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INOCULATION METHODS ON THE FIELD WITH SCLEROTINIA IN SUNFLOWER

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SUMMARY

Inoculation methods on the field to identify sunflower genotypes (Helianthus annus L.) that determine growth resistence or tolerance to head rot caused by Sclerotinia sclerotiorum (Lib) de Bary were tested. In an experiment conducted in 1982 a suspension with mycelium potato glucose agar at 2% (PGA 2%) was applied with a dropper and a hand sprayer on bracts and disk flowers, and with a syringe it was introduced in the parenchymatic tissue of the receptacle. Also mycelium pastilles of PGA 2% were applied on the dorsal surface of the capitulums, both covered and uncovered with a cloth bag. In 1983 and 1984 the treatments with syringe and mycelium pastilles were repeated. Other treatments were added, such as introducing sticks with mycelium into the dorsal surface of the capitulum, mycelium pastilles were introduced in the dorsal surface of the capitulum, covered and uncovered, by means of a hollow punch; and mycelium pastilles were placed in the internal surface of the bracts. Two pathogen isolates for each treatment were used. The experiments were carried out with an early selection of the variety "Issanka" and the hybrids "Cargill S400" and "Continental P75" in a randomized complete block design. The effectiveness of the treatments was estimated by the number and growth of the infections. In 1982 only the pastille techniques surpassed the others in number and size of infection. In 1983 the highest number of infections was obtained with the following techniques: mycelium pastilles applied with a hollow punch with covered and uncovered capitulums; mycelium pastilles with covered capitulums; sticks with mycelium; mycelium pastilles with uncovered capitulums and pastillles applied to the internal surface of the bracts. In 1984 the results of the first four techniques were significant. However, the analysis of each individual cultivar showed that the treatments had and unstable performance along the time, as well as with the isolates and hosts employed. The exception was the technique of the mycelium sticks which was the most appropriate because it was easy to apply and of a good stability when confronted with different environmental conditions.

INTRODUCTION

Sunflower head rot is a disease of great economic importance that under certain climatic conditions can cause a total crop failure. Purdy (1979) says that the disease is caused by "the most nonspecific omnivorous and successful of plant pathogens", Sclerotinia sclerotiorum (Lib.) de Bary.
In Argentina, head rot appears in all the sunflower growing area. In the south-east of Buenos Aires province the symptoms of the disease appear during the fructification stage to physiological maturity. The development of the disease is conditioned by very wet summers, high relative humidity, low temperatures and cloudy skies.
Even if there are several works where differences in susceptibility to head rot on Helianthus annuus L. and related species are mentioned (Leclerq, 1973;

Tourvielle de Labrouche, Guillaumin, Vear, and Lamarque, 1978; Sericys, 1981; Pierre and Regnault, 1982), no sunflower genotype with resistance or tolerance to Sclerotinia has been developed.
Although this work does not claim to solve this problem, it does provide an inoculation technique on the field that could help to identify sunflower genotypes with resistance to the development or tolerance to Sclerotinia in accord with Nelson's (1979) and Parlevliet's (1979) definitions.

MATERIALS AND METHODS

In 1982, 1983 and 1984 field trials were carried out at the Balcarce Agricultural Research Station (Argentina) as a complete randomized block design with four replications. The field plots were planted in three rows 5.10 m in length with 0,70 m spacing between rows and plants 0,30 m apart. Inoculum was applied on the central row.
To get suitable environmental conditions for the pathogen, seeds were sown later than usual for the area (January 21/1982, 7/1983 and 11/1984).
The hosts were: in 1982 the hybrid "Cargill S400", in 1983 an early selection of the variety "Issanka" and in 1984 the hybrid "Continental P75" together with the material used the previous years.
Fungus mycelium was employed for all the inoculation techniques.
It was obtained from sclerotia that had been dipped for 3 minutes in sodium hipochlorite, washed in sterile water and then germinated in Petri dishes containing 10 ml potato glucose agar at 2% (PGA 2%).
The dishes were kept for 8 days in an isolation chamber at a daily temperature between 12°C and 22°C.
Only one isolate of the parasite was used in 1982 and two in 1983 and 1984 (R1 and R3). Three plants chosen at random were inoculated with each isolate. A non-inoculated plant for each cultivar and each repetition was included as control.
Seven inoculation techniques were used in 1982. A suspension with mycelium at 2 % PGA was applied by means of a dropper, a syringe and a manual sprayer. A drop of the suspension was applied on the internal surface of the bracts (1) and between disk flowers. With a syringe 2 ml of the suspension were applied to the parenchymatic tissue in the hollow part of the receptacle (3). With a hand sprayer 2 ml of the suspension were sprayed on disk flowers (4) and to the internal surface of bracts (5). Also myclium pastilles at PGA 2% were used. They were applied to the dorsal surface of capitulums (the same area used by Vear et al. in 1977 and sericys in 1981). That area was first scraped with a penknife and then a pastille was adhered with Scotch-tape. In one treatment (6) capitulums were not covered and in another (7) they were covered with a cloth bag.
For the first five treatments a mycelium PGA 2% suspension obtained from three Petri dishes diluted in 0.5x 1 of distilled water and homogenized for three minutes was used. Mycelium pastilles were also produced in Petri dishes at PGA 2% and were extracted with a hollow punch at inoculation time.
Treatments (3), (6) and (7) produced lesions in the hosts.
In 1983 the following treatments were carried out: tissue pieces were extracted from the dorsal surface of capitulums with a hollow punch and in each lesion a mycelium pastille at PGA 2% was placed which was then covered with the tissue that had been extracted. In one treatment capitulums were not covered with a

