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A light and scanning-electron microscopic study of infection of tomato plants by virulent and avirulent races of Cladosporium fulvum

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Abstract

Infection of tomato plants by *Cladosporium fulvum* Cooke was studied using light and scanning-electron microscopy. Races 1.2.3 and 4 of *Cladosporium fulvum* were used, whereas tomato cultivars, carrying the Cf2 gene (susceptible to race 1.2.3 and immune to race 4) and the Cf4 gene (immune to race 1.2.3 and susceptible to race 4) served as differentials. No differences were observed in growth between compatible and incompatible combinations during germination, subsequent formation of runner hyphae and stomatal penetration. Runner hyphae did not show directional growth towards stomata.

Penetration usually occurred on the third or fourth day after inoculation. In compatible combinations the fungus grew intercellularly, often in close contact with spongy mesophyll cells. Under optimal conditions it did not cause visible damage to plant cells during early stages of infection. Under suboptimal conditions in winter, the host cells often reacted with callose deposition, but growth of the fungus did not appear to be inhibited. Ten to twelve days after inoculation conidiophores emerged through the stomata and produced conidia.

In incompatible combinations fungal growth was arrested one to two days after penetration and confined to stomata and surrounding cells. Very soon the host cells, in contact with the fungus, deposited extensive amounts of callose. Later these cells turned brown and collapsed. At the surface of the host cells, contacted by fungal hyphae, abundant extracellular material could be observed by scanning-electron microscopy.

Removing the epidermis of leaves before inoculation delayed the resistant response. On stripped leaves the rate of fungal growth was equal for both interactions up to ten days after inoculation, but the incompatible combination lacked sporulation.

Introduction

Cladosporium fulvum Cooke causes leaf mould of tomato (*Lycopersicon esculentum* Mill.). Many physiological races of the fungus are known (Hubbeling, 1966). In the cultivated tomato there are several genes for resistance, originating from wild tomato species (Kooistra, 1964; Hubbeling, 1968, 1971). A gene for gene relationship has been proposed (Day, 1956; Kooistra, 1964). The genes for resistance condition different reactions. Plants carrying resistance genes Cf2 and Cf4 react after infection by an avirulent race with very restricted lesions, not visible with the naked eye. This type of high resistance is called immunity (Hubbeling, 1971; Lazarovits and Higgins, 1976a, 1976b). In contrast, plants carrying resistance genes Cf1 and Cf3 permit some fungal growth and even some sporulation. Bond (1938) studied the combination tomato-*Cladosporium fulvum* by light microscopy. Since then, many new races have appeared and many genes for resistance were introduced by breeding.

In view of this, it would be of interest to study ultrastructural characteristics of different host-pathogen combinations. While this study was underway new histological and ultrastructural data on the interactions of *Cladosporium fulvum* race 1 with susceptible (Cf0 gene), resistant (Cf3 gene for resistance) and immune (Cf2, Cf4 genes for resistance) tomato plants were published (Lazarovits and Higgins, 1976a, 1976b). The present study is meant as an introduction to a physiological study on resistance and susceptibility of tomato plants to various physiological races of the fungus.

In this study, races 1.2.3 and 4 were used, whereas 'Vetomold' (carrying the Cf2 gene for resistance, susceptible to races 1.2.3 and immune to race 4) and 'Purdue 135' (carrying the Cf4 gene for resistance, immune to race 1.2.3 and susceptible to race 4) served as differentials. Later on, also near-isogenic lines of 'Moneymaker', carrying resistance genes Cf2 and Cf4, were used. Preliminary results of this study have been published elsewhere (De Wit and Hijwegen, 1976).

Materials and methods

Plants. Seed of tomato 'Purdue 135', 'Vetomold' and the near-isogenic lines of 'Moneymaker' carrying the resistance genes Cf2 and Cf4 (generously supplied by Mrs I. Boukema, IVT, Wageningen) were sown in trays with peat soil (Trio no. 17); seedlings were transplanted in pots (diameter 18 cm) after two weeks. Plants were kept in a greenhouse between 20 °C and 25 °C at 60% relative humidity. Light intensity was kept between 10000 and 15000 lux for 12 hours each day by shadowing the greenhouse in the summer with lime if necessary and supplementing with incandescent light from HPLR–400W lamps (Philips) during the winter.

The fungus. Monospore cultures of *Cladosporium fulvum* race 1.2.3 and race 4 (kindly supplied by Mr N. Hubbeling, IPO, Wageningen) were subcultured on potato-dextrose agar at 22 °C. Twice a year races were reisolated from susceptible tomato varieties, to ensure that they retained their virulence. Conidia from three-week-old cultures on potato-dextrose agar were used for inoculation.

