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A biochemical mechanism for the gene-for-gene resistance of tomato to Cladosporium fulvum

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Abstract

Model experiments were carried out with the tomato varieties 'Moneymaker' (no resistance genes), 'Leaf Mould Resister No. 1' (resistance gene Cf 1), 'Vetomold' (resistance gene Cf 2) and 'V 473' (resistance genes Cf 1 and Cf 2) and various physiological races of *Cladosporium fulvum*. Leaking of ^{32}P from labelled leaf disks was obtained on infiltration with high molecular weight excretion products from incompatible races of *C.fulvum* but not with those from compatible races. These products were obtained by Sephadex G-25 gel filtration of culture filtrates.

The observations are in line with our hypothesis that the gene-for-gene relation existing between tomato and *C.fulvum* is based on interaction of specific fungal excretion products with specific receptors in the host which may be located in the cell membrane. The presence of these fungal compounds is supposed to be controlled by four avirulence genes (A_1, A_2, A_3 and A_4) and that of the receptors by the four resistance genes (Cf 1, Cf 2, Cf 3 and Cf 4). Results obtained from experiments with tomatoes Cf 1, Cf 2 and Cf 1 Cf 2 suggest that leakage followed by the hypersensitivity reaction occurs when *C.fulvum* races possessing a specific avirulence allele penetrate into a host carrying the corresponding resistance allele.

It is not yet clear why growth of *C.fulvum* is stopped when leakage of the host tissue resulting in the hypersensitive reaction takes place. No compound toxic to *C.fulvum* is present or is formed in homogenates of tomato leaves.

Introduction

Tomato leaf mould caused by *Cladosporium fulvum* Cooke is an economically important disease in glasshouse cultures. By breeding and by introduction of resistance factors from wild *Lycopersicum* species, resistant varieties have gradually been obtained (Persiel, 1967; Williams, 1964). They show hypersensitivity upon inoculation with an incompatible race of *C.fulvum*. During this breeding work it was shown that resistance of *Lycopersicum esculentum* to *C.fulvum* is determined by four independent genes which have been called Cf 1, Cf2, Cf3 and Cf4 (Bailey and Kerr, 1964; Kooistra, 1964). The dominant allele of each of them determines resistance to certain races of the pathogen but is quite ineffective towards other races. To illustrate this fact, data have been summarized in Table 1, largely derived from Kooistra (1964) and Hubbeling (1966). The varieties are indicated by the dominant resistance alleles present; the tomatoes which are recessive to all resistance genes have been named cf. Since the authors cited could not yet designate the physiological races of the pathogen by distinctive genotypes, the races were given the indices of the resistance alleles of the tomato, which are ineffective towards them. The symbol

Table 1. Relation between the tomato varieties and races of the parasite *C.fulvum* studied. S: compatible; R: incompatible

Tomato varieties	Code, by dominant resistance genes	Races of *C.fulvum*					
		Indices as given by Kooistra (1964) and Hubbeling (1968):					
		0	1	2	1.2	3.4	1.2.4
		proposed genotype for avirulence:					
		$A_1A_2A_3A_4$	$a_1A_2A_3A_4$	$A_1a_2A_3A_4$	$a_1a_2A_3A_4$	$A_1A_2a_3a_4$	$a_1a_2A_3a_4$
Moneymaker[1] Leaf mould	cf	S	S	S	S	S	S
Resister No. 1	Cf 1	R	S	R	S	R	S
Vetomold	Cf 2	R	R	S	S	R	S
V 473	Cf 1 Cf 2	R	R	R	S	R	S
Vagabond[2]	Cf 1 Cf 2 Cf 4	R	R	R	R	R	S

[1] Tomatoes which are recessive for all resistance genes have been named cf.
[2] *Cf.* N. Hubbeling, 1970, Meded. Rijksfac. Landbouwwet. Gent 24: in press.

Tabel 1. Verband tussen bestudeerde tomatenvariëteiten en de rassen van C.fulvum. S: compatibel; R: incompatibel.

0 has been given to the race which can only attack tomatoes that are recessive to all resistance genes.

The complicated relation between host varieties and races of the pathogen in tomato has been recognized as a 'gene-for-gene' relation by Day (1954) in analogy to similar relations earlier described for other diseases, e.g. flax rust (Flor, 1942) and potato late blight (Black et al., 1953; Toxopeus, 1956). Such relations were later also described for apple scab (Boone and Keitt, 1957) and the rust and smut fungi (*cf.* Fincham and Day, 1963).

