SUSCEPTIBILITY OF DIFFERENT GARLIC (*Allium sativum* L.) TYPES TO *Sclerotium cepivorum* Berk.

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SUMMARY

The objective of this study was to confirm field observations on differences in susceptibility to white rot (*Sclerotium cepivorum* Berk.) shown by white, red and pink types of garlic cultivars planted in Mexico. Pink cultivars of garlic were the most susceptible followed by red and white cultivars. White rot induced the higher yield reductions on the pink cultivars (75 to 99 %), red varieties 65 to 90 %, and white cultivars 23 to 55 %. The white cultivars Perla and Blanco de Cortazar together with 'Massine' and 'Taiwan' types of red and pink cultivars, respectively, showed the lowest susceptibility to *S. cepivorum*.

ADDITIONAL KEY WORDS: cultivars, tolerance, white rot.

SUSCEPTIBILIDAD DE DIFERENTES TIPOS DE AJO (*Allium sativum* L.) A *Sclerotium cepivorum* Berk.

RESUMEN

El presente trabajo se realizó con la finalidad de confirmar las observaciones de campo respecto a que los genotipos de ajo (*Allium sativum* L.) de tipo blanco que se plantan en México, presentan menor susceptibilidad a la pudrición blanca (*Sclerotium cepivorum* Berk.) en comparación con ajos tipo morado y jaspeado. Los valores más altos de área bajo la curva del progreso de la enfermedad, tasa de incremento de enfermedad y la incidencia final de la pudrición blanca, en general los presentaron los ajos tipo jaspeado, seguidos de los morados y los más bajos correspondieron a los de tipo blanco. La reducción en el rendimiento causado por la enfermedad fue mayor en los ajos de tipo jaspeado, variando de 75 a 99 %, en los morados la reducción fue de 65 a 90 % y en los blancos de 23 a 55 %. Los cultivares de tipo Blanco Perla y Blanco de Cortazar destacaron por su menor susceptibilidad a *S. cepivorum* y, 'Massone' y 'Taiwan' en los tipo morado y jaspeado, respectivamente.

PALABRAS CLAVE ADICIONALES: cultivares, tolerancia, pudrición blanca.

INTRODUCTION

Mexico is the second main producer of garlic (*Allium sativum*) on the American Continent, after the United States, producing 65,257 t from 8,090 ha in 1996 (SAGAR, 1996). Garlic cultivars are classified according to the color of cloves, as red, pink and white types (Heredia, 1995). White rot (*Sclerotium cepivorum*) attacks garlic and reduces yield and quality in Mexico. The fungus survives as sclerotia that may survive up to 20 years (Coley-Smith *et al*., 1990). The interaction between *Allium* species and *S. cepivorum* is specific due to the presence of alkenyl and alkyl sulfoxides produced by the host, that simulate sclerotial germination (King and Coley-Smith, 1968; 1969). The high populations of sclerotia in soils together with their longevity, make it difficult to control the disease.

An important element in the management of the white rot consists in the knowledge of the behavior of garlic germplasm before the disease. In a trial conducted in a soil infested by *S. cepivorum* sclerotia, onion (*A. cepa*), leek (*A. porrum*), *A. fistulosum* and a garlic cultivar (*A. sativum*); garlic was the most susceptible specie (Coley-Smith and Esler, 1983), and garlic resistance was not been fully confirmed (Coley-Smith and Entwistle, 1988). Pérez *et al*. (1995) used, ^{60}Co irradiation to produce, red and white garlic with tolerance to *S. cepivorum*. Differences in the susceptibility to white rot have been observed between

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cultivars of garlic when planted in commercial plots. It is frequently stated that the white genotypes are less susceptible to this disease (Pérez *et al*., 1995; López *et al*., 1996).