Inoculation. a) Intact plants. Tomato plants of six to seven weeks old were used for inoculation. The fourth or fifth leaf was inoculated at the lower side by spraying with a conidial suspension in water (10^6 conidia/ml). After drying plants were incubated in plastic boxes. During the first day relative humidity was kept at 100% by keeping the boxes completely closed; afterwards, relative humidity was maintained between 85% and 100%, by slightly opening the boxes at day time and closing them at night. Temperature varied between 19 °C and 24 °C and light intensity between 10000 and 15000 lux.

b) Excised stripped leaves. In a few experiments excised leaves, with the cut ends of the petioles covered with moistened cotton wool, were placed in Petri dishes (diameter 15 cm) containing moistened filter paper and incubated in a climate room at 20–24 °C. Light intensity was 10000 lux for 16 hours a day, supplied by white fluorescent tubes (Philips 60 W). The lower epidermis of the leaves was removed prior to inoculation, so that conidia and germinating fungal hyphae were in direct contact with the mesophyll cells of the leaves.

Light, fluorescence and scanning-electron microscopy. Samples were taken at different times after inoculation. To assess germination and penetration of the fungus, epidermal strips of inoculated leaves were examined under the light microscope after staining in a solution of cotton blue in lactophenol.

To study growth of the fungus inside the leaf, leaf discs were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7 and dehydrated in an ethanol series. For ordinary light microscopy fixed leaves were stained in lactophenol-cotton blue and for fluorescence in 0.1% aniline blue in 1/15 M K_3PO_4, pH 12.2 for at least three hours. Observations were made under a Wild microscope after the method of Shimomura and Dijkstra (1975). Starch was stained with a solution of iodine and potassium iodide.

For scanning-electron microscopy, leaf discs were fixed in the same way as described for light microscopy (a few samples were postfixed in 2% OsO_4). After dehydration in ethanol leaf discs were gradually transferred to amyl acetate in a series of ethanol and amyl acetate mixtures. Discs were critical point dried (POLARON critical point apparatus) and coated with a thin layer of gold (Balzers coating unit with attachment for gold sputtering, type BAE 30I). They were then examined in a scanning-electron microscope (type Jeol JSM U_3 or Hitachi). In order to observe penetration and subsequent colonization of the leaf by the fungus the lower epidermis of inoculated leaves was removed before fixation. Penetration through the stomata was observed at the lower side of the stripped epidermis and colonization of the leaf in the stripped leaves themselves.

Results

Conidial germination and stomatal penetration. In Table 1 percentages of conidial germination and subsequent stomatal penetration by race 1.2.3 on both varieties are compared. With respect to germination and penetration there were no significant differences between compatible and incompatible interactions. The percentage

Table 1. Percentage of conidial germination (g) and stomatal penetration (p) by race 1.2.3 on 'Vetomold' (susceptible) and 'Purdue 135' (immune). Inoculations were carried out during winter (November-February) and summer (June-August).

Days after inoculation	Inoculated during winter				Inoculated during summer			
	'Vetomold'		'Purdue 135'		'Vetomold'		'Purdue 135'	
	g	p	g	p	g	p	g	p
2	55	2	47	1	43	7	47	4
3	77	2	52	3	51	10	58	7
4	91	6	79	5	76	33	67	35
5	91	11	82	13	83	39	88	46
6	85	19	88	15	90	74	87	61

Tabel 1. Percentage sporekieming (g) en penetratie (p) van fysio 1.2.3 op 'Vetomold' (vatbaar) en 'Purdue 135' (immuun). De inoculaties werden 's winters (november-februari) en 's zomers (juni-augustus) uitgevoerd.

In all figures, bar represent 10 μm.
Fig. 1. 'Vetomold' + race 1.2.3, five days after inoculation; runner hyphae passing by stomata.
Fig. 2. 'Purdue 135' + race 1.2.3, seven days after inoculation, hypha entering a stoma.
Fig. 3. 'Vetomold' + race 1.2.3, seven days after inoculation; hypha entering a stoma by a side branch.
Fig. 4. 'Purdue 135' + race 4, two days after inoculation, hypha passing over a stoma.