In the case of commercial apples little is known about the host genotype, but by the crossing of different races of *Venturia inaequalis* seven avirulence genes have been found to correspond with seven hypothetical resistance genes in apple (Boone and Keitt, 1957; Fincham and Day, 1963). Later work by Raa (1968a, 1968b) with resistant and susceptible seedlings obtained from seeds of a randomly pollinated resistant Antonovka hybrid of *Malus sylvestris* has strongly suggested that avirulence genes in *V. inaequalis* are responsible for the production of specific toxins, leading to the hypersensitivity reaction in the resistant host, but not in the susceptible host. The hypothesis was forwarded that the dominant allele of each of the seven avirulence genes produces a specific compound which only in apple possessing the corresponding resistance allele leads to a hypersensitive reaction owing to cell damage (Raa and Kaars Sijpesteijn, 1968).

It was suggested that the same principle might underlie the host-parasite relation in leaf mould of tomato (Kaars Sijpesteijn, 1969). This would implicate the occurrence of four avirulence genes (A) in *C.fulvum* corresponding to the four resistance genes (Cf) in the tomato in such a way that for instance any tomato carrying the dominant allele Cf1 is resistant to any physiological race carrying the avirulence allele A_1. The dominant alleles A_1, A_2, A_3 and A_4 of the four avirulence genes according to this

hypothesis effect the production and excretion of four different toxic compounds by the fungus, each of which evokes leakage of cell constituents, and as a consequence a hypersensitive reaction, in the host plant with the corresponding Cf allele. On the other hand a tomato will be susceptible to a race if A alleles and Cf alleles do not correspond. In Table 1 the hypothetical genotype with regard to avirulence genes is given for certain races of this haploid fungus.

Since tomatoes of known genotype and *C. fulvum* races of known virulence were available an attempt was made to confirm this hypothesis.

Materials and methods

Organisms. Seeds of the different homozygous tomato varieties were obtained from the Institute for Horticultural Plant Breeding in Wageningen and plants were grown in the greenhouse. For all experiments healthy mature leaves were used. The races 0, 1, 2, 1.2, 3.4 and 1.2.4 of *C. fulvum* were obtained from the Institute of Phytopathological Research in Wageningen, the numbers of the isolates being 63–26, 66–11, 63–29, 63–43, 63–24, and 67–2, respectively. The cultures were kept on potato-dextrose-agar slants at 24°. Shake cultures were grown at 24°C in the following medium: 1% casamino acids (Difco, technical grade), 0.3% yeast extract (Difco), 4% glucose and 0.0025M potassium phosphate buffer (pH 6.0) in tap water. Pathogenicity of the cultures used was checked by inoculation of appropriate tomato varieties. Results of these tests were in full confirmation of the disease pattern given by Kooistra (1964) and Hubbeling (1968).

Chemicals. Tomatidine was prepared from tomatine (Fluka A.G.) by acid hydrolysis (Sato et al., 1951). The purity was checked by R_F-value and melting point. ^{32}P-labelled orthophosphate was obtained from Philips-Duphar, Radioactive Chemicals, Petten (The Netherlands).

Techniques. To separate low and high molecular material Sephadex G-25 gel filtration fractions were prepared as follows: Culture filtrate of shake cultures of *C. fulvum* was filtered through cotton wool or filter paper and applied to a column (3.8 × 91 cm) filled with Sephadex G-25 medium grade (Pharmacia, Uppsala, Sweden). The void volume (Vo) of the column was 330 ml as determined with Blue Dextran 2000, the total volume (Vt) was 1030 ml. The maximum sample volume was 300 ml. Gel filtrations were performed with glass distilled water at 4°C. The fractions were collected in an LKB 7000 UltroRac Fraction collector (LKB, Stockholm, Sweden) and freeze-dried (Cenco-Virtis freeze-drier, Gardiner, N.Y., USA). Protein was determined by the Folin method (Lowry et al., 1951).

To obtain ^{32}P-labelled tomato tissue, leaves were cut off and placed in a flask with water containing ^{32}P-orthophosphate carrier-free (10μC/ml). The leaves were illuminated for about 2 hours with a 250 W lamp and a light current of air was passed over them to increase uptake. When after about 2 h the leaves showed sufficient radioactivity as measured with a portable GM counter, disks were cut from interveinal areas by means of a 8 mm diameter corkborer. These were distributed at random over the infiltration vessels to obtain equal radioactivity in all flasks. After washing three times with water the disks were placed into 5 ml of buffer solution (Tris-HCl, 10^{-2} M,

pH 6.0) containing 2% (w/v) of the freeze-dried fraction 1 (cf. Fig.1). The liquid was slowly infiltrated in vacuo in a specially designed glass vessel to warrant the complete removal of air from the disks. Samples (0.1 ml) were removed immediately after infiltration and after different intervals. They were dried on aluminium planchets under a 250 W lamp before counting in triplo with a Philips GM counter.

