T1974PATH02

GROWTH OF PLASMOPARA WITHIN SUSCEPTIBLE AND RESISTANT SUNFLOWERS PLANTS

F. MONTES and W. E. SACKSTON
(Spain)

Large-seeded varieties of sunflowers have been grown in Spain for many years for confectionery use. Small-seeded varieties with high oil content developed in the USSR, were grown on about 1200 ha in 1964. By 1973 the sunflower area in Spain was well over 300,000 ha.

Downy mildew (*Plasmopara halstedii* (Farl.) Berl. et de Toni), although probably present earlier, was not recognized on sunflowers in Spain until 1972 (R. Jimenez Diaz, unpublished). Its distribution in that year, and subsequent occurrence over a wide area, make it a serious threat in Spain, as in most other sunflower producing countries throughout the world (Cohen and Sackston 1974).

Resistance to the pathogen has been discovered in sunflower lines of North American origin (Vranceanu and Stoenescu 1970, Vear and Leclercq 1971, Zimmer and Kinman 1972). The biology of the pathogen, and relatively low value of the crop per unit area, make the production of resistant varieties the only practical method of control of the disease at this time.

Natural infection in the field is erratic, and is very much influenced by environmental factors which may vary greatly from year to year. It is therefore necessary to test varieties, plant breeders'lines, and segregating progenies using artificial inoculations. Results of such inoculations on seedlings have sometimes proved confusing. The pathogen may sporulate on the cotyledons, or occur in root and hypocotyl tissues of lines which remain free of the usual systemic symptoms under both natural and artificial infection.

In this paper we report on preliminary efforts at Córdoba to determine differences in reaction among various tissues of susceptible and resistant sunflower lines to inoculation with the mildew pathogen, and the significance of such differences.

MATERIAL AND METHODS

The susceptible variety used in most experiments was Peredovik. The cytoplasmic male sterile line CMS HA 89 produced by M. L. Kinman in Texas and multiplied at Córdoba, was a susceptible control in some experiments, and was the susceptible parent in crosses with different sources of resistance. Various resistant lines were tested. HA 61, produced by M. L. Kinman and resistant to both the Red River race and the European isolates of downy mildew, and HIR-34, produced by P. Leclercq in France, and resistant to European isolates, were used in most experiments (Zimmer and Kinman 1972, Vear and Leclercq 1971). The mildew inoculum used was an isolate from infected plants in a farm near Córdoba.

Plants were grown in a soil-sand-peat mixture in pots in a plastic greenhouse with no control of environment ; in a glasshouse with a low level of supplementary light from flourescent tubes ; or in a small improvised growth cabinet in a laboratory. Temperature in the growth cabinet was maintained at about 18°C by room heat and the flourescent lights in the winter, and by two room air-conditioners in the laboratory in the summer. Day length was 15 to 16 hours. Light intensity was about 8500 lux provided by a mixture of flourescent tubes and incandescent bulbs.

Four methods of inoculation were used :

Germinated seeds. Sunflower seeds were germinated in moist perlite at 20°C for 2 to 3 days. The germinated seeds were immeresed in a suspension of zoosporangia in deionized water, at a concentration of 70,000 per ml, at 18°C for 5 hours. They were then sown in the soil mixture in pots, and incubated in the growth cabinet for 12 days. The pots were then covered with plastic bags to maintain a saturated atmosphere for 2 to 3 days at 18°C to induce sporulation.

Root dip. Plants of various ages were removed carefully from the growing medium, the roots were washed, and were immersed in a suspension containing 45,000 zoosporangia per ml, for 24 hours at 18°C. They were then repotted in the soil mixture and incubated in the growth cabinet at 18°C for 15 days.

Paper square. Filter paper squares 1 mm diameter soaked in a suspension of 400.000 zoosporangia per ml were placed on cotyledons or stem apices, the plants were covered with plastic bags and incubated for six days in the growth cabinet (Cohen and Sackston 1973).

