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LIGNIFICATION, A POSSIBLE MECHANISM OF ACTIVE RESISTANCE AGAINST PATHOGENS[1]

Lignificatie als mogelijk afweermechanisme tegen ziekteverwekkers

BY

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INTRODUCTION

BUTLER & JONES (1955), in their well-known book, state: "...when lignin infiltration occurs, solution of the wall is usually much more difficult and cutin and suberin deposits are apparently refractory" (p. 132) and: "In many parasites (not in all) attacking the wood of plants the hyphae can only pass from cell to cell through the pits in the walls, the rest of the lignified wall offering an impassable barrier" (p. 139).

Furthermore, describing the disease called "shot hole", a disease of stone fruits, the authors mention that the injured tissue is bordered by a barrier of cells with lignified walls, apparently checking development of the parasite, so that only a limited area is affected. In most cases the injured area with the lignified cells drops away, producing a "shot hole"; in some cases, however, abscission does not occur and a layer of cork is deposited on the side turned to the affected tissue. The most acceptable conclusion is, that in this case there is an active reaction of the plant to the parasite, resulting in the lignification of some layers of cells, thus checking the parasite's expansion.

EL-DIN-FOUAD (1956) in anatomical investigations on the nature of the plant – parasite relationship in cucumber – cucumber scab (caused by *Cladosporium cucumerinum* Ell. & Arth.) found only lignification of the dead cells in the resistant variety 'Mabro'; wounding alone also gave this effect. After wounding, susceptible plants did not react in this way. BEHR (1949), however, reports that on making wounds in cucumber fruits or petioles of a susceptible variety with a scalpel and subsequent inoculation with *Cladosporium cucumerinum* no infection results, although some growth of hyphae occurs. On anatomical investigation of the wounded tissue it was found that the surrounding cells were lignified. When using milder methods of inoculation, infection succeeded.

Apparently the unsuccessful inoculation is due to the fact that a defense mechanism is already operating in the plant as a consequence of the wounding.

PIERSON & WALKER (1954), also investigating the plant – parasite relationship in cucumber – *Cladosporium cucumerinum*, in susceptible and resistant hosts, found that infection of resistant plants caused a thickening of the cell walls surrounding the infection place. Unfortunately they did not react upon lignin in these thickened cell walls. However, according to REINDERS (1952) lignification is accompanied by a considerable thickening of cell walls, so that it is

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quite probable that also in this case thickening may be ascribed to lignification as a response of the host to the parasite. From the above it seems probable that the presence of lignin offers great difficulties to the invading hyphae of many parasites.

The aim of this investigation was primarily to determine whether the resistance-increasing effect of phenylserine against *Cladosporium cucumerinum* found by VAN ANDEL (1958) could be attributed to lignification of cell walls.

MATERIAL AND METHODS

Young cucumber seedlings of the susceptible variety 'Lange gele Tros' were grown in partially sterilized sand at about 20 °C, and, after 5–6 days, used as such or placed in water or a solution of 0.01 M L-threo-β-phenylserine during two days. Subsequently the plants were sprayed with a suspension of spores of *Cladosporium cucumerinum* and placed in a greenhouse at 18–20 °C and high humidity.

After five days the plants were examined anatomically by stripping off the epidermis and the underlying cell layers of the hypocotyls. Then they were stained in cotton blue or phloroglucinol HCl.

In other experiments seeds of the susceptible variety 'Lange gele Tros' and the resistant variety 'Vios' were put on water-soaked filter paper and placed in the dark at 23 °C for five days. Half of each lot of seedlings was sprayed with spores of *Cladosporium cucumerinum* and both inoculated and uninoculated plants were placed in the dark at 17 °C during five days. Afterwards the plants were examined anatomically in the way already described.

RESULTS

A first experiment was achieved with plants grown in sand. Susceptible 'Lange gele Tros' plants were inoculated by brushing the hypocotyls with a suspension of *Cladosporium cucumerinum* spores in water (control) or a 0.01 M L-threo-β-phenylserine solution. In order to provide wounds for a good penetration the brushing was done fairly roughly. Afterwards the plants were placed at 18–20 °C and high humidity. However, neither control nor phenylserine-treated plants became diseased. On anatomical investigation of strips of hypocotyls a great number of patches of cells with lignified walls were found in both cases.

It had to be determined whether the lignification was indeed due to brushing or occurred independently as a natural reaction of the plants under investigation. To gain more evidence regarding this subject the following experiment was performed.