Radioactivity initially present in the leaf disks was calculated as the sum of the radioactivity removed with the samples and of the radioactivity finally present in the vessel. The latter was determined after ashing the leaf disks together with the residual liquid. Owing to poor solubility of the ash in water or dilute HCl these results are subject to slight variation. Leakage of ^{32}P was expressed as percentage of total leakable ^{32}P.

Extraction of tomatine from tomato leaves was carried out by drying the leaves in an oven at 60 °C and subsequent extraction with 2% acetic acid. After 48h the material was filtered through cheese cloth. The solution was made alkaline with NH_4OH and the resulting precipitate was centrifuged, washed and taken up in methanol. After evaporation a grey powder was obtained.

Cell-free sap of tomato tissue was obtained by a specially designed press. Leaves were pressed between PVC disks and the sap immediately centrifuged (5 min., 10000 g). The sap was sterilized by milli-pore filtration (0.8 μm).

Fungicidal activity was determined by the common spore germination test and by the roll culture method (Pluijgers and Kaars Sijpesteijn, 1966).

Results

Leakage of tomato tissue caused by Sephadex G-25 fractions of C.fulvum

Model experiments were designed to study the effect of excretion products of the various races on the tissues of the different host varieties. Several procedures were tried to study this interaction. Most promising results were obtained by studying the leakage of labelled phosphate from leaf disks infiltrated with high molecular weight fractions of culture filtrate of *C.fulvum*. These fractions were prepared by Sephadex gel filtration.

Gel filtration. Culture filtrates of the *C.fulvum* races $A_1A_2A_3A_4$, $a_1A_2A_3A_4$, $A_1a_2A_3A_4$, $a_1a_2A_3A_4$ and $a_1a_2A_3a_4$ (*cf.* Table 1) were freed from low molecular weight material by filtration over Sephadex G-25 medium grade. The elution pattern (280 nm) as recorded by the Uvicord spectrophotometer (Fig.1) displayed no qualitative differences for the races studied. The absorbancy varied with the incubation period.

Of every run the void volume fraction (fraction 1, mol.wt. $\geqslant$ 1000) was collected and freeze-dried. A greyish powder was obtained and stored in a sealed flask at −30 °C until use. The total protein content varied from 20–50% (w/w) depending on the race of *C.fulvum* and the incubation period. Maximum protein content in the culture was reached by the various races after 18–30 days. The addition of yeast extract to the culture medium stimulated growth but did not increase the total amount of protein in fraction 1, although the absorption of this fraction was higher if yeast extract had been added to the medium.

Leakage of ^{32}P from leaf disks of tomato infiltrated with Fraction 1 of culture filtrates. Leaves of the various tomato varieties were cut off and allowed to take up water

Fig. 1. Characteristic elution pattern of Sephadex G-25 gel filtration of culture filtrate of *C.fulvum* race $A_1a_2A_3A_4$. Sample volume 225 ml.

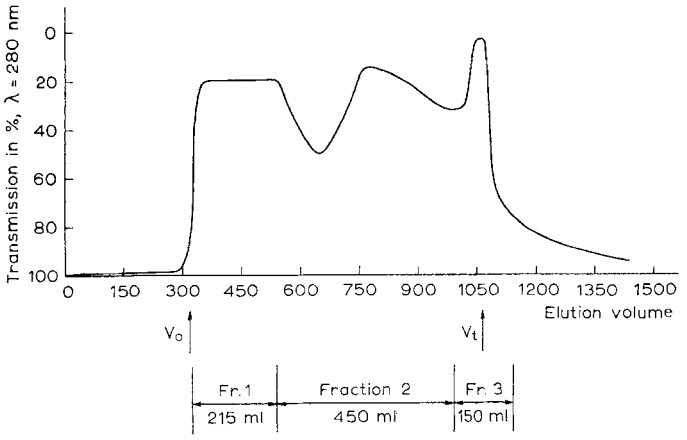

Fig. 1. Karakteristiek elutiepatroon van een Sephadex G-25 gelfiltratie van cultuurfiltraat van C.fulvum fysiologisch ras $A_1a_2A_3A_4$. Opgebracht: 225 ml.

containing per ml 10μC orthophosphate-^{32}P. After sufficient uptake of radioactivity, leaf disks were prepared.