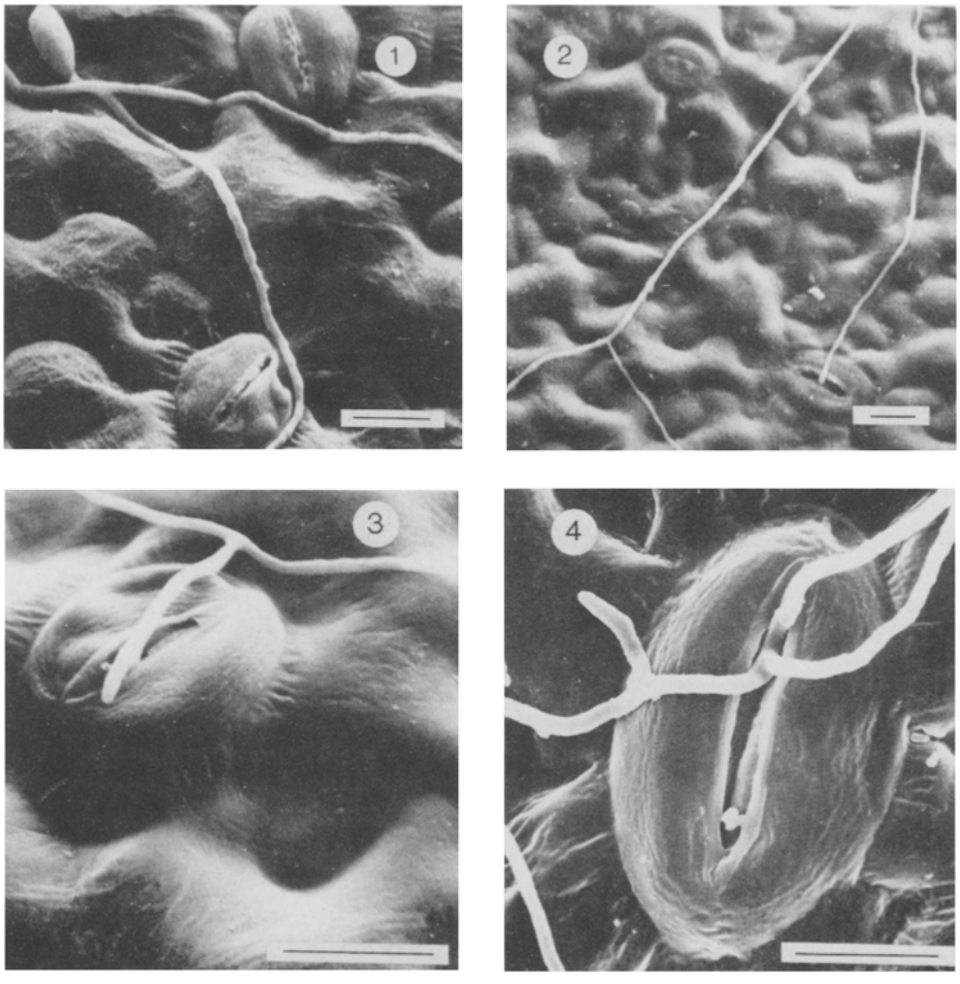

Fig. 1. 'Vetomold' + fysio 1.2.3, vijf dagen na inoculatie; loophyfen die langs huidmondjes groeien.
Fig. 2. 'Purdue 135' + fysio 1.2.3, zeven dagen na inoculatie; hyfe penetreert een huidmondje.
Fig. 3. 'Vetomold' + fysio 1.2.3, zeven dagen na inoculatie; hyfe penetreert een huidmondje m.b.v. een vertakking.
Fig. 4. 'Purdue 135' + fysio 4, twee dagen na inoculatie; hyfe, die over huidmondje heengroeit.

of penetration of germinated conidia was higher during summer than during winter. During winter plants were supplied with artificial light. For race 4 results were similar. Although penetration occurred through stomata only, there was no directional growth of runner hyphae towards stomata (Fig. 1). Scanning-electron microscopy revealed that germinated hyphae grew over and along stomata already one day after inoculation but penetration was usually observed not earlier than two to three days after inoculation (Fig. 2 and 3). Very often hyphae were growing over stomata without entering them (Fig. 4). Possibly, during the first days of incubation, when relative humidity was near 100%, conidial germination was favoured, but penetration prevented, because most stomata were closed under these circumstances. Variable relative humidity did stimulate stomatal opening, which was necessary for fungal penetration. The hyphae penetrated mostly without formation of an appressorium, directly or by means of a side branch (Fig. 2 and 3). In incompatible interactions it was often seen that the fungus grew out of the stoma again, which it had entered (Fig. 5). In successful penetrations of incompatible combinations often extracellular material was deposited on the surface of the stoma (Fig. 5). This extracellular material may extend into the stomatal cavity as could be observed at the inner side of the stripped epidermis (Fig. 6).

Fungal growth and plant response inside the leaf in compatible and incompatible interactions. On the leaf surface no differences in growth between virulent and avirulent races were observed, but as soon as the fungus had entered the stoma, histological differences between both combinations could be detected.

Light microscopy. Epidermal and spongy mesophyll cells around stomata, which were penetrated stained more intensely with cotton blue in the incompatible combination. Very often, the penetrated stoma itself did not contain starch and was coloured brown in the incompatible interactions as a result of a hypersensitive response. This reaction was rarely seen in compatible interactions.