A number of plants was divided into two groups, one group was left untreated, the other was brushed with water. After three days both groups of plants were examined in the way described previously. The control group showed no lignification, whereas the treated plants did. So it is very probable that in this case lignification was due to mechanical injury caused by brushing. These results are in close agreement with those of BEHR (1949), who found lignification upon wounding and who also found that inoculations in a "mild" way were by far more successful than under "drastic" conditions of severe wounding.

As the procedure followed thus far did not allow of any conclusion about the

role of phenylserine in lignin formation, the method was modified. Plants were placed with the roots in either distilled water or L-threo-β-phenylserine 0.01 M at 20 °C during two days; after that they were sprayed with spores and transferred to a temperature of 18–20 °C at high humidity. After five days control plants were heavily infected, whereas L-threo-β-phenylserine gave 100% protection. On investigation the cells of the control plants showed no lignin formation; in the treated plants some patches of lignified cells were found, but from other anatomical data it was concluded that in this case lignification was not the cause of protection, but probably a reaction due to chemical injury caused by phenylserine.

Because of these results it seemed worth-while to investigate the importance of this process with respect to natural resistance which occurs in some cucumber varieties.

For this purpose plants grown on filter paper in the dark were used. Susceptible plants grown in this way were usually very heavily diseased whereas the resistant ones were only slightly affected. Moreover the epidermis with underlying cortical parenchyma cells from these plants was easier to strip off. As susceptible variety 'Lange gele Tros' was used, as resistant variety 'Vios'.

The results were as follows:

1. 'Lange gele Tros': not inoculated: no lignification,
2. 'Lange gele Tros': inoculated: no lignification,
3. 'Vios': not inoculated: no lignification,
4. 'Vios': inoculated: lignification.

So only the resistant inoculated plant shows lignin formation and the most acceptable view is that following an invasion of the parasite a process comes into action which inhibits the parasite in its progress. Lignification starts in all cases in the cell layers just beneath the epidermal layer; epidermis cells themselves don't become lignified.

It is very difficult to give the direct proof that lignification and infection occur at the same place as the stains for fungus (cotton blue) and for lignin (phloroglucinol HCl) cannot be used at the same time.

Discussion

From the above some evidence is obtained that besides the more common phenomena of cork formation and cell necrosis as mechanisms of defense against parasites lignification may be another way of active resistance in host – parasite relationships.

It is not improbable that besides cucumbers also other plants show a reaction of this kind. Possibly also the increase in resistance against *Fusarium oxysporum* f. *lycopersici* of tomatoes upon cutting the roots (Keyworth & Dimond, 1952) can be ascribed to a similar mechanism.

One of the enzymes which is required for lignification is peroxidase. In recent years some publications have appeared on the fate of peroxidase in infected plants. The general reaction seems to be a stimulation in the first phase of attack as well in susceptible as in resistant plants, but only in resistant plants the high level is maintained. Of course an increase of the enzyme alone would not be sufficient, also a substrate for the enzyme should be there. In some cases

the resistant plant might differ from the susceptible one by a higher peroxidase content as well as a greater ability of the neighbouring cells to synthesize enough substrate for the creation of a barrier against the progressing hyphae.

Investigations concerning the dynamics of phenolic compounds – especially of the lignin precursors – are in progress.

SAMENVATTING

In de literatuur zijn aanwijzingen te vinden, dat in bepaalde gevallen een schimmelaantasting tot staan gebracht kan worden door verhouting van de wanden van een aantal cellagen rondom de infectieplaats.

Onderzocht werd of de door VAN ANDEL (1958) gevonden bescherming van komkommers tegen *Cladosporium cucumerinum* – na toediening van L-threo-β-phenylserine – toe te schrijven zou zijn aan een sterkere verhouting van de celwanden. Geconcludeerd werd, dat dit niet het geval is.

Door dit onderzoek werd echter de aandacht gevestigd op de mogelijke betekenis van dit mechanisme voor de resistentie van bepaalde komkommerrassen. Om dit na te gaan werden geïnoculeerde en niet geïnoculeerde kiemplanten van het vatbare ras 'Lange gele Tros' en het resistente ras 'Vios' anatomisch onderzocht. Alleen in het geïnoculeerde resistente ras werd verhouting van schorsparenchymcellen waargenomen. Lignine-vorming kan dus inderdaad van belang zijn als mechanisme van actieve resistentie tegen pathogenen.

In de laatste jaren zijn enige publikaties verschenen, waaruit blijkt dat de activiteit van het enzym peroxydase, dat de vorming van één van de tussenprodukten bij de vorming van lignine katalyseert, toeneemt wanneer infectie van de plant optreedt. Het verschil tussen vatbaar en resistent zou in bepaalde gevallen kunnen berusten op het vermogen van de cellen rondom de infectieplaats, om in niet of wel voldoende mate het benodigde substraat te synthetiseren.

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