The reaction of garlic to white rot may depend on the amount and time of a stimulus produced by the roots, or to tissue resistance. In studies to evaluate the resistance to white rot of *Allium* species, the reliability of results obtained depends, among other things, on the sampling technique, the pathogenicity of *S. cepivorum,* and the temperature conditions (Entwistle *et al.*, 1990; Brix and Zinkernagel, 1992). A reduced response by *Allium* to *S. cepivorum* could result from a weak stimulation of sclerotial germination, or to tissue resistance (Zinkernagel and Brix, 1990, Brix and Zinkernagel, 1992).

This paper describes investigations into the reactions to *S. cepivorum* of different cultivars of garlic.

MATERIALS AND METHODS

Source of Inoculum

Sclerotia from a naturally infested soil in Cortazar, Guanajuato Mexico, were obtained by wet-sieving (Utkhede and Rahe, 1979) and transferred to potato-dextrose-agar (PDA). Mycelium from the colonies was transferred to Erlenmeyer flasks containing barley seeds so as to produce large quantities of sclerotia (van der Meer *et al.,* 1983). Viability on agar was 96 % and germination in a sterile soil exposed to one atmosphere with 0.6 mg of diallyl disulfide per g of soil (Entwistle and Smith, 1994) was of 87 %.

Prior to the trials for the comparison of susceptibility of garlic genotypes, the pathogenicity of sclerotia produced in the barley preparation was corroborated. For this purpose, in rectangular garden plots containing soil treated with methyl bromide (454 $g \cdot m^{-3}$), 1,000 sclerotia per linear m were inoculated to a depth of 10 cm, in three 1 m rows with an inter-row separation of 25 cm. They were covered with a layer of soil and cloves-"seeds" of garlic cv. Celayense were planted to a depth of 5 cm and a clove to clove separation of 5 cm. By the end of 142 days a 100 % incidence of plants with white rot was registered.

Experiment 1

The study was established in autumn-winter (1995-1996 in the Bajío Experimental Field of the National Institute for Forest and Agricultural Research (Instituto Nacional de Investigaciones Forestales y Agropecuarias) Celaya, state of Guanajuato, Mexico. The red cultivars used were Pocitas, Criollo del Bajío, Massone, Vikingo I, Pata de Perro, and Criollo de Oaxaca. The pink type cultivars used were Tacatzcuaro, Celayense and Taiwan; and the white type cultivars used were Perla, Blanco de Cortazar and Blanco de Egipto. In 1.0 m wide by 30 cm deep rectangular garden plots, clay soil previously treated with methyl bromide (454 $g \cdot m^{-3}$ of soil) was deposited. A rectangular plot (1 x 10 m) was prepared with inoculum and another one without inoculum. In the first one, 1,000 *S. cepivorum* sclerotia per linear m to a depth of 10 cm were added in every row, with an inter-row distance of 25 cm. After this, they were covered with a layer of soil and a clove-"seed" of the material, having a 20-plants per row for every cultivar, sown 5 cm over sclerotia. Twelve garlic cultivars were planted in every rectangular garden plot, and plots were replicated three times. The experimental design used was that of random blocks with a split plots distribution, where the big plot corresponded to the inoculum levels (with and without inoculum) and the small plot corresponded to the garlic germplasm. The incidence of the disease was recorded weekly and the weight of healthy and diseased cloves recorded at the end of the experiment, these yield expressions were expressed in $kg \cdot ha^{-1}$. White rot incidence was calculated as, the area under the disease progress curve (AUDPC) (Campbell and Madden, 1990) and the rate of disease increase according to Weibull's epidemiological model (Campbell and Madden, 1990); and Tukey's mean comparison test $P \leq 0.05$ were calculated. Soil temperatures at a depth of 10 cm were recorded by obtained, thus registering a maximal average temperature of 20.1 ºC and a minimal average temperature of 13.4 ºC. Day-degrees were calculated, using a threshold temperature of 5 ºC, both for *S. cepivorum* and garlic (Poinselot, 1980), using the double sine method (Higley *et al*., 1986).

