T1978PATH11

INOCULATION OF SUNFLOWERS WITH ASCOSPORES OF CLEROTINIA SCLEROTIORUM

By

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Introduction

Attack by Sclerotinia sclerotiorum appears at present to be one of the major problems for the sunflower crop. No complete resistance against this parasite is known in Helianthus annuus or closely related species, but there are significant differences in the reactions of different genotypes to field attack and in the rate of extension of Sclerotinia mycelium on their capitula (Vear and Guillaumin, 1977). However, mycelial extension only involves part of the infection cycle, excluding all of the entry process. If the best use is to be made of partial resistances, characteristics having some resistance at each stage of the infection cycle must be combined. Therefore a detailed knowledge of the infection process of sunflowers by Sclerotinia sclerotiorum is very necessary.

This paper reports the use of a number of inoculation techniques to study the process of infection of different sunflowers by ascospores and to try to define more satisfactory breeding tests than those at present employed.

Materials and Methods

In order to obtain large quantities of ascospores a collection technique was developed. Figure 1 shows the arrangement by which sclerotia with apothecia are enclosed under plastic cups, with the soil surface covered by a sheet of polythene and then aluminum foil. Ejection of ascospores is obtained by lifting the cup suddenly and then replacing it. The cloud of spores settles on the cup walls and the foil. This can be repeated over several days. If the cups and foil are kept dry, the spores remain viable up to two months. A suspension is obtained by washing the cup with a 2% saccharose solution, 0.5 ml of this suspension (containing about 50 spores/mm^3) was used for each inoculation as droplets or with a syringe.

To obtain high concentrations of dry spores, apothecia are cut from sclerotia and placed inverted on Petri disces. This method has the disadvantage that each apothecia can be used only once. The spores were then placed on circles of blotting paper 5 cm in diameter, giving for each inoculation 100,000 to 5 million spores.

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Four sunflower genotypes were inoculated with a scospores of Sclerotinia sclerotiorum in the form of powder and a suspension, with depostiion of drops or inoculation by syringe. Inoculaticns at pre-, mid-, and post-flowering were made on stems and capitula; for the latter organ, on both dorsal and floral surfaces in the internal parenchyma. Inoculations with agar pastilles containing mycelium, in general use at Clermont-Ferrand, were also made, for the purpose of comparison with the ascospore trials.

The capitula inoculations showed that the internal lacuntae parenchyma are susceptible to infection from the time of capitulum formation. In contrast, the external tissues form a barrier to infection. On the dorsal surface, this barrier remains effective throughout the vegetition period but in the case of applications on the tubular florets, susceptibility appeared dramatically at about August 15th. However, from inoculations made up to six weeks earlier, the pathogen appears capable of maintaining a latent infection in the florets until the period of susceptibility.

The infection route in stems was not determined, but gradient of susceptibility was observed: the area of maximum susceptibility moves upwards during the summer but becomes generalized at the end of the season.

The four sunflower genotypes showed considerable differences in susceptibility. The two relatively resistant hybrids appeared to have different reactions: resistance to progression of the pathogen in the tissues or mechanical resistance to disruption of stems and capitula.

These results suggest that it may be possible to use ascospores in breeding tests of resistance to Sclerotinia.

FIGURE 2 - % success of inoculations with ascospere suspensions into the stem at different levels.

