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LIMITED MANIFESTATION OF RESISTANCE IN SUNFLOWERS TO DOWNY MILDEW

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INTRODUCTION

The presence of *Plasmopara halstedii* /Farlow/ Berlese et de Toni in resistant sunflowers has already been described (4) and experimentally underlined (1,3,5) using various kinds of artificial inoculations. According to these observations the pathogen was able to develop to a limited extent within root, hypocotyl, and stem tissues of symptomless plants belonging to one of the resistant lines tested. In addition, preliminary experiments carried out by the author (11) indicated that the occurrence of mycelium and oospores of the pathogen within resistant sunflower seedlings often resulted in hypocotyl lesions on such plants.

Recently it was reported (8) that on cotyledons of certain sunflower genotypes containing a resistance gene against *P. halstedii* the fungus showed sporulation two weeks after inoculation.

On the other hand, light— and electron microscopical investigations (15,16) pointed out that in case of resistant interaction the encysted zoospores were unable to penetrate into the host and hypersensitive degeneration of the host cells in contact with these spores occurred.

The present paper reports comparative studies on various aspects of resistance and susceptibility of sunflowers to *P. halstedii* for giving a tentative characterization of both interactions.

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MATERIALS AND METHODS

The sunflower cultivars, Chakinskiĭ 269 (susceptible) and Remil (resistant) were used in this study. The inoculum of *P. halstedii* was obtained from field collections representing the main sunflower growing areas of Hungary, and was maintained on susceptible sunflowers in the greenhouse.

To determine the possible sites of penetration inoculations were made at different times from seed germination to the 6-leaf-stage of the seedlings. Inoculation techniques included the WSI-method, dipping of single plant organs into or spraying them with a spore suspension containing 10^4-10^5 sporangia/ml, or placing drops of this suspension on the plant surface. Inoculated tissues were fixed and prepared for both light—, and scanning electron microscopical (SEM) observations by using various procedures (10, 14). The SEM observations were performed on a JSM-15 instrument.

To make further comparisons between susceptible and resistant interactions, internal development of *P. halstedii*, its ability to produce reproductive organs within or on the host plants, as well as the appearance of tissue degeneration associated with fungal invasion were recorded as described previously (10,12,13).

RESULTS AND DISCUSSION

The downy mildew pathogen, *P. halstedii* was able to infect the young seedlings of both cultivars. However, penetration and internal development of the fungus, as well as plant response varied greatly depending on the mode of inoculation and type of the host. The main differences between susceptible and resistant interactions proved to be as follows:

1. *The possible sites of penetration.* Inoculations made at various sites and ages of sunflower seedlings of both cultivars are compared in Table 1. It is shown that susceptible sunflowers could be infected by *P. halstedii* at each part of the seedlings, whereas penetration took place exclusively on roots, hypocotyls, and cotyledons of the resistant plants. Observations with the SEM indicated that, at least in case of hypocotyl-inoculation, the pathogen entered the host thorough the cuticle rather than through the stomata and that no difference in the mode of penetration could be detected in case of both cultivars

2. *The extent of internal development of the pathogen.* From

Table 2 it is clear that the pathogen equally colonized root and hypocotyl tissues of both kinds of sunflowers but mostly was unable to invade the epicotyl of resistant plants (13). Mycelium was observed only in the cortical parenchyma of resistant seedlings, whereas in case of susceptible cultivar parenchymatic tissues of both cortex and pith were found to be invaded. The different invasion of tissues of the two cultivars combined with certain structural features of the cotyledonary node (diaphragm) may partly explain why epicotyls of the resistant seedlings remain free from the pathogen despite of its occurrence in hypocotyl tissues of the same plants. Similar conclusions have already been drawn from other investigations (5).

3. *The frequency and intensity of fungal sporulation.* Once infection has established and the moisture conditions became favourable, the fungus sporulated freely on the invaded roots and hypocotyls regardless of their susceptibility to *P. halstedii.* On the other hand sporulation on cotyledons of inoculated susceptible seedlings was abundant, but the fungus usually failed to sporulate on cotyledons of resistant plants.