cloth bag (1) and in the other they were covered (2); a syringe with a 4 ml of the suspension per capitulum was introduced in the parenchymatic tissue of the receptacle (3); mycelium PGA 2% pastilles were applied to the internal surface of the bracts (4) and also the treatments of the year before with mycelium pastilles on the dorsal surface of the head, uncovered (5) and covered (6). Also sticks with mycelium PGA 2% were introduced in the dorsal surface of the capitulums at a depth of about 1,5 cm (7) (the same area used by Iliescu in 1975 and Vear et al. in 1977). The sticks were first placed in test tubes 25 mm in diameter and 150 mm in length. These test tubes contained PGA 2% with mycelium of the pathogen.

The treatments applied in 1983 were repeated in 1984.

Innoculations were carried out at the end of the flowering period in 1982 and 1984 and at seed setting in 1983.

Data were recorded seven days after inoculation, except for Cargill S400 and Continental P75 hybrids where data were registered 9 days after inoculation in 1984.

In all the tests the number of infections was determined and the average size or extent between the larger and the smaller diameter of each necrotic spot was evaluated. A variance analysis for each experiment and the differences between means were determined with the test of the least significant difference (lsd).

RESULTS AND DISCUSSION

In 1982 the treatments with pastilles surpassed the other techniques and the control both in number and in the size of the infections produced (Table 1). The pastille treatment where the heads were covered was more effective than where they were uncovered, due to the increased relative humidity and diminished light intensity. This was probably generated by the cloth bags that favored the infection (Lamarque, 1980). The lack of effectiveness of the treatments applying the suspension with a dropper or a hand sprayer could be explained by the low relative humidity found the 8 hours that followed the inoculations (Lamarque, 1980; Lamarque and Rapilly, 1981). Another cause of failure could be the destruction of the mycelium structure by the action of the homogenizer unabling the growth of new mycelium in the host. This last explanation would be supported also by the ineffectivity of the treatment with a syringe since the suspension was applied in this case on parenchymatic tissue which has enough humidity. In agreement with Tourvieille de Labrouche et al. this tissue is also easily infected by the pathogene.

In 1983 it was possible to generate with isolate R1 the greatest number of infections with the mycelium pastilles applied with a hollow punch, the technique with mycelium pastilles on covered capitulums and the technique with mycelium sticks. On the other hand the only ineffective treatment with isolate R3 was when it was applied as a suspension with a syringe. The maximum mycelial development with R1 was reached with mycelium pastilles applied with a hollow punch and with mycelium pastilles on covered capitulums, while with R3 the maximum development was reached with the techniques above mentioned and with pastilles applied with a hollow punch on covered heads and with mycelium sticks (Table 2).