To assess maximum leakability of ^{32}P-containing compounds from these disks, they were infiltrated in vacuo with an aqueous solution of 1% chloroform. Tris-HCl buffer (10^{-2}M, pH 6.0) served as a control. At regular intervals samples were taken from the liquid around the disks and radioactivity was measured to assess leaking. In experiments with different tomato varieties treatment with chloroform caused very rapid leakage until about 78% of the ^{32}P initially present in the disks had leaked out. Thereafter no further leakage took place suggesting that approximately 22% of the labelled phosphate was chemically or physically bound in the cell and could not leak out. Control leakage caused by the buffer solution proceeded always slowly as can be seen from the Fig.2, 3 and 4. Buffers of various composition were tested but, with the techniques used, it proved impossible further to decrease leakage.

In the following experiments leakage of ^{32}P will be expressed as percentage of total leakable ^{32}P. The latter value was the maximum leakage obtained with 1% chloroform.

The same method was used to assess the leakage caused by fraction 1 preparations of the various culture filtrates. Leaf disks of the varieties 'Leaf Mould Resister No.1' and 'Vetomold' were infiltrated with this fraction prepared from race $a_1A_2A_3A_4$ and from race $A_1a_2A_3A_4$ dissolved in Tris-HCl buffer. As shown in Table 1 the pattern of susceptibility of these two varieties to the two races is opposite.

Leakage after different time intervals is presented in Fig.2A and 2B. These results show that the rate of leakage of tomato tissue by a preparation from a compatible race does not significantly exceed the leakage caused by the buffer alone. In contrast the rate of leakage of the same tomato varieties caused by preparations from an incompatible race greatly exceeds the control leakage. These observations strongly support the hypothesis forwarded in the Introduction.

Fig. 2. Leakage of ^{32}P from tomato leaf disks infiltrated with fraction 1 of culture filtrate of two races of *C.fulvum*. ●——● race $A_1a_2A_3A_4$; ▲——▲ race $a_1A_2A_3A_4$; ■—— ■ buffer control. A: Tomato variety 'Leaf Mould Resister No. 1' (Cf 1); B: Tomato variety 'Vetomold' (Cf 2). R: incompatible combination; S: compatible combination.

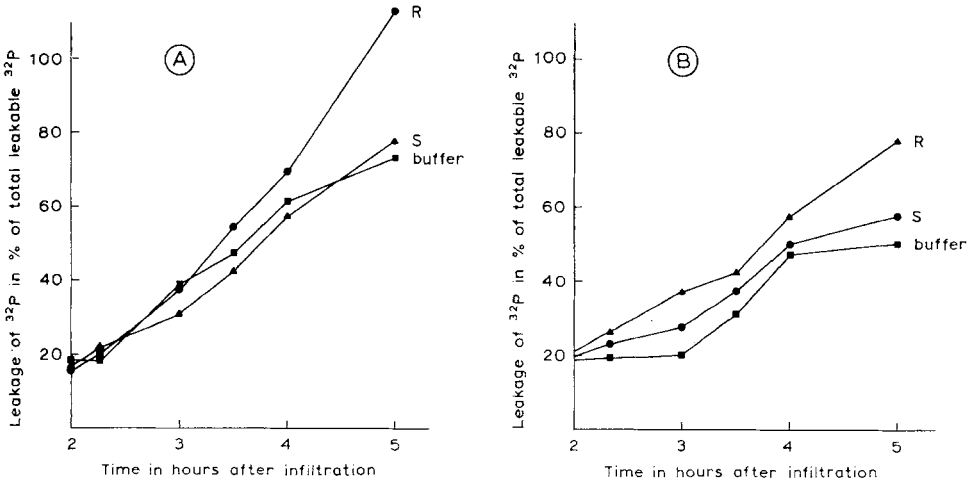

Fig. 2. Lek van ^{32}P uit tomatenbladschijfjes na infiltratie met fractie 1 van het cultuurfiltraat van twee fysiologische rassen van C.fulvum. ●——● *fysio $A_1a_2A_3A_4$;* ▲——▲ *fysio $a_1A_2A_3A_4$;* ■—— ■ *buffercontrole. A: Tomatenras 'Leaf Mould Resister No. 1, (Cf 1); B: Tomatenras 'Vetomold' (Cf 2). R: incompatibele combinatie; S: compatibele combinatie.*

Further experiments with other host varieties and races of the pathogen were in full agreement with the above results.

Tomato variety V 473 carrying the resistance genes Cf 1 as well as Cf 2 was treated with Fraction 1 from the following fungi $A_1A_2A_3A_4$, $a_1A_2A_3A_4$, $a_1a_2A_3A_4$ and $a_1a_2A_3a_4$. The host-parasite relation between these fungi and the plant is given in Table 1. As is shown in Fig.3, leakage in the resistant combinations (R) largely exceeds that in the compatible combination (S) and the control. The former combination gave a leakage of 70–77 %of the total leakable ^{32}P compounds against 43–50% in the latter combination and 45% in case of the control.