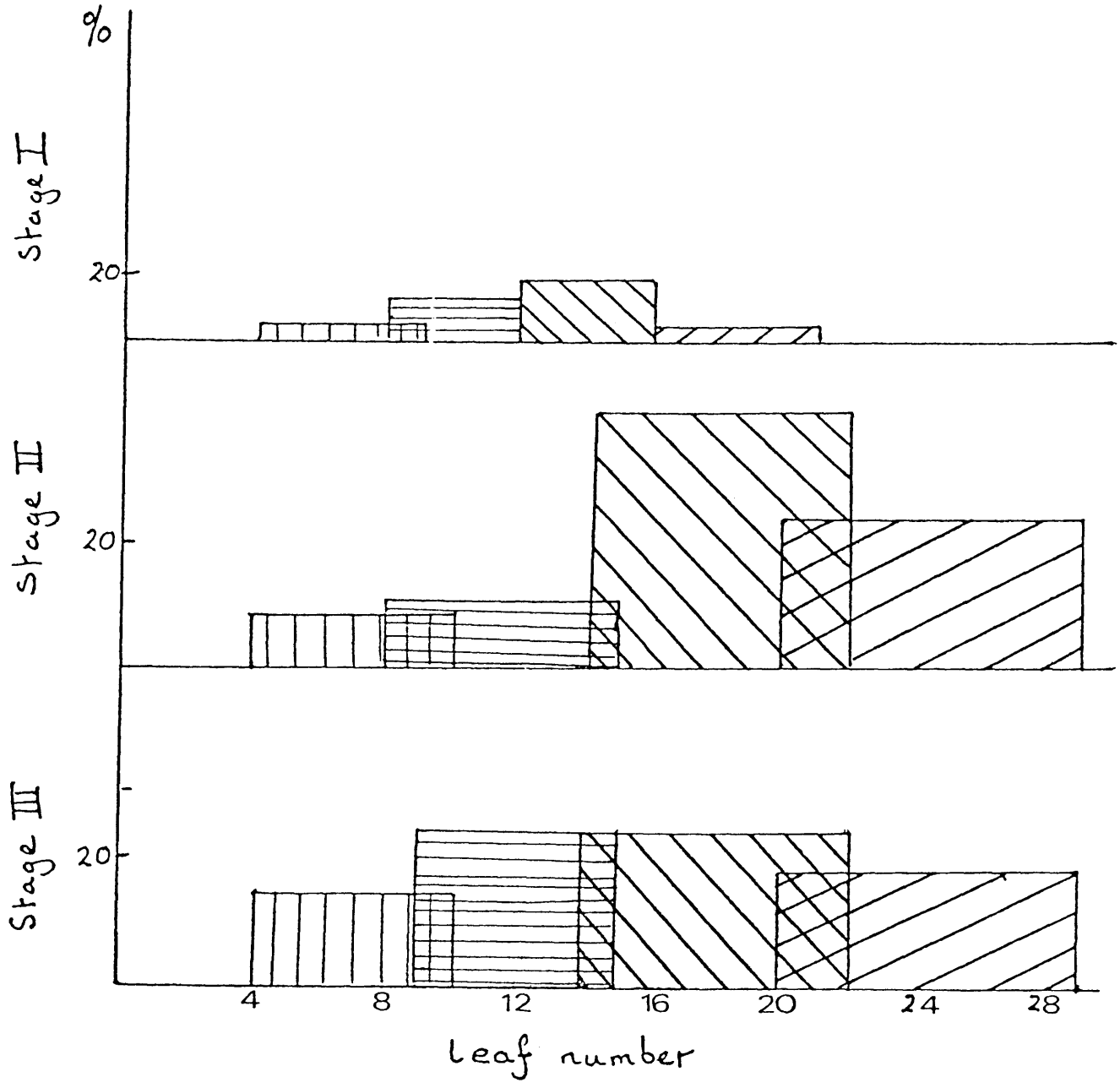
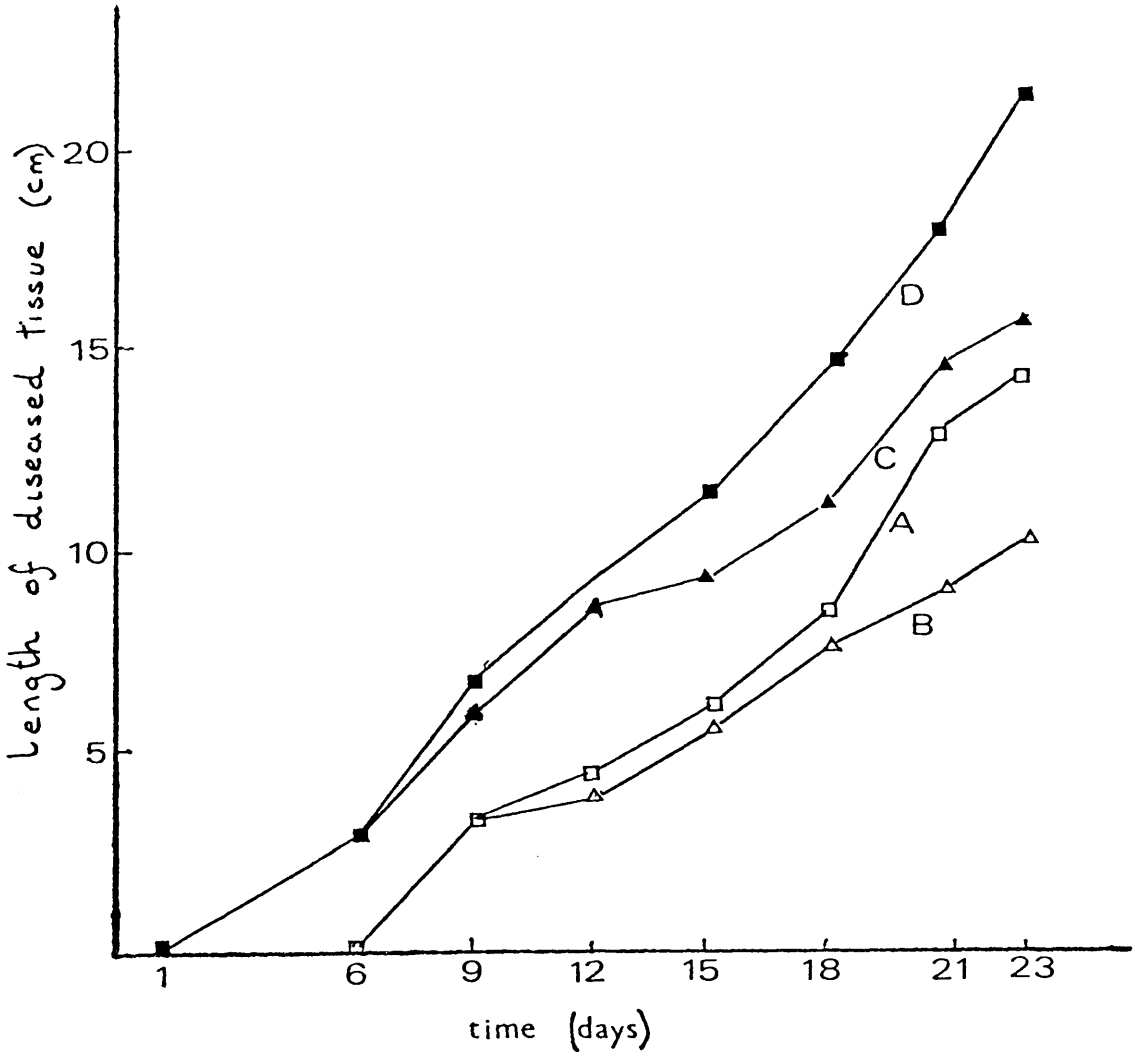