In fixed leaves, fungal growth inside the leaf could be examined rather easily. Four to five days after inoculation, that means one to two days after penetration, fungal growth in incompatible interactions was arrested while in compatible interactions it was abundant. After penetration the fungal hyphae were distinctly thickened in compatible as well as in incompatible interactions. Fungal growth inside the leaf was exclusively intercellular. After fungal contact the spongy mesophyll cells reacted differently in the two types of interactions. In incompatible interactions spongy mesophyll cells turned brown after hyphal contact, a phenomenon rarely seen in compatible interactions.

Fluorescence microscopy. a) Intact leaves. Fluorescence microscopy was used to investigate whether compatible and incompatible combinations reacted differently with regard to callose deposition. Callose deposition is often regarded as a reaction of plants to wounding (Currier, 1957; Nims et al., 1967). In fungal, bacterial and virus diseases, callose formation has been suggested to be correlated with incompatibility (Heath, 1971, 1972, 1974; Shimomura and Dijkstra, 1975). Usually it is deposited as a thick layer between cell membrane and cell wall and is often looked upon as a response occurring before hypersensitive cell death.

Fig. 5. 'Purdue 135' + race 1.2.3, seven days after inoculation; hypha entering and leaving a stoma again; extracellular material present at the stoma (arrow).
Fig. 6. 'Purdue 135' + race 1.2.3, fourteen days after inoculation; inner side of stripped epidermis; short penetrated hypha; note extracellular material (arrow).
Fig. 7. 'Purdue 135' + race 1.2.3, callose deposition (C) around penetrated stomata (S) observed under fluorescence microscope.
Fig. 8. 'Purdue 135' + race 4, seven days after inoculation, hypha (H) in close contact with spongy mesophyll cells.

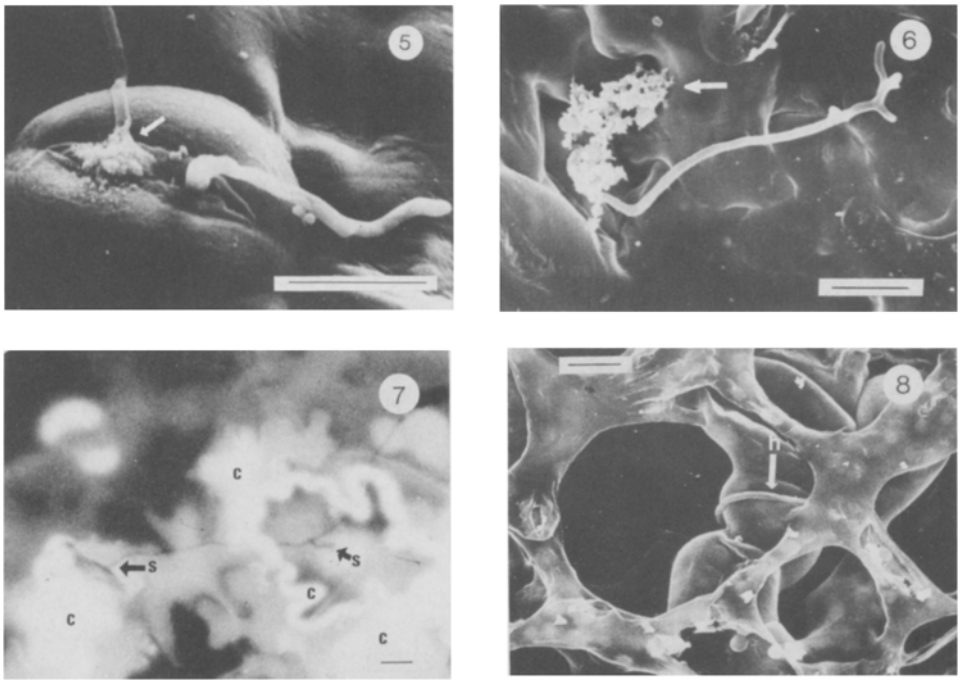

Fig. 5. 'Purdue 135' + fysio 1.2.3, zeven dagen na inoculatie; hyfe die huidmondje gepenetreerd heeft en weer verlaat; extracellulair materiaal op huidmondje aanwezig (pijltje).
Fig. 6. 'Purdue 135' + fysio 1.2.3, veertien dagen na inoculatie; binnenkant gestripte epidermis; korte gepenetreerde hyfe; let ook op aanwezig extracellulair materiaal.
Fig. 7. 'Purdue 135' + fysio 1.2.3; callose afzetting (C) rond gepenetreerde huidmondjes (S) waargenomen d.m.v. fluorescentie microscopie.
Fig. 8. 'Purdue 135' + fysio 4, zeven dagen na inoculatie; hyfe (H) in nauw contact met sponsparenchymcellen.

