del capítulo, causada por *Sclerotinia sclerotiorum* (Lib.) de Bary, es una enfermedad importante para el girasol (*Helianthus annuus* L.). El desarrollo de híbridos con una adecuada resistencia genética es necesario para reducir las pérdidas de rendimiento. El objetivo de este estudio fue encontrar una técnica efectiva para inocular capítulos de girasol con *Sclerotinia sclerotiorum* para identificar y seleccionar genotipos resistentes y susceptibles.

Un experimento factorial fue conducido para evaluar la efectividad de diferentes procedimientos de inoculación. Los factores fueron color de bolsa, tratamiento con agua, tipo de inóculo, y tasa de inoculación.

Sobre la base de los resultados de este estudio, el procedimiento de inoculación recomendado a investigadores que deseen seleccionar líneas o híbridos para resistencia a la podredumbre del capítulo causada por Sclerotinia en girasol sería asperjar los capítulos al comienzo de floración con 5 cm^3 de una suspensión de ascosporas que contenga 5000 ascosporas por mililitro y cubrir los capítulos con bolsas de papel marrón inmediatamente. La aplicación adicional de agua después de la incoulación no sería necesaria. Las mediciones de la infección podrían comenzar 35 días después de la inoculación.

MÉTHODES D'INOCULATION DES CAPITULE DE TOURNESOL AVEC *Sclerotinia sclerotiorum*

RÉSUMÉ

La pourriture blanche, causée par Sclerotinia sclerotiorum (lib.) de Bary, est une des principales maladies du tournesol (*Helianthus annuus* L.). Le développement d'hybrides résistants est nécessaire pour réduire la perte en rendement. L'objectif de cette étude était de trouver une technique efficace d'inoculation des capitules de tournesol avec *Sclerotinia sclerotiorum* dans le but de identifier et sélectionner les génotypes résistants et susceptibles.

Une expérience factorielle a été conduite pour évaluer l'efficacité de procédures d'inoculations différentes. Les facteurs à l'étude étaient couleur du sac, traitement de l'eau, type d'inoculum, et quantité inoculée.

D'après nos résultats, la procédure recommandée aux chercheurs voulant sélectionner des lignées ou hybrides de tournesol résistantes au Sclerotinia serait d'asperger les têtes en début de floraison avec 5 cm^3 d'une suspension d'ascospores contenant 5000 ascospores par millilitre et de couvrir les capitules d'un sac en papier marron immédiatement après l'inoculation. L'addition d'eau après inoculation ne serait pas nécessaire. Les mesures d'infection pourraient commencer au plus tôt 35 jours après inoculation.