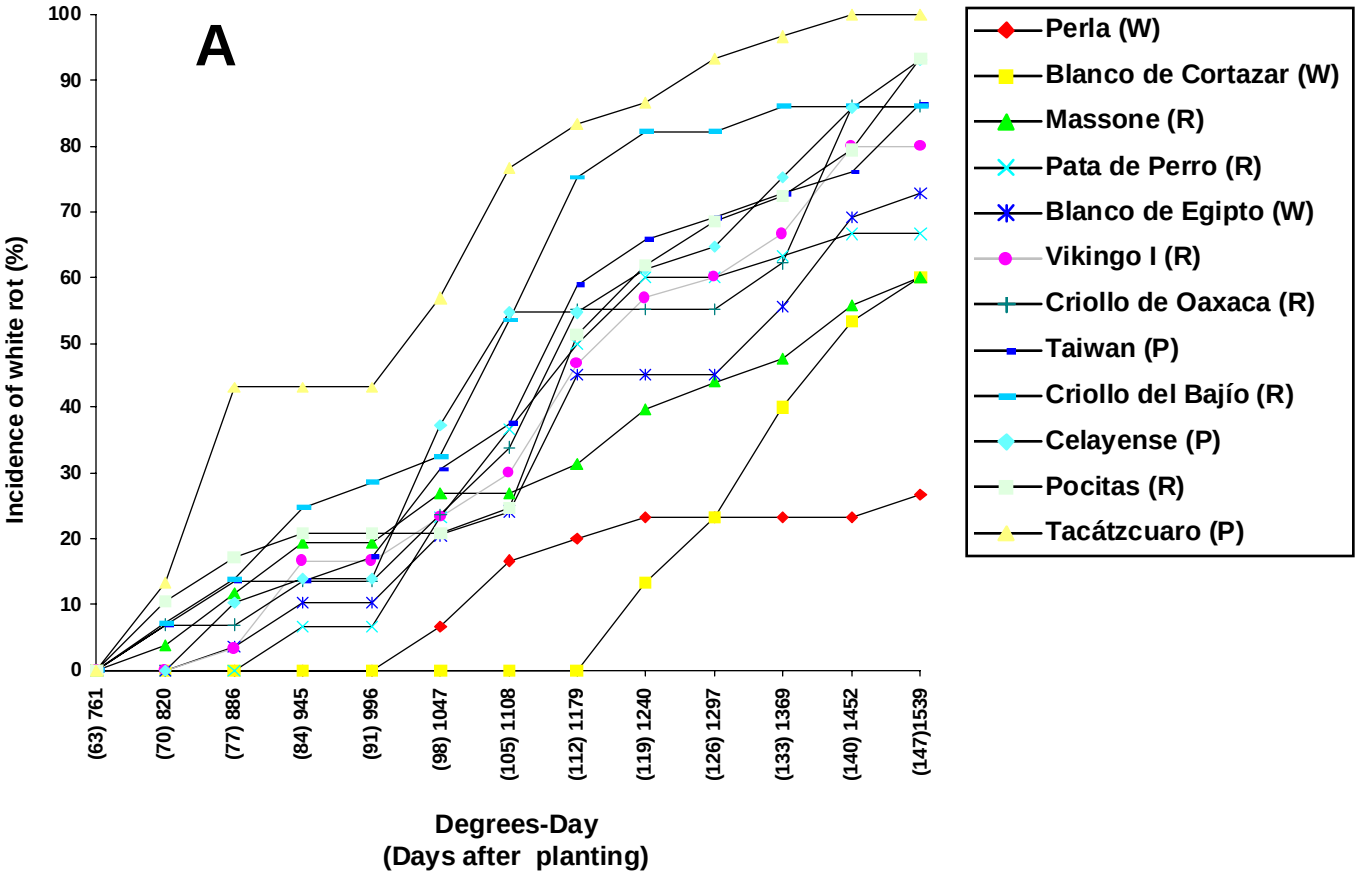
Experiment 2

The study was repeated in autumn-winter 1996-1997 with six garlic cultivars: ('Massone', 'Pata de Perro': red, 'Tacatzcuaro': pink, and 'Perla', 'Blanco de Cortazar' and 'Blanco de Egipto', white). These cultivars were selected because of their differential response to disease in the first trial. Other wise the details were similar to experiment 1.