In Table 2 callose deposition in leaves of 'Vetomold' and 'Purdue 135' after inoculation with race 1.2.3 and 4 are compared. The experiment was carried out during winter. From this table it can be concluded that callose deposition was not specific for the incompatible interaction. In both combinations callose was deposited profusely. In incompatible interactions fungal growth was limited to the fluorescent area. In compatible interactions the fungus outgrew the fluorescent areas; the growing mycelium did not give rise to further callose formation. Therefore, callose formation seemed to be an early reaction of the host after initial fungal contact, ir-

Table 2. Callose deposition in incompatible and compatible combinations. Experiments were carried out during winter (November–February).

	Host-parasite combinations											
	4 days after inoculation				6 days after inoculation				8 days after inoculation			
	A*	B	C	D	A	B	C	D	A	B	C	D
Number of penetrations	11	6	5	–	13	15	11	16	13	20	6	6
Length of penetrated hypha, expressed in cell diameter	1/4–2	1/2–2	1/2–2	–	1/4–3	1/2–5	1/2–10	1/2–3	1/4–3	1/2–∞**	1/2–∞	1/2–3
Number of penetrations with callose deposition	10	4	3	–	5	5	5	11	21	0	2	3
Percentage of penetrations with callose deposition	91	67	60	–	38	33	45	62	70	0	33	50

*A = 'Purdue 135', inoculated with race 1.2.3.
B = 'Vetomold', inoculated with race 1.2.3.
C = 'Purdue 135', inoculated with race 4.
D = 'Vetomold', inoculated with race 4.
– = no observations.
** ∞ = beyond limit of microscopic field (= 300 μm diam.).

Tabel 2. Callosevorming in incompatibele en compatibele combinaties. De experimenten werden 's winters (november–februari) uitgevoerd.

respective of the type of interaction involved. This experiment was repeated during summer with the same cultivars and with two near-isogenic lines of 'Moneymaker' carrying Cf2 and Cf4 genes for resistance. Results of the latter experiment are presented in Table 3. Here, callose formation was almost specific for the incompatible interaction. In Fig. 7 a typical appearance of callose deposition in an incompatible interaction, as seen under the fluorescence microscope, is shown.

b) Stripped leaves. Stripped leaves, inoculated with conidia of both races fluoresced already 12 hours after inoculation. Fluorescence had increased at 24 hours and 48 hours after inoculation. The non-inoculated stripped leaves did not show appreciable fluorescence.

Although results in Table 3 suggested that callose deposition might be causally related to resistance, the experiments with stripped leaves showed that callose deposition seemed to be a consequence rather than a cause of resistance.

On stripped leaves of immune reacting combinations fungal growth was not inhibited. Hence, after removal of the epidermis a resistant leaf became more or less susceptible.

Scanning-electron microscopy. a) Intact leaves. SEM studies provided some more details about fungal growth especially inside the leaf. Inside the leaf the fungus could be observed by removing the lower epidermis at different times after inoculation. The fungus grew exclusively intercellularly without formation of haustoria. In compatible interactions fungal growth was abundant, in many cases in close contact with spongy mesophyll cells (Fig. 8 and 9), but in others not at all. As far as could be observed in SEM, host cells did not react after fungal contact. Nine to ten days after inoculation formation of stroma could be observed beneath the stomata. Later on, conidiophores emerged through stomata (Fig. 10) and produced conidia.

In incompatible interactions mycelium was rarely observed between the spongy mesophyll cells. As could be seen at the inner side of stripped epidermis, the length of the penetrated hyphae varied to a certain extent, but never exceeded a length of four to five times the diameter of an epidermal cell (Fig. 6 and 11). The hyphae in incompatible interactions were often swollen and curled. Sometimes amorphous material was deposited around the penetrated hyphae (Fig. 11). Hyphae in contact with mesophyll cells appeared to be collapsed; the mesophyll cells were often also collapsed or showed abnormal extracellular material at the surface of the cell wall (Fig. 12). This extracellular material occurred as 'bubbles' and was probably produced by the host cells after fungal contact. It was most frequently found on the spongy mesophyll cells, but also at the inner side of epidermis cells, which were contacted by a hypha.

b) Stripped leaves. As could be concluded from these experiments, compatibility or incompatibility became apparent after the fungus had entered the tomato leaves. In the next experiments the epidermis was removed very gently before leaves were inoculated with conidia. There was no difference in mycelial growth on stripped leaves of immune and susceptible reacting plants. The mesophyll cells, which were contacted by hyphae of germinated conidia did not appear to be collapsed in both combinations. However, the so-called 'bubbles' occurred very frequently, mainly in the incompatible interactions (Fig. 13).