The variety Moneymaker which is recessive to all resistance genes was also treated with fraction 1 in a concentration which caused leakage in incompatible combinations. As Fig.4 shows, with none of the *C.fulvum* races $A_1A_2A_3A_4$, $a_1A_2A_3A_4$ or $a_1a_2A_3A_4$ a difference was found with the leakage caused by the buffer.

Hence fraction 1 of the culture filtrate of an incompatible race causes leakage of ^{32}P from the leaf cells after infiltration. In incompatible combinations of host and pathogen leakage is consistently higher than in compatible combinations or after the tissues have been infiltrated with the buffer only. The compounds which bring about this extra leakage in a resistant combination are only present in the fraction 1 obtained by Sephadex G-25 filtration of a culture filtrate (Fig.1) since the fractions 2 and 3 in buffer did not cause any extra leakage of resistant tomato leaf tissue. Some typical experiments have been described. Other experiments with the same combinations yielded closely comparable results.

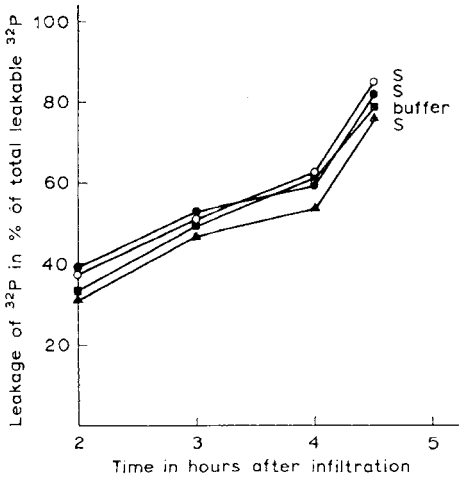

Fig. 3. Leakage of ^{32}P from leaf disks of tomato variety 'V 473' (Cf 1 Cf 2), infiltrated with fraction 1 of culture filtrate of *C.fulvum* races. ○——○ $A_1A_2A_3A_4$; ●——● $a_1A_2A_3A_4$; △——△ $a_1a_2A_3A_4$; ▲——▲ $a_1a_2A_3a_4$; ■——■ buffer control. R: incompatible combination; S: compatible combination.

Fig. 3. Lek van ^{32}P uit bladschijfjes van het tomatenras 'V 473' (Cf 1 Cf 2) na infiltratie met fractie 1 van het cultuurfiltraat van verschillende fysiologische rassen. ○——○ $A_1A_2A_3A_4$; ●——● $a_1A_2A_3A_4$; ▲——▲ $a_1a_2A_3A_4$; ▲——▲ $a_1a_2A_3a_4$; ■——■ *buffercontrole. R: incompatibele combinatie; S: compatibele combinatie.*

Fungitoxicity of tomatine and its aglycon tomatidine and the host-parasite combination C.fulvum-tomato

Leakage of host cells may explain the hypersensitive reaction in the incompatible combination; however, this does not explain why growth of the pathogen is limited. Since tomatine, a glycosidic constituent of the cell content (ca. 0.5% of the dry wt.) is toxic to some fungi, it seemed possible that, by leaking, this compound, or the aglycon tomatidine or other conversion products, might come into contact with the fungus and thus cause growth inhibition.

By our routine roll-culture method the fungitoxic activity of tomatine and of tomatidine for the races $A_1A_2A_3A_4$, $A_1A_2a_3a_4$ and $a_1a_2A_3a_4$ was evaluated. Even a concentration of 1000 ppm did not inhibit growth; also spore germination tests were negative. After 48 h in 100 ppm of tomatine all spores had germinated and there was no difference in growth between the control and the highest concentration of tomatine and tomatidine tested (100 ppm).

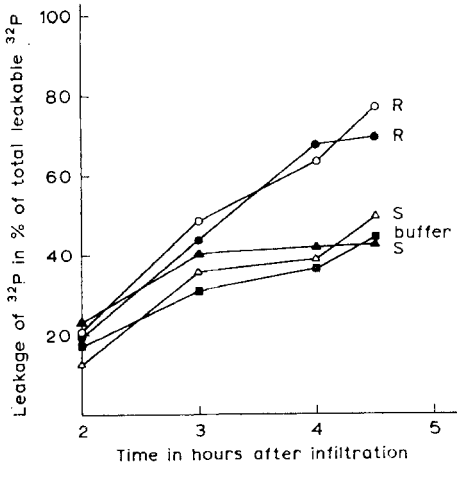

Fig. 4. Leakage of ^{32}P from leaf disks of tomato variety 'Moneymaker'. (cf), infiltrated with fraction 1 of culture filtrate of *C.fulvum* race. ○——○ $A_1A_2A_3A_4$; ●——● $a_1A_2A_3A_4$; ▲——▲ , $a_1a_2A_3A_4$; ■——■ buffer control. R: incompatible combination; S: compatible combination.