RESULTS

Temperature conditions prevailing during the development of trials favoured the development of *S. cepivorum* and the garlic host. Thus soil temperatures at a depth of 10 cm were a maximum of 20.1 ºC and a minimum 13.4 ºC. Symptoms of chlorosis and wilt first appeared after 820 and 742 degrees-day in both experiments (Figure 1 and 2).

The three types (colors) of garlic differed in the rate of development of white rot symptoms (Table 1 and 2). Thus the rate of white rot development was slowest in the white cultivars, and highest in the pink cultivars (Figure 1 and 2). Likewise, the highest values of the AUDPC for the epidemiological variables, disease rate (b^{-1}) and the final incidence of disease in general were shown by the pink



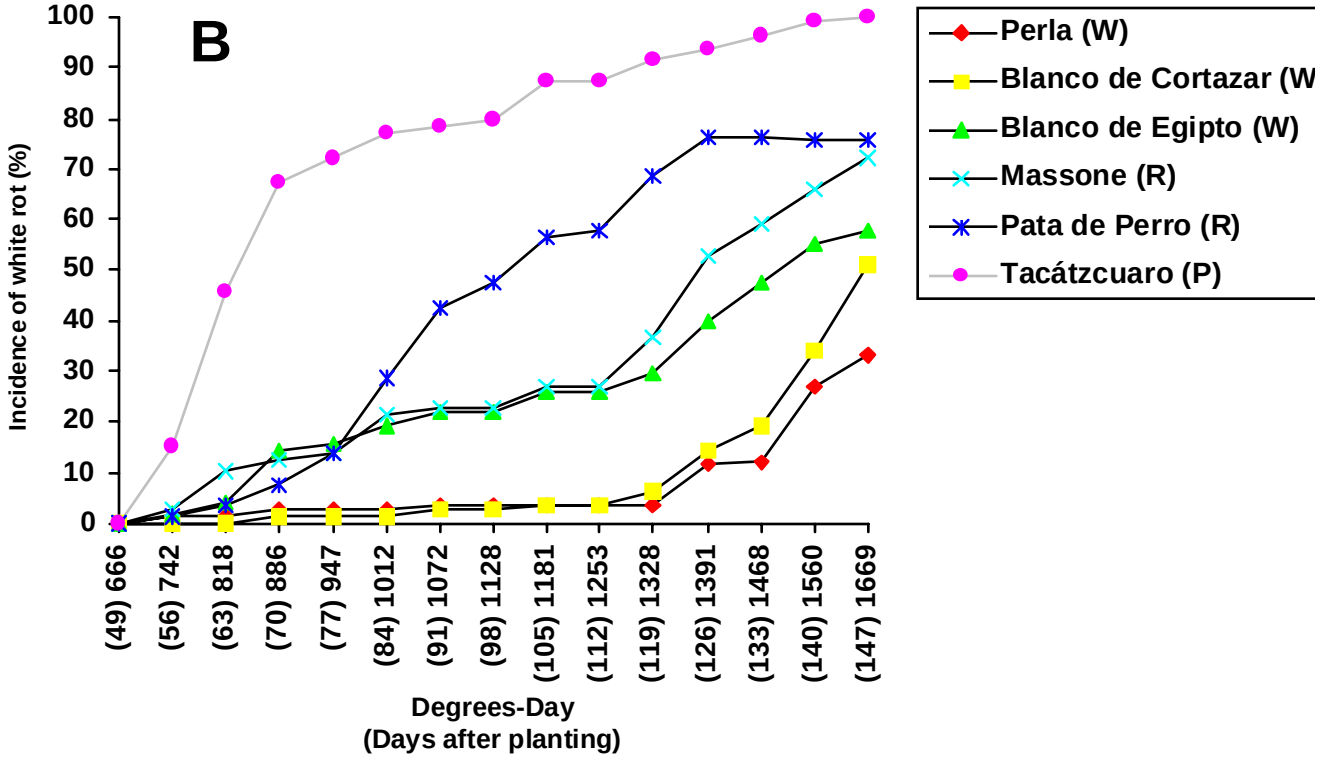


Figure 1. Progresse or white rot in garlic cultivars of the Pink type (P), Red type (R) and White type (W). Celaya, Gto. A) Cycle 1995-1996, B) Cycle 1996-1997.