FIGURE 3 - Extension rate of diseased area after stem inoculation at stage II.

A = INRA 4701 B = CIC 61 x BC 251 C = Peredovik D = CPM2 x Rha 266



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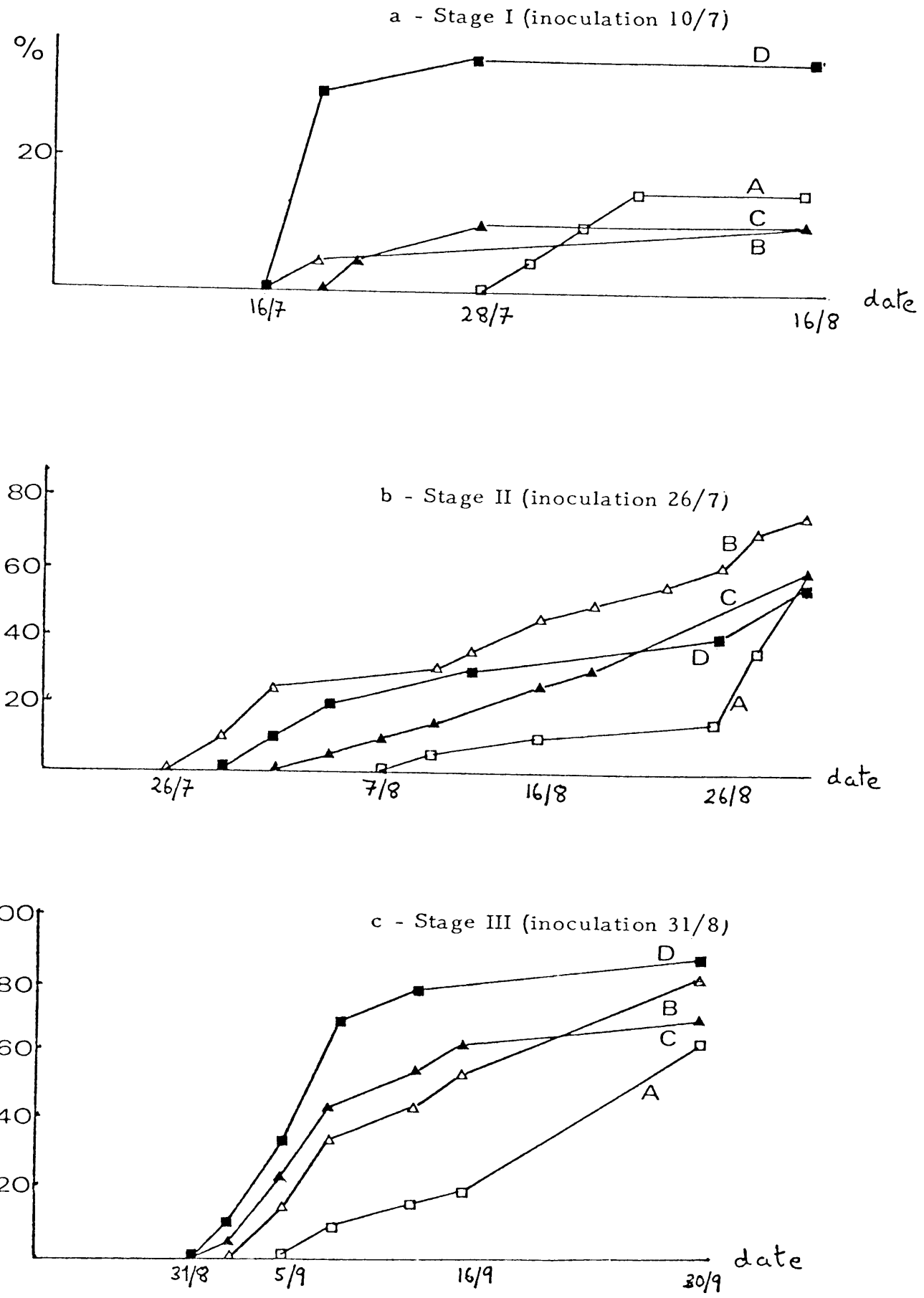
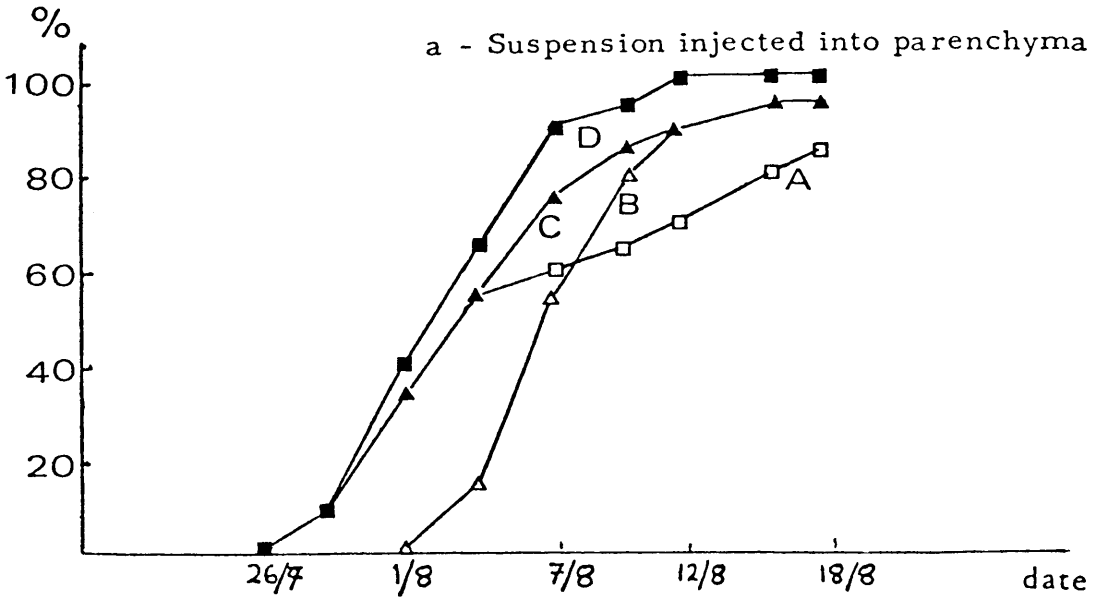
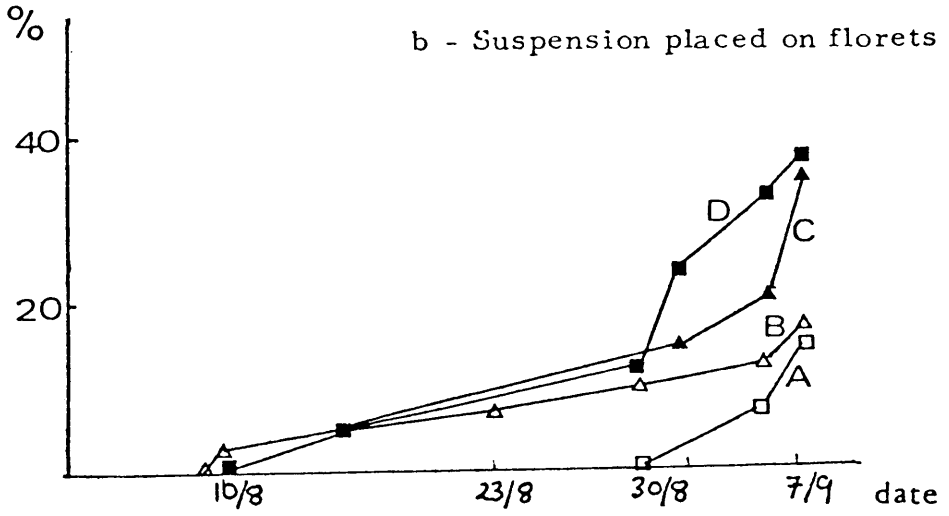