Injection. Plants of various ages were inoculated by injecting a drop of suspension containing 70.000 spores per ml into the hypocotyl or the stem with a hypodermic syringe. Inoculated plants were incubated in a greenhouse.

At appropriate intervals after inoculation, external symptoms and the presence and intensity of the pathogen on cotyledons and leaves were noted. Plants were then sectioned into discs 5 mm thick, these were incubated on moist filter paper in petri dishes at 18°C for 5 to 6 days, and examined for sporulation of the pathogen.

RESULTS AND DISCUSSION

GERMINATED SEED INOCULATIONS

Many selections and crosses from the sunflower breeding program at Córdoba have been tested for reaction to inoculation of germinated seeds with *P. halstedii*. The results of two such experiments are given in table 1. The results are fairly consistent for those lines and crosses included in both experiments. The variability may be attributed in part to poor control of environmental conditions because of frequent power failures and similar difficulties.

Table 1

Reaction of sunflower lines and crosses to inoculation of germinated seeds with Plasmopara halstedii

Sunflower line	Number of plants		
	Inoculated	With sporulation (and mean intensity)	
		Cotyledons	Epicotyl discs
Susceptible			
CMS-HA 89 (1)[1])	53	50 (5)[2])	50
CMS HA 234 (1)	29	17 (5)	3
Peredovik (2)	12	12	
Resistant			
CM 29 (1)	38	0	0
CM 90 RR (1)	31	1 (2—3)	0
CM 90 RR (2)	15	2	
HIR 34 (1)	54	2 (1—2)	0
HIR 34 (2)	9	0	
HA 60 (2)	16	6	
HA 61 (2)	17	0	
AD 66 (2)	8	1	
CMS HA 89×CM 29 (1)	52	8 (1—2)	0
CMS HA 89×CM 29 (2)	15	1	
CMS HA 89×HIR 34 (1)	37	6 (2)	
CMS HA 89×HIR 34 (2)	18	2	
CMS HA 89×HA 60 (2)	12	5	
CMS HA 89×HA 61 (1)	40	1 (2)	0
CMS HA 89×HA 61 (2)	17	0	
CMS HA 89×AD 66 (1)	34	3 (2—3)	0
CMS HA 89×AD 66 (2)	16	0	
CMS HA 234×CM 29 (1)	50	8 (1)	1
CMS HA 234×CM 90 RR (1)	10	1 (2)	0
CMS HA 234×HIR 34 (1)	49	7 (1—2)	0
CMS HA 234×HA 61 (1)	34	2 (1)	0

[1]) Experiment (1) and experiment (2) respectively.
[2]) Intensity rated on a scale from 1 = few sporangiophores, barely visible, to 5 = surface covered with sporangiophores.

Although the pathogen sporulated on the cotyledons of several plants among the resistant lines, it did not penetrate to the epicotyl of any resistant seedlings sectioned in these, or in our other experiments. Delanoe (1972) working with the resistant variety INRA 7702 observed no symptoms on the cotyledons of seedlings inoculated by the germinated seed method, and found mycelium of the pathogen only in the roots and lower portion of the hypocotyl. She suggested that resistance in this variety might be physiological and located in the upper portion of the hypocotyl.

ROOT DIP INOCULATIONS

Plants of Peredovik, HIR 34, and HA 61, were inoculated by the root dip method 11 days after sowing, when the first true leaves were 1 to 2 mm long, and were incubated at 18°C for 15 days. The plants were then sectioned into discs which were placed in moist chambers. The results are given in table 2.