garlic cultivars, in the first place, with the red ones in second place and the lowest ones corresponded to the white garlic cultivars (Table 1 and 2). Because of their lower susceptibility to *S. cepivorum,* 'Perla' and 'Blanco de Cortazar' were outstanding in the white types; for the red ones, 'Massone' was also outstanding and 'Taiwan' was notorious in the pink group (Table 1 and 2).

Even when the red genotypes commonly showed an intermediate performance to susceptibility, it was also evident that in this type of garlic there was a larger heterogeneity in the response to the attack of *S. cepivorum*; for example, 'Criollo del Bajio' had a very susceptible performance while 'Massone' had a similar susceptibility to that of some white type genotypes (Table 1).

In both experiments, the yield reduction (%) in garlic plants grown in *S. cepivorum* infested soils was larger in the pink group of garlic, with a variation ranging from 75 to 99 % and with an average decrease of 89 %. In the red cultivars decrease had a variation in the range of 65 to 99 % with an average value of 73 %. In the white genotypes showed a decrease in the range of 23 to 55 % with an average of 42 % (Table 1 and 2). Within the highly susceptible red garlic cultivars, Tacatzcuaro was the most damaged cultivar and it showed the lowest yield. Within the red cultivars, 'Criollo de Oaxaca' and 'Pocitas' were the most affected by white rot. Within the white genotypes, 'Blanco de Egipto' was the one which yield was mostly affected (Table 1 and 2).

The correlation analysis between AUDPC, disease increase rate, final accumulated incidence of diseased plants and yield decrease percentage, indicated an inversely proportional relation between disease and yield (Table 3).

DISCUSSION

The AUDPC analysis, the disease increase rate, and the final accumulated incidence of garlic plants affected by *S. cepivorum*, made possible the characterization of the response of garlic genotypes to white rot (table 1 and 2). A direct significant correlation was found between the disease incidence expressed in AUDPC, the rate of increase and the final incidence of white rot and the yield of garlic at harvest (Table 3).

The lowest susceptibility to white rot of the white types of garlic, compared to that of the red types of garlic was previously reported by Bristow (1982). However, Coley-

TABLE 1. Incidence of white rot as area under the disease progress curve (AUDPC), disease-increase rate (b^{-1}) (Weibull model), determination coefficient of b^{-1} (r^2), final incidence of white rot and yield of varieties of different types of garlic. Celaya, state of Guanajuato. México. 1995-1996.