4. *The occurrence, timing, and intensity of hypocotyl lesions.* Though hypocotyl lesions caused by *P. halstedii* occurred on both susceptible and resistant sunflowers, such symptoms appeared earlier and were more severe on the resistant seedlings, at least up to the end of the second week of incubation.

As pathogenesis progressed, however, the difference became less evident and finally the susceptible seedlings died (12). Similar results were observed microscopically. In Table 3 it is shown that by the fourth day of incubation mycelium was easy to recognize in parenchymatic tissues of both cultivars. At the same time many of the invaded cells of resistant seedlings started to show granular and yellowish appearance indicating cell degeneration, while similar alterations among the invaded susceptible cells occurred much later (Table 3).

From the results obtained it is assumed that Plasmopara-resistant sunflowers used in this study respond to infection with a hypersensitive reaction. A similar reaction was found of root epidermal cells of a resistant sunflower line following inoculation with *P. halstedii* (15). Nevertheless, according to these observations, necroses of the host cells at the penetration site of encysted zoospores were in all cases accompanied with degeneration of the fungus itself, and as a result, no penetration took place on the inoculated resistant roots.

As resistance of sunflowers used in this study came from HA-61, a line possessing the Pl_2 and Pl_3 genes against *Pl. halstedii* (9,17), it

seems likely that any resistance controlled by these genes may be characterized as an incomplete one being restricted to the plant parts above the cotyledonary node.

Although the cause of this phenomenon is still unknown, some experimental data obtained during this study and described earlier (1,3,5) suggest that at least two, or more factors including anatomical and biochemical features of the host are responsible for the incomplete resistance.

Practically, affected plants of a resistant cultivar showing no disease symptoms in the field may serve as a source of inoculum in the soil by supplying it with zoosporangia and/or oospores of *P. halstedii*. Besides, new pathogenic forms of the fungus may arise providing a possibility to overcome this resistance (6,7,11).

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TABLE 1

Sites of penetration on sunflower seedlings resistant and susceptible to Plasmopara halstedii

Plant age at time of inoculation	Site of inoculation	Evidence of penetration	
		susceptible	resistant
Germinating seed	radicle	+	—
	hypocotyl	+	—
	cotyledon	+	— */
Young seedling	root	+	—
	hypocotyl	+	—
	cotyledon	+	— */
	true leaf	+	—
	growing point	+	—

*/ penetration seldom occurred

TABLE 2

Systemic development of Plasmopara halstedii *within sunflower seedlings in relation to susceptibility* */

Cultivars	Plant response	% seedlings with mycelium in			
		hypocotyl			
		tap root	underground	aboveground	epicotyl
Chakinskii 269	S	94	89	94	83
Remil	R	100	96	96	7

S = susceptible, Resistant = resistant

*/ Inoculations performed by the WSI METHOD

TABLE 3

Tissue necrosis associated with fungal development in sunflower seedlings resistant and susceptible to Plasmopara halstedii

Days after inoculation	Chakinskii 269			Remil		
	Appearance of visible symptoms	Presence of fungal elements	Establishment of tissue degeneration	Appearance of visible symptoms	Presence of fungal elements	Establishment of tissue degeneration
2	—	—	—	—	—	—
4	—	+	—	—	+	+ +
6	—	+	—	+	+	+ + +
8	+	+	—	+	+	+ + +
10	+	+ +	+	+	+ +	+ + +
12	+	+ +	+	+ +	+ +	+ + +
14	+ +	+ +	+ +	+ +	+ +	+ + +
16	+ + +	+ +	+ +	+ +	+ +	+ + +
18	+ + +	+ +	+ + +	+ + +	+ +	+ + +
20	+ + +	+ +	+ + +	+ + +	+ +	+ + +

Legend: visible symptoms: + = slight discolouration; ++ = pale brownish lesions; +++ = deep-brown, necrotic lesions

presence of fungal elements: + = mycelium; ++ = mycelium with oospores

establishment of tissue degeneration: + = shriveling of tissues; ++ = single cell necrosis; +++ = tissue necrosis