FIGURE 4 - Percentage of plants lodged after inoculation of ascospore suspensions in the stem

A = INRA 4701 B = CIC 61 x BC 251 C = Peredovik D = CPM2 x Rha 266





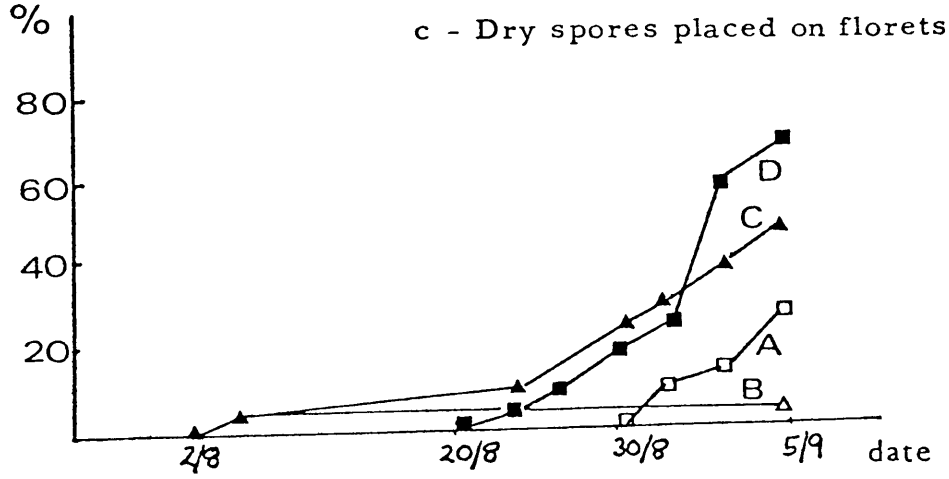


FIGURE 5 - Percentage of capitula destroyed after inoculation by ascospores at stage 11.