Cultivars and types of garlic	AUDPC	Disease reduction (%) [y]	b^{-1}	r^2	Final incidence (%)	Yield (kg•ha^{-1})		
						Inoculated	Not inoculated	Reduction[x] (%)
'Tacatzcuaro' (P)	777.9 a[z]	-	0.2128 a	0.94	100 a	115 g	14,145 a	99
'Criollo del Bajio' (R)	612.2 ab	21.3	0.1476 b	0.95	86 abc	1,321 def	6,133 d	78
'Celayense' (P)	517.1 bc	33.5	0.1284 bc	0.96	93 ab	1,038 cde	13,050 a	92
'Taiwan'(P)	501.3 bc	35.4	0.1192 bcd	0.97	86 abc	2,354 bc	9,507 b	75
'Pocitas' (R)	490.0 bc	37	0.1149 bcd	0.90	93 ab	706 fg	7,452 c	90
'Criollo de Oaxaca' (R)	457.6 bc	41.2	0.1121 cde	0.95	86 bc	690 fg	2,871 f	76
'Vikingo I' (R)	439.6 c	43.7	0.1085 cde	0.97	80 bcd	1,486 cdef	4,367 e	66
'Pata de Perro' (R)	406.6 c	47.7	0.1016 cde	0.94	67 de	1,789 bcde	5,260 de	66
'Blanco de Egipto' (W)	368.3 c	52.7	0.0952 cde	0.94	73 cde	1,929 bcde	4,327 e	55
'Massone' (R)	355.0 c	54.4	0.0772 e	0.95	60 e	2,029 bcd	5,738 d	65
'Blanco de Cortazar' (W)	159.8 d	79.4	0.0843 de	0.97	60 e	2,411 b	5,294 de	54
'Perla' (W)	149.8 d	80.7	0.0409 f	0.85	27 f	3,448 a	4,487 e	23
C.V. (%)	13.1		10.7		6.9	18.7	6.5	

[z]Means in every column with the same letter are statistically the same according to the Tukey's test at a $P \leq 0.05$
[y] Regarding with Tacatzcuaro cultivar that was the most susceptible one.
[x] Regarding with the production obtained in not inoculated plants.
P: pink; R: red; W: white; C.V.: coefficient of variation.

TABLE 2. Incidence of white rot as area under the disease progress curve (AUDPC), disease-increase rate (b^{-1}) (Weibull model), determination coefficient of b^{-1} (r^2), final incidence of white rot and yield of cultivars of different types of garlic. Celaya state of Guanajuato. México. 1996-1997.

Cultivars and types of garlic	AUDPC	Disease reduction (%) [y]	b^{-1}	r^2	Final incidence (%)	Yield (kg·ha^{-1})		
						Inoculated	Not inoculated	Reduction[x] (%)
'Tacatzcuaro' (P)	958.2 a[Z]	-	0.2951 a	0.94	100.0 a	412 e	15,840 a	97
'Pata de Perro' (R)	593.7 b	38.1	0.1083 b	0.95	76.25 b	2,039 cd	6,479 bc	68
'Massone' (R)	409.7 bc	57.3	0.0778 b	0.93	72.0 bc	1,318 d	6,073 bc	78
'Blanco de Egipto' (W)	351.6 c	63.3	0.0592 b	0.91	58.0 bc	2,179 c	4,789 d	54
'Blanco de Cortazar' (W)	116.0 d	87.9	0.0662 b	0.96	51.0 cd	3,224 b	5,712 c	44
'Perla' (W)	101.6 d	89.4	0.0607 b	0.93	33.0 d	5,263 a	6,886 b	24
C.V. (%)	23.5		27.1		13.2	15.6	4.9	

[z]Means in every column with the same letter are statistically the same according to the Tukey's test at a $P \leq 0.05$
[y]Regarding with Tacatzcuaro cultivar that was the most susceptible one.
[x]Regarding with the production obtained in not inoculated plants.
C.V.: coefficient of variation; P: pink; R: red; W: white.

TABLE 3. Correlations between AUDPC, disease-increase rate (b^{-1}), final accumulated incidence of white rot (I_f) and yield decrease (Rr) in two trials of cultivars garlic. Celaya state of Guanajuato. Mexico. 1995-1996 and 1996-1997.

Variables	b^{-1}		I_f		Rr	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
AUDPC	0.93**	0.89*	0.86**	0.96**	0.87**	0.91*
b^{-1}			0.84**	0.83*	0.84**	0.76^{NS}
I_f					0.95**	0.98**

[NS], * and ** not significant, significance at a $P \leq 0.05$ and $P \leq 0.01$.