On stripped leaves two types of hyphae could clearly be distinguished:

Fig. 9. 'Purdue 135' + race 4, ten days after inoculation, hyphae in close contact with spongy mesophyll cells.
Fig. 10. 'Purdue 135' + race 4, twelve days after inoculation; young conidiophores emerging from a stoma.
Fig. 11. 'Purdue 135' + race 1.2.3, fourteen days after inoculation; inner side of stripped epidermis; penetrated hypha (H) in stoma (S) short and swollen; note amorphous material around hyphal tips (arrow).
Fig. 12. 'Purdue 135' + race 1.2.3, twelve days after inoculation; collapsed mycelium and extracellular material present at the cell wall of mesophyll cells (arrow).

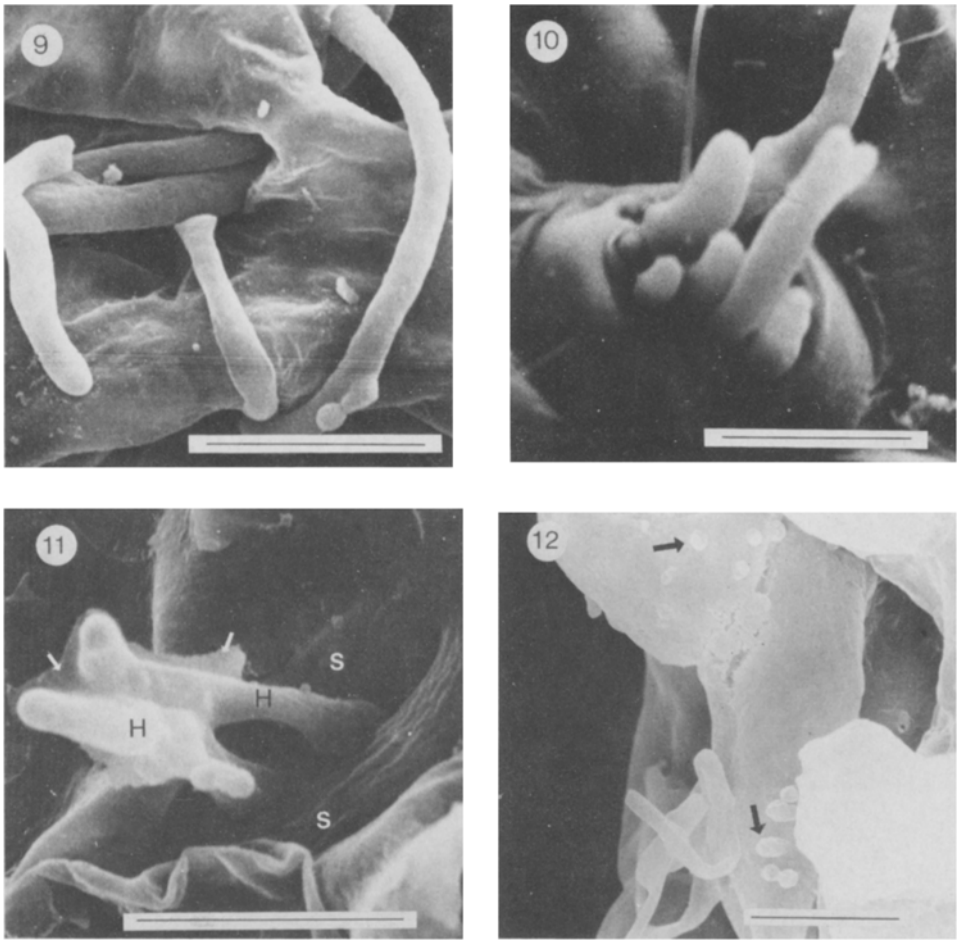

Fig. 9. 'Purdue 135' + fysio 4, tien dagen na inoculatie; hyfen in nauw contact met sponsparenchymcellen.
Fig. 10. 'Purdue 135' + fysio 4, twaalf dagen na inoculatie; jonge conidioforen, die uit een huidmondje komen.
Fig. 11. 'Purdue 135' + fysio 1.2.3, veertien dagen na inoculatie; binnenkant gestripte epidermis; korte gezwollen hyfe (H) in huidmondje (S); let ook op amorf materiaal rond de hyfe-toppen (pijltje).
Fig. 12. 'Purdue 135' + fysio 1.2.3, twaalf dagen na inoculatie; abnormaal verwrongen mycelium en extracellulair materiaal aanwezig op de celwand van sponsparenchymcellen (pijltje).

Table 3. Callose deposition in incompatible and compatible combinations. Experiments were carried out during summer (June–August).