In 1984, isolates R1 and R3 together with the following techniques: pastilles

applied with a hollow punch on covered and cuncovered capitulums, mycelium pastilles on covered capitulums, and mycelium sticks were the most efficient ($p \leq 0.05$) by the number of infections they produced (Table 3).
With R1 all the treatments developed a good mycelial growth except the suspension applied with a syringe. With R3 mycelial development was not appropriate in the treatment with suspension applied with a syringe and with pastilles applied on the internal surface of bracts.
In 1983 and 1984 (Tables 2 and 3) there were no significant differences between the treatments with pastilles applied with a hollow punch with the capitulums covered and uncovered because mycelium developed in similar relative humidity and light intensity. The pastilles were placed at a depth of 1 cm from the dorsal surface of the capitulum and were covered with the same volume of extracted tissue as a hermetic lid. On the other hand, when using pastilles with mycelium they were placed on a superficial lesion of the capitulum and here there were differences in the relative humidity and radiance between the treatments with covered and uncovered capitulums.
The relationship of each cultivar with all the techniques was analized in the experiment carried out in 1984. It was found that the mycelium stick technique was the only one that was effective in all of the three cultivars and with both pathogen isolates. The stability of this technique is probably a consecuence of the close contact between mycelium and parenchymatic tissue that somehow protects the host-pathogen relationship from environmental variations. This result disagrees with that obtained by Vear et al. in 1977 but is in accord with what was observed by Illiescu et al. in 1976.
There were no significative differences between the isolates ($P \leq 0.05$). This behaviour disagrees with laboratory observations where both rate of groth and sclerota production were higher in R3 than in R1. In accord with Chivers, (1929, mentioned by Purdy, 1979) not always is there a relationship between the differences found in vitro and the pathogenic capacity of the isolates.

CONCLUSIONS

The techniques in which a dropper, a hand sprayer or a syringe were used, were ineffective in the conditions described. The techniques with pastilles and mycelium pastilles applied with a hollow punch present difficulties in their application and instability of behaviour which make their use unadvisable. The technique with the mycelium sticks is the most appropriate because of its stability in different environmental conditions and it is easy to apply.

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TABLE 1 – Mean response of the hybrid Cargill S400 to various inoculation methods on the field with Sclerotinia sclerotiorum (Lib.) de Bary.

INOCULATION METHODS				INFECTIONS NUMBER	INFECTIONS SIZE (mm)
Mycelium suspension 2% PGA	Dropper	– Bracts	(1)	0,000	0,000
		– Disk Flowers	(2)	0,015	0,083
	Syringe	– Receptacle	(3)	0,050	0,163
	Hand sprayer	– Disk flowers	(4)	0,015	0,050
		– Bracts	(5)	0,018	0,055
Mycelium pastilles	capitulum dorsal surface	– not covered	(6)	0,812	4,133
		– covered	(7)	0,965	4,973
Control			(8)	0,070	0,258
		lsd 0,05		0,110	0,592

TABLE 2 – Mean response of the early selection variety Issanka to different inoculation methods on the field with two Sclerotinia sclerotiorum (Lib.) de Bary isolates.

INOCULATION METHODS				INFECTIONS–ISOLATES R1 NUMBER	R1 SIZE	R3 NUMBER	R3 SIZE
Mycelium pastilles hollow punch	– dorsal surface of capitulums	– uncovered	(1)	0,778	3,835	0,695	3,845
		– covered	(2)	0,642	3,012	0,472	2,275
Mycelium suspension syringe	– receptacle		(3)	0,082	0,208	0,000	0,000
	– bracts		(4)	0,415	1,288	0,445	1,582
Mycelium pastilles	– dorsal surface of capitulums	– uncovered	(5)	0,608	2,528	0,418	1,902
		– covered	(6)	0,890	5,388	0,695	3,005
Mycelium sticks	– dorsal surface of capitulums		(7)	0,780	3,280	0,778	3,708
Control			(8)	0,000	0,000	0,000	0,000
		lsd 0,05		0,277	2,035	0,406	1,658

TABLE 3 – Mean response of the early selection variety Issanka and hybrids Cargill S400 and Continental P75 to different inoculation methods on the field with two Sclerotinia sclerotiorum (Lib.) de Bary isolates.

INOCULATION METHODS				INFECTIONS ISOLATES R1 NUMBER	R1 SIZE (mm)	R3 NUMBER	R3 SIZE (mm)
Mycelium pastilles hollow punch	– dorsal surface of capitulums	– uncovered	(1)	0,712	3,572	0,681	3,999
		– covered	(2)	0,748	3,893	0,654	3,770
Mycelium suspension syringe	– receptacle		(3)	0,110	0,466	0,303	0,798
	– bracts		(4)	0,229	0,869	0,258	1,018
Mycelium pastilles	– dorsal surface of capitulums	– uncovered	(5)	0,195	1,039	0,258	1,152
		– covered	(6)	0,664	3,654	0,579	3,702
Mycelium sticks	– dorsal surface of capitulums		(7)	0,796	4,580	0,870	5,527
Control			(8)	0,020	0,083	0,020	0,083
		lsd 0,05		0,542	3,774	0,528	4,380