Fig. 4. Lek van ^{32}P uit bladschijfjes van het tomatenras 'Moneymaker' (cf) na infiltratie met fractie 1 van het cultuurfiltraat van verschillende fysiologische rassen van C.fulvum. ○——○ $A_1A_2A_3A_4$; ●——● $a_1A_2A_3A_4$; ▲——▲ $a_1a_2A_3A_4$; ■——■ *buffercontrole. R: incompatibele combinatie; S: compatibele combinatie.*

In addition shake cultures were performed with two races of *C.fulvum* in different concentrations of tomatine or tomatidine. Growth was estimated by the mycelium dry weight after 14 days; even with 2000 ppm no inhibition was found.

To investigate whether possibly another fungitoxic product was present in tomato plants or formed after decompartmentalization of the host cells, spore germination of *C.fulvum* was investigated in freshly harvested cell sap. To this end sap from the varieties 'Moneymaker' (cf) and 'Vagabond' (Cf 1 Cf 2 Cf 4) was filter-sterilized immediately after pressing and spores of *C.fulvum* $A_1A_2A_3A_4$ and $A_1A_2a_3a_4$ were added at different time intervals. 'Moneymaker' is susceptible for both races of the fungus and the variety 'Vagabond' is resistant to both. No growth inhibition was found in any of the combinations after 14 days incubation. In contrast, the sap of tomato tissue appeared to be a satisfactory medium for *C.fulvum*.

These experiments show that neither tomatine nor tomatidine or another compound from tomato is directly fungitoxic or fungistatic to any of the physiological races of *C.fulvum*. It therefore is unlikely that tomatine, tomatidine or another fungicide are involved somehow in the resistance mechanism of tomatoes towards *C.fulvum*.

Discussion

Any biochemical explanation of the resistance mechanism must be in accordance with the genetic data.

The results summarized in Table 2 show that there is a strong correlation between the occurrence of the hypersensitivity resistance reaction in experiments in vivo and of extra cell leakage in model experiments involving infiltration of fungal products. In compatible combinations leakage did not exceed the leakage caused by a control buffer solution. These observations strongly support the hypothesis forwarded in the introduction to explain the host-parasite relation in leaf mould of tomato, as far as

Table 2. Combinations tested and results obtained in the leakage experiments. S: compatible; R: incompatible; l: leakage exceeds that of control; n: no leakage in excess of the control; –: not investigated.

Tomato varieties	Genotype for resistance (single set of genes)	Races of *C.fulvum*				
		Indices as given by Kooistra (1964) and Hubbeling (1968)				
		0	1	2	1.2	1.2.4
		Proposed genotype for avirulence:				
		$A_1A_2A_3A_4$	$a_1A_2A_3A_4$	$A_1a_2A_3A_4$	$a_1a_2A_3A_4$	$a_1a_2A_3a_4$
Moneymaker	cf 1 cf 2 cf 3 cf 4	S/n	S/n	–	S/n	–
Leaf Mould Resister No. 1	Cf 1 cf 2 cf 3 cf 4	–	S/n	R/l	–	–
Vetomold	cf 1 Cf 2 cf 3 cf 4	–	R/l	S/n	–	–
V 473	Cf 1 Cf 2 cf 3 cf 4	R/l	R/l	–	S/n	S/n

Tabel 2. Onderzochte combinaties en verkregen resultaten bij de uitlekproef. S: compatibel; R: incompatibel; l: uitlek overtreft die van de controle; n: uitlek niet groter dan de controle; – niet onderzocht.

the genes Cf1, Cf2, A_1 and A_2 and their products are concerned. Results with the other genes will be the subject of a later publication.

Hence the dominant Cf alleles of the genes for resistance may be responsible for the presence of specific receptors in the cell membranes which in combination with specific compounds of the fungus lead in the model experiment to leaking of the cells, and in vivo to a hypersensitive reaction. The production of these specific fungal compounds is supposed to be controlled by specific avirulence genes (A_1, A_2, A_3 and A_4) of the fungus. An incompatible combination results when in host and parasite corresponding Cf alleles and A alleles are present. This is particularly illustrated by the reverse both of host reaction and of degree of leakage of 'Leaf Mould Resister' and 'Vetomold' when treated with race $A_1a_2A_3A_4$ instead of with race $a_1A_2A_3A_4$. This experiment also proves that the compound produced as a result of the A_1 allele differs from the one produced by the A_2 allele.