	Host-parasite combinations											
	4 days after inoculation				6 days after inoculation				8 days after inoculation			
	A*	B	C	D	A	B	C	D	A	B	C	D
Number of penetrations	30	30	30	30	30	30	30	30	30	30	30	30
Length of penetrated hypha, expressed in cell diameter	1/4–3	1/4–3	1/4–3	1/4–3	1/4–3	1/4–3	1/2–10	1/2–10	1/4–3	1/4–3	1/2–∞**	1/2–∞
Number of penetrations with callose deposition	2	1	0	0	18	19	1	0	22	25	1	3
Percentage of penetrations with callose deposition	6	3	0	0	60	63	3	5	73	83	3	10

*A = 'Purdue 135', inoculated with race 1.2.3.
B = 'near-isogenic line Cf4', inoculated with race 1.2.3.
C = 'Vetomold', inoculated with race 1.2.3.
D = 'near-isogenic line Cf2', inoculated with race 1.2.3.
** ∞ = beyond limit of microscopic field (= 300 μm diam.).

Table 3. Callosevorming in incompatibele en compatibele combinaties. De experimenten werden 's zomers (juni–augustus) uitgevoerd.

Fig. 13. 'Purdue 135' + race 1.2.3, nine days after inoculation; extracellular material present (arrow); abundant growth of mycelium on stripped leaf.
Fig. 14. 'Purdue 135' + race 1.2.3, nine days after inoculation; runner hyphae (R) and thickened hyphae (T) on stripped leaf.

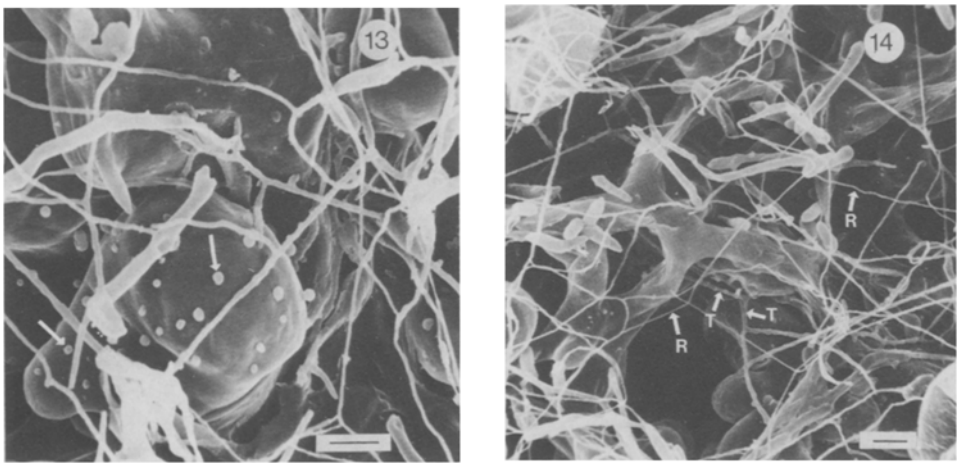

Fig. 13. 'Purdue 135' + fysio 1.2.3, negen dagen na inoculatie; extracellulair materiaal aanwezig (pijltje); overvloedige groei van mycelium op gestript blad.
Fig. 14. 'Purdue 135' + fysio 1.2.3, negen dagen na inoculatie; loop-hyfen (R) en verdikte hyfen (T) op gestript blad.

1) Thin runner hyphae, which are normally only produced at the leaf surface;
2) Hyphae with two to three times the diameter of runner hyphae, normally only produced inside the leaf immediately after penetration of a stoma (Fig. 14).

Although under natural circumstances thick hyphae were formed immediately after runner hyphae had penetrated a stoma, on stripped leaves where hyphae of germinated conidia were in direct contact with mesophyll cells, runner hyphae were formed first. The thickened hyphae were observed only a few days after inoculation of the stripped leaves. They were in close contact with the spongy mesophyll cells. Thus, it may be concluded that contact with mesophyll cells was not the only stimulus that triggered formation of thick hyphae.

Discussion

On the leaf surface *Cladosporium fulvum* behaved very similarly to *Cercospora beticola* (Rathaiah, 1976). There was no directional growth of the runner hyphae towards the stomata and penetration occurred three to four days after inoculation. Rathaiah found, that for successful penetration stomata need not to be open. Zoospores of fungi like *Pseudoperonospora humuli* and *Plasmopara viticola* show a positive chemotaxis to stomata (Royle and Thomas, 1973) and rust hyphae show directional growth of germ tubes towards stomata (Heath, 1974). Light, fluorescence and scanning-electron microscopy revealed no differences between virulent and avirulent races of *C. fulvum* with respect to conidial germination and stomatal penetration. This is in agreement with observations described for other host-pathogen

combinations (Skipp and Deverall, 1972; Skipp and Samborski, 1974).

Cell browning and cell collapse are suggested to be a consequence rather than a cause of the initial resistant reaction. Similar conclusions were drawn by other workers for other host-pathogen interactions (Ogle and Brown, 1971; Király et al., 1972; Mayama et al., 1975).