Table 2

Reaction of susceptible and resistant sunflowers to inoculation of roots and hypocotyl base with Plasmopara halstedii

Sunflower line	Days after seeding	Number of plants				
		Inoculated	Sporulation (and mean intensity) on discs from			
			Roots	Collar	Upper hypocotyl	Epicotyl
Peredovik	11[1])	18	17(1)[3])	17(2)	18(3)	1(3)
	30[2])	12	9(1)	8(1)	5(1)	2(1)
HIR 34	11	17	11(1)	12(1)	6(1)	0
	30	7	2(1)	2(1)	0	0
HA 61	11	20	16(1)	15(1)	6(1)	0
	30	5	2(1)	2(1)	0	0

[1]) Plants were 9—10 cm tall, with first true leaves about 1—2 mm long.
[2]) Plants were 12—20 cm tall, with four true leaves present.
[3]) Intensity rated on a scale from 1=few sporangiophores, barely visible, to 5=surface covered with sporangiophores. The pathogen was not observed on any sections of uninoculated control plants of any of the three varieties.

The pathogen sporulated sparsely on discs from the roots and collar (root-hypocotyl transition zone), and profusely on discs from the hypocotyl at the insertion of the cotyledons of Peredovik (table 2). In only one plant of this variety was sporulation observed on epicotyl tissues. Limited infection through the roots of suspectible seedlings has been reported by ohters (Cohen and Sackston 1973). Although the pathogen sporulated on discs from the roots and collar regions of most seedlings of the resistant HIR 34 and HA 61, it reached the upper hypocotyl in relatively few plants, and did not penetrate to the level of the cotyledons in any.

Plants of the three varieties were similarly inoculated by the root dip method 30 days after seeding, when they had four true leaves. The pathogen reached the cotyledon level in about half the plants of Peredovik, and the epicotyl in two of the 12 plants. Although sporulation was observed on discs from the root and collar regions of some HIR 34 and HA 61 plants, in no case did the pathogen reach the upper hypocotyl in the older plants.

PAPER SQUARE INOCULATIONS

Inoculations were made only on the susceptible variety Peredovik. In one experiment, inoculum squares were placed on the cotyledons or on the apex of plants 10 days after seeding, when the true leaves were 1 to 2 mm long. After 6 days of incubation, the plants were sectioned and the discs were placed in moist chambers. In some of the apically inoculated plants sporulation was observed on only the first, or on the first and second discs; in others it developed on nine discs, having grown downwards 4.5 cm in 6 days. In half the plants inoculated on the cotyledons, the pathogen was confined to the cotyledons; in others it reached the epicotyl and grew up to the stem apex within 6 days.

In another experiment, plants inoculated when 10 days old and incubated under plastic bags for 6 days, were transferred to a greenhouse for 35 days. They were then sectioned and the discs were incubated in moist chambers.

Most of the plants apically inoculated showed systemic mildew symptoms, and the pathogen was found in all parts of the plant. In one plant without symptoms, the pathogen was observed only on discs from the hypocotyl. In plants inoculated on the cotyledons, typical systemic symptoms developed in some, and the fungus was recovered from all tissues. No symptoms developed in others; the fungus grew out only from the hypocotyl discs of the symptomless plants.

Plants in another experiment were inoculated apically; on the first true leaves; and on the cotyledons, 25 days after seeding when four to six true leaves were present. Individual plants were sectioned 3, 6 and 13 days after inoculation, and the discs were incubated in moist chambers. Infection was low, ranging from five of 15 plants inoculated apically, to eight of 13 plants inoculated on the cotyledons. In plants inoculated apically, the pathogen had moved downwards in the stem 1 to 2 cm within 6 days after inoculation, and 7 to 8 cm in 13 days. In plants inoculated on the true leaves, the pathogen was observed only at the point of inoculation 3, 6, and 13 days later, in all but one plant. In that one plant, however, it had grown throughout the inoculated leaf and had penetrated 4 cm upward in the stem in 6 days. In inoculated cotyledons the pathogen was present only in the inoculated area after 6 days. After 13 days it had grown throughout the cotyledons, but did not penetrate to their insertion on the stem.