The indices used in the literature to indicate the various races obviously designate the indices of those avirulence genes which are present in the recessive form. For example, race 1.2 should have the genotype $a_1a_2A_3A_4$ and race 0 the genotype $A_1A_2A_3A_4$.

In the analogous case of the apple pathogen *V. inaequalis* strong indications for the presence of a series of such avirulence genes could be obtained by crossing various races of the pathogen. In the case of *C. fulvum* this is impossible because a sexual stage is lacking. However, Day (1957) observed that by ultraviolet irradiation of an avirulent isolate of *C. fulvum* virulence to a certain tomato variety could be obtained. This mutation to virulence is a strong indication in favour of the existence of avirulence genes.

Occurrence of spontaneous mutations of *C. fulvum* can explain the fact that a tomato variety which was regarded as completely resistant could become subject to attack by a 'new race' of the pathogen (Kooistra, 1964). In this 'new race' obviously a dominant avirulence allele has mutated into a recessive allele unable to produce a compound which causes the hypersensitive reaction in the host.

The Cf 1 and Cf 2 genes in tomato appear to express selective properties of the cell ectoplast to react with the various compounds excreted by fungal races carrying the various A genes. The behaviour of the latter compounds on the Sephadex G-25 column indicates that they possess a molecular weight $\geqslant 1000$; they may be of protein nature.

The concentration of the fraction 1 in the infiltration liquid is rather high (2%, w/v); 20–50% (w/w) of it consist of proteins, the remainder possibly being polysaccharides. Presumably only a minor part of the extra-cellular material will be involved in the leakage process. The lowest concentration of fraction 1 required in the infiltration fluid to show the leakage effect has not yet been assessed.

Plants carrying the Cf 1 allele for resistance react on infection with an incompatible race A_1 by chlorotic areas without a sharp boundary with healthy tissue. Plants carrying the Cf 2 allele for resistance react with a more restricted pinpoint spot on a race with avirulence allele A_2. Chlorosis becomes visible in a later stage. A brown dry spot is seen in the centre of the chlorotic areas. Microscopic observations have revealed that in this case chlorosis is located only immediately around the invading hyphae. Bond (1938) has shown that both in a resistant and in a susceptible plant *C. fulvum* penetrates via the stomata but in the resistant combination mycelium is

not abundant; the hyphae are deformed and show large vacuoles. Similar symptoms are described for other fungi in a starvation stage or on an unsuitable medium (Egawa et al., 1968).

It appears at present difficult to indicate why, after the hypersensitive reaction of the tissue has taken place, the fungus stops growing and deteriorates as described by Bond (1938). Fungicides were not found to be present or formed in homogenates of the plant, unlike in the case of apple and Venturia (Raa, 1968a; Raa, 1968b). However, possibly such compounds arise as a result of the leakage. Also the drying out of the hypersensitive area may be a factor adverse to growth. Further investigations are required to elucidate this point.

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Samenvatting

Een biochemisch mechanisme voor de gen-om-gen resistentie van tomaten tegen Cladosporium fulvum

Modelproeven werden uitgevoerd met de tomatenvariëteiten 'Moneymaker' (geen resistentiegenen), 'Leaf Mould Resister No. 1' (resistentiegen Cf 1), 'Vetomold' (resistentiegen Cf 2), 'V 473' (resistentiegen Cf 1 en Cf 2) en verschillende fysiologische rassen van *Cladosporium fulvum*. Bladponsjes van radioactief gemerkte bladeren (gemerkt met ^{32}P) werden geïnfiltreerd en geïncubeerd met cultuurfiltraatfracties (fractionering over Sephadex G-25) van *C. fulvum*. Waargenomen werd dat ponsjes, behandeld met cultuurfiltraat van een niet compatibele *C. fulvum* een grotere uitlek van radioactief gemerkt materiaal te zien gaven dan in de gevallen waarin een compatibele schimmel werd gebruikt.

Deze waarnemingen stemden overeen met onze hypothese dat de gen-om-gen relatie die bestaat tussen tomaten en *C. fulvum*, gebaseerd is op een interactie van specifieke schimmelprodukten met specifieke receptoren in de plantencellen, mogelijk in de membranen. De produktie van de specifieke stoffen door de schimmel zou worden bepaald door vier avirulentiegenen (A_1,A_2,A_3 en A_4), en de aanwezigheid van de specifieke receptoren in de plantencel door de vier resistentiegenen Cf 1, Cf 2, Cf 3 en Cf 4.