In plants inoculated during summer, callose deposition was observed almost exclusively in incompatible interactions, as described also by Lazarovits and Higgins (1976a, 1976b). Inoculations carried out during winter showed callose deposition in nearly equal amounts in both interactions. Also when plants were inoculated with conidia of two to three month-old cultures, callose deposition was the same for both interactions. Hubbeling (personal communication) observed, that susceptible plants produced many necrotic spots when inoculated with conidia of old cultures. Thus, callose deposition is influenced by external conditions. As soon as a runner hypha had penetrated a leaf its diameter increased two to three-fold. It is not known how the fungus is feeding itself inside the leaf. According to Van Dijkman (1972) the fungus does not produce polygalacturonases or cellulases in significant amounts in vitro. This observation was confirmed by us, except for C_x-cellulase, which was produced by the fungus when grown on several media, including cell walls of tomato mesophyll cells. The fungus is probably living on substances leaching from the mesophyll cells into the intercellular space. It is known that sugars and other photosynthetic products synthesized in the mesophyll cells leach into the free space and are transported to the small veins by so-called transfer cells (Kursanov and Brovchenko, 1970). The extracellular material at the surface of mesophyll cells, found in incompatible interactions was also reported by Lazarovits and Higgins (1976a, 1976b). They observed this extracellular material more frequently in resistant plants carrying the Cf3 gene for resistance than in plants carrying the Cf2 and Cf4 genes for resistance. In our case this extracellular material was found in plants carrying the Cf2 and Cf4 genes for resistance. According to Lazarovits and Higgins (1976a) the main components of this material were polyphenols.

There was a significant difference in mycelial growth in incompatible interactions between intact leaves and leaves from which the lower epidermis had been stripped off prior to inoculation. For stripped leaves expression of immunity appeared to be delayed in time. Fungal growth was rather abundant and the only difference between compatible and incompatible interactions was lack of sporulation in the incompatible combinations. It seems possible that the early resistant response is triggered in the penetrated stoma, after recognition of the fungus by the guard cells, because very often these guard cells were the first to react after fungal contact.

The question how fungal growth is inhibited in the incompatible interaction remains still to be solved. These problems will be the subject of further studies.

Samenvatting

Een licht- en raster-elektronenmicroscopisch onderzoek van infectie van tomateplanten met virulente en avirulente fysio's van Cladosporium fulvum

Infectie van tomateplanten met *Cladosporium fulvum* Cooke werd onderzocht met gebruikmaking van licht- en raster-elektronenmicroscopie. Fysio's 1.2.3 en 4 van

Cladosporium fulvum werden gebruikt, terwijl tomatecultivars met resistentiegen Cf2 (vatbaar voor fysio 1.2.3 en immuun voor fysio 4) en resistentiegen Cf4 (immuun voor fysio 1.2.3 en vatbaar voor fysio 4) als 'differentials' werden gebruikt. Na inoculatie werden geen verschillen in groei van de schimmel gemeten tussen compatibele en incompatibele combinaties tijdens kieming, vorming van loop-hyfen en daaropvolgende penetratie van huidmondjes. Er kon geen gerichte groei van de loophyfen naar de huidmondjes worden waargenomen (Fig. 1). De penetratie vond meestal plaats op de derde of vierde dag na inoculatie (Fig. 2).

In compatible combinaties groeide de schimmel intercellulair in nauw contact met sponsparenchymcellen (Fig. 9). Onder optimale omstandigheden werden noch de waardplantcellen, noch de hyfen beschadigd gedurende de eerste tien dagen na inoculatie terwijl ook callosevorming achterwege bleef (Tabel 3). Onder suboptimale omstandigheden werd door de waardplanten callose gevormd, maar de groei van de schimmel werd hierdoor niet geremd (Tabel 2). Tien tot twaalf dagen na inoculatie groeiden conidioforen door de huidmondjes naar buiten (Fig. 10), waarna sporulatie volgde. In incompatiblele combinaties werd een tot twee dagen na penetratie van het huidmondje de groei van de schimmel volledig geremd, zodat deze slechts beperkt bleef tot het huidmondje en de omringende cellen. Spoedig na deze remming werden door de waardplantcellen, in de omgeving van de schimmel, grote hoeveelheden callose gevormd (Fig. 7; Tabel 3). Later werden deze cellen bruin en verschrompelden. Aan de buitenkant van de waardplantcellen, in de omgeving van de schimmel kon met behulp van de elektronenmicroscoop veel extracellulair materiaal worden waargenomen (Fig. 13). Verwijdering van de epidermis vóór inoculatie vertraagde de resistentie-reactie. De groeisnelheid van de schimmel op bladeren waarvan de beneden-epidermis verwijderd was, was voor beide combinaties gelijk tot tien dagen na inoculatie. Bij de incompatibele combinaties werd nooit sporulatie waargenomen, daarentegen wel bij de compatibele.

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