TABLE 1. Percent Success of inoculation of ascospore suspensions into the stems of 4 sunflower genotypes in the field

Plant Stage	Incubation Period (Days)	Percent Attack of Genotypes			
		INRA 4701	CIC61 BC251	Peredovic	CPM2 Rha266
I	20	15	10	10	35
	45	30	30	25	60
II	20	60	75	60	55
	35	70	85	85	85
III	20	5	40	40	70
	25	65	85	70	90

The 4 sunflower genotypes were INRA 4701, a relatively resistant hybrid variety, Peredovik, the susceptible population variety and 2 experimental hybrids, CPM2 x Rha266 which is susceptible in the field and with the mycelial test and CIC61 x BC251 whose parents are relatively resistant to the mycelial test.

The inoculations were made at three stages:

I - 10 days before flowering
II - Mid-flowering: 15 days after stage I
III - 3 weeks post-flowering

When aqueous inoculum was deposed externally the sunflower organ was enclosed in opaque polythene for one week.

Results and Discussion

A. Stem Inoculations

External applications of drops of ascospore suspension in the axils of senescent petioles failed completely, so it was not possible to determine the route of stem infection. In contrast, inoculation of the suspension with a syringe into the pith showed considerable success from the preflowering stage onward.

Inoculations were made at four levels on each plant and Figure 2 shows that the maximum susceptibility (defined by the % success of inoculation) climbs along the stem with age, but becomes generalized towards maturity. This midstem susceptibility can be compared with the most frequent natural attacks of sunflower stems. One may note that, at the different stages, the maximum susceptibility remains at the level of the largest leaves. Perhaps the availability of photosynthetic products plays a role here.

Table 1 shows the percentage success of inoculation of the four genotypes. Infection took a quite long time to appear and was greatest at stages II and

III. INRA 4701 was generally least infected and COP2 x Rha266 the most infected. The relative resistance of the genotypes can be appreciated in two ways. Figure 3 shows the extension rate of the diseased area following successful inoculation. The greatest resistance to extension appears with CIC61 x BC251. In contrast if resistance is appreciated by the percentage lodging of stems after attack (Figure 4), INRA 4701 shows the greatest resistance. Its stem strength must be an important factor.

B. Capitulum Inoculations

a) Injections of scopspore suspensions into the capitulum parenchyma: Table 2 shows that these injections were at least partially successful at stage I, although natural attacks are not generally observed at this time. The differences between cut heads and plants in the field is probably due to different lengths of time before final observations. From capitulum formation onwards it appears that the parenchyma of all varieties is susceptible, and that while pathogen extension rates may vary, any differences in resistance to establishment do not occur at this level.

TABLE 2. Percent success of inoculations with ascopore suspensions into the capitula of 4 sunflower genotypes.

		Percent attack of genotypes			
	Plant Stage	INRA 4701	CIC61 BC251	Peredovik	CPM2 Rho266
Cut heads (9 days)	I	15	5	25	35
	II	45	65	65	95
	III	100	100	100	100
Plants in field (20 days)	I	65	45	55	65
	II	89	95	95	100
	III	85	90	95	85

b) External inoculations:

1. On the dorsal surface of capitula no success was obtained with application of ascospore suspensions. This confirms our results over several years, and indicates that the dorsal surface does not constitute an entry route for Sclerotinia ascospores.

2. The application of drops of ascospore suspensions into florets gave varying success. Table 3 shows the results in the field. It can be noted that the young flower buds do not appear to be susceptible, but most importantly, that the percentage of attacks increases considerably between 20 and 35 days after inoculation. Since, in solution, ascospores germinate within 4 hours it must be possible for the pathogen to remain in a latent form, perhaps as a small colony in the florets, until such time as the stage of the plant permits extension, after flowering. It may be noted that at stage III there was as much success after 20 days as at stage II after 35 days. These two observations were in fact at the same date (about September 1).