INJECTION INOCULATIONS

Plants of Peredovik, HIR 34, and HA 61 were inoculated 40 days after seeding, in the 10 to 14 true leaf stage. Inoculum was injected into the hypocotyl, or into the first stem internode, with a 25 gauge hypodermic needle. Four, 10, and 15 days after inoculation plants were sectioned, and the discs were incubated in moist chambers. The results are given in table 3.

Table 3

Reaction of susceptible and resistant sunflowers to inoculation with *Plasmopara halstedii* by hypodermic injection

Sunflower line	Zone inoculated	Days after inoculation	Number of plants		
			Inoculated	Sporulation (and mean intensity)	Spread in plant (cm)
Peredovik	Hypocotyl	4	4	4(1)[1]	0.5[2]
		10	4	4(4—5)	8
		15	5	5(5)	10
	Internode	4	5	5(1)	0.5
		10	5	5(2—3)	18
		15	9	9(4—5)	33
HIR 34	Hypocotyl	15	3	2(1)	3
	Internode	15	7	7(2—3)	7
HA 61	Internode	15	5	4(1)	2

[1] Intensity rated on a scale from 1=few sporangiophores, barely visible, to 5=surface covered with sporangiophores.
[2] The distance spread represents the maximum in each treatment, occurring in only one or two plants in some cases.

No external symptoms were observed in any of the inoculated plants. The pathogen was much more abundent in tissues of Peredovik than in the resistant plants, and it spread upwards much faster than downward.

Spread of the pathogen from the point of inoculation in the hypocotyl, past the cotyledons into the epicotyl, was observed in only one plant of Peredovik, and was relatively slow. Spread of the pathogen from the point of inoculation in the first internode was limited in all three varieties. It did not penetrate downward past the cotyledons into the hypocotyl in any of the varieties. In Peredovik it spread upward to the third internode of two plants, and the fourth internode of one, in 15 days. In resistant plants mycelium did not penetrate beyond the first internode, and was sparse. The mycelium occurred mostly in the cortical parenchyma, and rarely in the pith.

CONCLUSIONS

Although the work reported is largely preliminary, certain conclusions may be drawn from it.

1. The isolate of *P. halstedii* used has the limited pathogenicity of the „European" race.

2. Plants of a susceptible variety become less susceptible with increasing age of tissues, regardless of the point or method of inoculation, as reported earlier by others.

3. The relative resistance observed in older tissues of susceptible sunflower appears to be different from that in young tissues of resistant varieties.

4. The pathogen is able to develop to a limited extent when inoculated into stem, as well as hypocotyl, tissues of resistant plants.

5. Although the hypocotyl-stem transition area at the cotyledons presents a barrier to the pathogen in both susceptible and resistant varieties, it can overcome this barrier in the susceptible but not in the resistant plants.

6. Stem internodes apparently serve as a barrier to the pathogen in resistant varieties, but less so in susceptible ones.

REFERENCES

1. Cohen, Y. and W. E. Sackston, 1973, *Factors affecting infection of sunflowers by Plasmopara halstedii,* Can. J. Bot. 51 : 15—22.
2. Cohen, Y. and W. E. Sackston, 1974, *Seed infection and latent infection of sunflowers by Plasmopara halstedii,* Can. J. Bot. 52 : 231—238.
3. Delanoe, D., 1972, *Biologie et épidémiologie du mildiou du tournesol,* Informations Techniques CETIOM 29 (4) : 1—49.
4. Vear, F. et P. Leclercq, 1971, *Deux nouveaux gènes de résistance au mildiou du tournesol,* Ann. Amélior. Plantes 21 (3) : 251—255.
5. Vrânceanu, V. and F. Stoenescu, 1970, *Imunitate la mana florii-soarelui, condiţionată monogenic,* Probleme Agricole 2 : 34—40.
6. Zimmer, O. E. and M. L. Kinman, 1972, *Downy mildew resistance in cultivated sunflower and its inheritance,* Crop. Sci. 12 : 749—751.