Smith and Entwistle (1988), reported that the white cultivar Thwaite Grange was susceptible to white rot and not resistant as previously reported (Bristow, 1982). The difference of results probably was due to the high density of sclerotia used by Coley-Smith and Entwistle (1988), since they used 500 sclerotia in the root mass when transplanting garlic in the field, and in a second year of test the same level of sclerotia was applied. Brix and Zinkernagel (1992) suggested standardizing the quantity of sclerotia to give an incidence close to 100 % and hence, to compare the genotypes under an inoculum density that enables the separation of their reaction to the disease. However, we consider that the results obtained by different researchers, regarding with the behavior of a certain host genotype, will be comparable only if the amount of sclerotia reaching an incidence percentage close to 100 % is standardized, which would be used as the reference for all the researchers conducting susceptibility tests among garlic cultivars.

In our study, the amount of inoculum (approximately 50 sclerotia per every clove-"seed" of garlic with a germination of 87 %), was similar to that used by Brix and Zinkernagel (1992), 40 sclerotia per "seed" with a germination superior to 70 %. This germination was enough to attain an incidence close to the 100 % in susceptible genotypes. In our case, we obtained a 100 % of plants infected with white rot in the highly susceptible cv. Tacatzcuaro. Because of this, we consider that the susceptibility differences observed in the different tested cultivars are highly reliable. The results obtained showed that the white garlic cultivars Perla and Blanco de Cortazar were less susceptible to *S. cepivorum*, while 'Tacatzcuaro' was highly susceptible. Therefore we suggest to use the formerly mentioned cultivar as the susceptible germplasm to be used as reference in the worldwide level.

We do not know if the lowest susceptibility to a *S. cepivorum* shown by the white cultivars of garlic corresponds to a stimulating resistance or to a tissue resistance. Sánchez *et al.* (1998), stated that red genotype 'Pocitas 750-4' was less susceptible to white rot and, because the tissue of 'Pocitas 750-4' showed the same susceptibility as that of other ones to the macerating activity of the fungus, they consider that the later emergence of this genotype, as well as its lower root volume, in contrast with the highly susceptible red type 'Chileno Santa Martha', could partially explain its lower susceptibility to white rot.

Results obtained clearly showed differences in the susceptibility to *S. cepivorum* of the white, pink, and red garlic germplasm, thus confirming prior observations in different commercial plots in garlic producing areas in

Mexico, where some white garlic genotypes have shown lower susceptibility.

Further, studies are being conducted to try to understand the mechanisms responsible for the lower susceptibility shown by these garlic genotypes.

CONCLUSIONS

The white garlic cultivars known as 'Perla' and 'Blanco de Cortazar', showed the lowest susceptibility to S. cepivorum, followed by 'Massone' and 'Taiwan' from the red and pink types.

White rot induced the highest yield reductions on the pink cultivars (75 to 99 %), red cultivars 65 to 90 %, and white cultivars 23 to 55 %.

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LITERATURE CITED

ANONYMOUS. 1996. Anuario estadistico de los Estados Unidos Mexicanos. Centro de Estadística. Secretaría de Agricultura y Ganadería y Desarrollo Rural. D.F., México. 733 p.

BRISTOW, A. 1982. Breaking through the garlic barrier. The Garden 107: 337.

BRIX, H. D.; ZINKERNAGEL, V. 1992. Screening for resistance of *Allium* species to *Sclerotium cepivorum* with special reference to non-stimulatory resistance. Plant Pathology 41: 308-316.

CAMPBELL, L. C.; MADDEN, V. L. 1990. Introduction to Plant Disease Epidemiology**.** John Wiley & Sons, Inc. New York, USA. 532 p.

COLEY-SMITH, J. R.; MITCHEL. C. M.; SANSFORD, E. C. 1990. Long-term survival of sclerotia of *Sclerotium cepivorum* and *Stromatinia gladioli.* Plant Pathology 39: 58-69.

COLEY-SMITH, J. R.; ENTWISTLE, A. R. 1988. Susceptibility of garlic to *Sclerotium cepivorum*. Plant Pathology 37: 261-264.

COLEY-SMITH, J. R.; ESLER, G. 1983. Infection of cultivars of onion, leek, garlic, and *Allium fistulosum* by *Sclerotium cepivorum*. Plant Pathology 32: 373-376.