De waarnemingen, verkregen uit de proeven met de tomaten Cf 1, Cf 2 en Cf 1 Cf 2, doen vermoeden dat de uitlek een gevolg is van een overgevoeligheidsreactie die optreedt, indien een fysio van *C. fulvum*, dat een specifiek avirulentie allel bezit, een gastheer binnendringt die beschikt over een bijpassend resistentie allel.

Het is tot nu toe niet duidelijk waarom de groei van *C. fulvum* stopt indien uitlek op gaat treden als gevolg van de overgevoeligheidsreactie. In homogenaten van tomatenbladeren werd geen stof gevonden die de groei van *C. fulvum* remt.

References

Bailey, D. L. & Kerr, E. A., 1964. *Cladosporium fulvum* race 10 and resistance to it in tomato. Can. J. Bot. 42: 1555–1558.

Black, W. B., Mastenbroek, C., Mills, W. R., & Peterson, L. C., 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. Euphytica 2:173–178.

Bond, T. E. T., 1938. Infection experiments with *Cladosporium fulvum* Cooke and related species. Ann. appl. Biol. 25:277–307.

Boone, D. M. & Keitt, G. W., 1957. *Venturia inaequalis* (Cke.) Wint. XII. Genes controlling pathogenicity of wild-type lines. Phytopathology 47:403–409.

Day, P. R., 1954. Physiologic specialization of *Cladosporium fulvum* in Engeland and Wales. Pl. Path. 3:35–39.

Day, P. R., 1957. Mutation to virulence in *Cladosporium fulvum*. Nature, Lond. 179:1141–1142.

Egawa, H., Tsuda, M., Ueyama, A., & Matuo, T., 1968. Formation of abnormal mycelium of *Fusarium roseum* Link on a modified Czapek-D-amino acid medium. Experientia 24:403–404.

Fincham, J. R. S. & Day, P. R., 1963. Fungal genetics. Blackwell Scient. Publ. Oxford, Great Britain. p.239.

Flor, H. H., 1942. Inheritance of pathogenicity in *Melampsora lini*. Phytopathology 32:653–669.

Hubbeling, N., 1966. Identificatie van nieuwe fysio's van *Cladosporium fulvum* en selectie op resistentie daartegen bij de tomaat. Meded. LandbHogesch. Opzoek Stns Gent 21:925–930.

Hubbeling, N., 1968. Attack of hitherto resistant tomato varieties by a new race of *Cladosporium fulvum* and resistance against it. Meded. Rijksfac. Landbouwwet. Gent 23:1011–1017.

Kaars Sijpesteijn, A., 1969. Aspects of natural disease resistance. Meded. Rijksfac. Landbouwwet. Gent 24:379–391.

Kooistra, E., 1964. Recent experiences of breeding leaf mould resistant tomatoes. Euphytica 13: 103–109.

Lowry. O. H., Rosebrough, N.J., Farr, A. L. & Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. biol. Chem. 193:265–275.

Persiel, F., 1967. Resistenz und Resistenzzüchtung gegen die Braunfleckenkrankheit, *Cladosporium fulvum* Cooke, bei Tomaten. Z. PflZücht. 57:325–360.

Pluijgers, C. W. & Kaars Sijpesteijn, A. 1966. The antifungal activity of thiosemicarbazides in vitro and their systemic activity against cucumber scab *Cladosporium cucumerinum*. Ann. appl. Biol. 57:465–473.

Raa, J., 1968a. Polyphenols and natural resistance of apple leaves against *Venturia inaequalis*. Neth. J. Pl. Path. 74, Suppl. 1: 37–45.

Raa, J., 1968b. Natural resistance of apple plant to *Venturia inaequalis* – A biochemical study of its mechanism. Thesis Univ. Utrecht, the Netherlands.

Raa, J. & Kaars Sijpesteijn, A., 1968. A biochemical mechanism of natural resistance of apple to *Venturia inaequalis*. Neth. J. Pl. Path. 74:229–231.

Sato, Y., Katz, A. & Mosettig, E., 1951. Degradation of tomatine. J. Am. Chem. Soc. 73:880–881.

Toxopeus, H. J., 1956. Reflections on the origin of new physiologic races in *Phytophthora infestans* and the breeding for resistance in potatoes. Euphytica 5:221–237.

Williams, W., 1964. Genetical principals and plant breeding. Chapter XI. Blackwell Scient. Publ. Oxford, Great Britain.

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