TABLE 3. Percent success of inoculations by deposition of droplets of ascospore suspensions on the floral surface of the capitula of 4 sunflower genotypes in the field.

Plant Stage	Incubation Period (days)	Percent attack of genotypes			
		INRA 4701	CIC61 BC251	Peredovik	CPM2 Rho266
I	20	0	0	0	0
	35	0	0	0	0
II	20	0	10	10	10
	35	5	20	45	40
III	20	25	13	31	19
	35	62	50	81	

3. Inoculum in the form of dry spores gave rather similar results to those with suspensions, although with some additional success at stage I. Perhaps the dry spores can remain viable until the florets have opened. As with the aqueous inoculum, there was a considerable increase in the percentage success of stage II inoculation between 20 and 35 days, again probably due to a sudden increase in the susceptibility of the plants about 4 weeks after flowering. In this case, the inoculum could have been maintained as dry spores or as a small mycelial colony. However, an additional factor appears to be involved. The inoculum placed on florets at anther dehiscence gave much greater success than that on already pollinated florets. Although in the first case there was more pollin than in the second, the results at stage III suggest that pollin is not necessary. One could suggest that at the time of anther dehiscence and stigma opening, the florets have greater humidity than later, permitting better germination of ascospores and the formation of small latent colonies.

c. Varietal Differences: Measurements of extension of Sclerotinia after successful inoculation of the capitulum parenchyma confirm observations following mycelial inoculation that there are significant differences between genotypes. In 4 of 5 comparisons both INRA 4701 and CIC61 x BC251 were highly significantly better than CPM2 x Rha266 and in 3 of 5 these genotypes were better than Peredovik. INRA 4701 appeared significantly less resistant (5% level) than CIC61 x BC251 in only one comparison. Thus from the point of view of resistance to pathogen extension these two genotypes appear comparable.

However, Figure 5 shows the pattern of capitulum destruction of the four genotypes after the different inoculations at stage II. It can be seen that, as with the stem, INRA 4701 has a mechanical resistance to tissue destruction, even when these are invaded by Sclerotina.

Concerning resistance to pathogen installation CIC61 x BC251 and INRA 4701 appear better than Peredovik and CPM2 x Rha266, but only with dry spore inoculum is there any significant difference in percentage infection which suggests that CIC61 x BC251 may have more resistance to installation than INRA 4701.

TABLE 4. Percent success of inoculations by deposition of dry ascospores on the floral surface of the capitula of 4 sunflower genotypes in the field.

Plant Stage	Floret Stage	Incubation Period (Days)	Percent attack of genotypes			
			INRA 4701	CIC61 BC251	Peredovic	CPM2 Rho266
I		35	20	0	15	0
II	Anther dehiscence	20	5	5	20	20
		35	30	5	50	70
	Fertilized Ovule	20	0	0	0	0
		35	10	0	20	0
III	with added pollen	20	20	20	10	45
	without added pollen	20	5	70	30	40

Conclusions

Sclerotinia appears to infect sunflower capitula through the florets with extension into the parenchymatons tissues as the plant matures. It is still not known what constitutes the barrier between the small mycelial colony in the florets or other structures and the parenchyma which, at least with very slight wounding, is susceptible at the time of flowering. It could be the seeds and their surrounding bracts or the external edge of the parenchyma. This remains a very important question to ellucidate.

Although none was very evident with the four genotypes used, one might expect there to be a genetic variability in sunflowers for at least certain of these characters. Therefore, while we can suggest that the inoculation method of ascospore suspensions into the parenchyma may be a satisfactory replacement, more rapid and less sensitive to climate, for our mycelial test for Sclerotinia resistance, development of a test with external inoculation of florets is necessary. It would repeat almost exactly the whole natural infection cycle and would thus aid the selection of sunflowers with combinations of partial resistance to pathogen installation, pathogen extension and tissue destruction.

References

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