ENTWISTLE, A. R.; SMITH, E. J. 1994. Methods for research on *Allium* white rot (*Sclerotium cepivorum*), pp. 16-21. *In*: Proceedings of the Fifth International Workshop on *Allium* White Rot. Entwistle, E. R.; Velero-Vara, J. M. (eds.) Cordoba, Spain. Session 3.

ENTWISTLE, A. R.; GREEN, R. K.; SPENCE, J. N.; MEAD, A. 1990. Screening accessions of *Allium cepa* from the vegetable gene bank, Wellesbourne for response to *Sclerotium cepivorum,* pp. 210-218. *In*: Proceedings of the Fourth International Workshop on *Allium* White Rot. Entwistle, A. R.; Mattush, P. (eds.). Braunschweig, Germany.

HEREDIA, Z. A. 1995. Guía para cultivar ajo en el Bajío. Folleto para productores Núm. 1. INIFAP-CIRCE-CEBAJ. Celaya, Guanajuato. México. 24 p.

HIGLEY, L. G.; PEDIGO, L. P.; OSTLIE, K. R. 1986. DEGDAY: A program for calculating degree-days and assumptions behind the degree-day approach. Environmental Entomology 15: 999-1016.

KING, J. E.; COLEY-SMITH, J. R. 1968. Effects of volatile products of *Allium* species and their extracts on germination of sclerotia of *Sclerotium cepivorum* Berk. Annals of Applied Biology 61: 407-414.

KING, J. E.; COLEY-SMITH, J. R. 1969. Production of volatile alkyl sulfides by microbial degradation of synthetic alliin and alliin-like compounds in relation to germination of sclerotia of *Sclerotium cepivorum* Berk. Annals of Applied Biology 64: 303-314.

LÓPEZ F., L. C.; VALADEZ, M., C.; MACIAS V., L. M. 1996. Respuesta de genotipos de ajo morado y blanco a *Sclerotium cepivorum* Berk. en combinación con aplicaciones de tebuconazol a la semilla y al suelo. Memorias XXIII Congreso Nacional de Fitopatología. Guadalajara, Jalisco. México. Resumen 18.

PÉREZ M., L.; SALINAS J., G.; SÁNCHEZ P., J. R. 1995. Trial regional de adaptación y rendimiento de materiales de ajo *Allium sativum* L. tolerantes a la pudrición blanca *Sclerotium cepivorum* Berk., generados por irradiación. Revista Mexicana de Fitopatología 13: 18-25.

POINSELOT, R. P. 1980. Horticulture principles and practical applications. Prentice Hall, Inc. New Jersey, USA. 532 p.

SÁNCHEZ P., J. R.; ZAVALETA-MEJÍA, E.; MORA A., G.; PÉREZ M. L. 1998. Pathogenicity of four isolates of *Sclerotium cepivorum* in three genotypes of garlic (*Allium sativum* L.). pp. 45-49. *In*: Proceedings of the Sixth International Workshop on *Allium* white rot. Pérez, M.L.; Entwistle, A. (eds.). Irapuato, Guanajuato, México. En prensa.

UTKHEDE R. S, RAHE J. E, 1979. Wet-sieving flotation technique for isolation of *Sclerotium cepivorum* propagules from muck soil. Phytopathology 69: 295-297.

VAN DER MEER, Q. P.; VAN BENEKOM, J. L.; VAN DER GIESSEN, A.C. 1983. Screening for resistance to white rot caused by *Sclerotium cepivorum* Berk. in onions (*Allium cepa* L.) and leek (*Allium porrum* L.). Euphytica 32: 697-701.

ZINKERNAGEL, V.; BRIX H. D. 1990. Genetic control.- A survey, pp. 195-201. *In*: Proceedings of the Fourth International Workshop on *Allium* White Rot. A. R. Entwistle, A. R.; Mattusch, P. (eds.) Braunschweig, Germany.