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SHORT COMMUNICATION

THE OCCURRENCE OF ACID HYDROLYZABLE PHENOLICS IN RELATION TO *FUSARIUM* WILT DISEASE IN TOMATO PLANTS[1]

Het voorkomen van in zuur hydrolyseerbare fenolische verbindingen in verband met de Fusarium-*verwelkingsziekte bij tomaten*

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Infection of a susceptible tomato variety led to an accumulation of acid hydrolyzable phenolic acids. This accumulation doesn't support the view in which the phenolic acids are hold to be responsible for the resistance against the wilting disease.

Decrease in lignification and vascular discolouration are known to occur in combination with the *Fusarium* wilt disease in tomato plants. Lignin formation is considered to take place via synthesis of p-hydroxy-phenylpropanoids, such as ferulic acid, sinapic acid and their corresponding alcohols as precursors (BRAUNS & BRAUNS, 1960). The decrease in lignification can be explained according to DAVIS *et al.* (1953), by conversion of phenolic compounds into melanins. β-Glycosidases have been shown to be produced by the host and also by *Fusarium*, both in liquid culture and in the host. When the host cells become disorganised through the action of the pathogen, the phenolic glycosides are hydrolyzed and then the free phenols are thought to be oxidized by the host's phenoloxidase to melanins.

Due to the activity of glycosidases in diseased cells one might expect to find increased amounts of phenolic acids in wilt-diseased plants, as compared with either healthy or resistant reacting plants. MENON & SCHACHINGER (1957), however, came to opposite results, although ROHRINGER *et al.* (1958) did report an accumulation of phenolic acids in diseased susceptible tomato plants.

A variety of simple phenolic acids is known to occur in plants, either in the form of glycosides and esters or as free acids. In the course of this investigation the need arose to identify these various compounds in order to establish more specifically any differences.

The pathogen used was a strain of *Fusarium oxysporum* f. *lycopersici* Sacc., kindly supplied by Dr. F. GROSSMANN. Two varieties of tomato plants were used, 'Bonner Beste' as the susceptible variety and 'Moneymaker' as the resistant one. After growing the seedlings under greenhouse conditions for about three weeks, they were inoculated at their 4th – 5th leave stage by dipping the roots in a homogenized suspension of *F. oxysporum* f. *lycopersici*. After inoculation the plants were kept at 26 °C and a high relative humidity.

Extracts of the stems only were made by grinding them in cold 90% methanol in a "Virtis 23" homogenizer and keeping this suspension overnight at 4 °C.

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The next day the plant material was extracted successively with cold 90% methanol, 80% ethanol, boiling 96% ethanol and acetone, respectively. Chlorophyll pigments could be extracted by partition into carbon-tetrachloride. The solvent-soluble extracts were all added together; they constitute the so-called soluble fraction. The insoluble fraction consists of the plant material left after the successive extractions.

Acid hydrolysis in 2N HCl of the soluble and insoluble fraction was carried out according to IBRAHIM & TOWERS (1960). One major modification was introduced by carrying out the ether extraction continuously for 2 × 24 hours instead of 2 × 6 hours. The phenolic acids were separated by means of two-dimensional paper-chromatography and detected by ultraviolet light (2537 Å), ammonia vapors and by spraying with diazotised-sulfanilic acid/sodium carbonate. By means of this method we could detect six phenolic acids: p-coumaric acid, ferulic acid, sinapic acid, p-hydroxy-benzoic acid, vanillic acid and syringic acid.

As Table 1 shows, the largest amounts were found in the diseased susceptible variety in the soluble fraction, indicating that an accumulation of phenolic acids

TABLE 1. Distribution of acid hydrolyzable phenolics in a wilt-susceptible ('Bonner Beste') and a wilt-resistant ('Moneymaker') tomato variety.

Voorkomen van na zuurhydrolyse aantoonbare fenolzuren in een vatbare ('Bonner Beste') en een resistante ('Moneymaker') tomatevariëteit.

Phenolic acids in:	'Bonner Beste'				'Moneymaker'			
	acid hydrolyzed				acid hydrolyzed			
	soluble fraction		insoluble fraction		soluble fraction		insoluble fraction	
	H	D	H	D	H	D	H	D
p-coumaric acid/*p-cumaarzuur*	t	t	–	t	t	t	–	t
ferulic acid/*ferulazuur*	t	++	–	–	t	+	–	+
sinapic acid/*sinapinezuur*	–	–	+	t	–	–	–	–
p-hydroxy-benzoic acid/ *p-hydroxybenzoëzuur*	t	+	–	–	–	+	+	+
vanillic acid/*vanillinezuur*	t	++	+	+	t	+	–	t
syringic acid/*syringinezuur*	–	t?	–	–	–	–	+	–
Fenolzuren in:	*G*	*Z*	*G*	*Z*	*G*	*Z*	*G*	*Z*
	oplosbare fractie		*onoplosbare fractie*		*oplosbare fractie*		*onoplosbare fractie*	
	in zuur hydrolyseerbare				*in zuur hydrolyseerbare*			
	'Bonner Beste'				*'Moneymaker'*			

The intensity of the coloured spots was taken as a rate for the amounts of phenolic acids.
De intensiteit der op het chromatogram gekleurde vlekken werd beschouwd als een maat voor de hoeveelheid fenolzuren.

Relative amounts are indicated as: –, t (trace), +, ++ / *Relatieve hoeveelheden zijn aangeduid als:* –, t *(spoor)*, +, ++
H/G = healthy / *gezond* D/Z = diseased / *ziek*

takes place after infection. In the resistant variety, infection did not considerably affect the amounts of phenolic acids in the soluble and insoluble fractions. A quantitative determination of the various compounds will be needed before final conclusions can be drawn.Contrary to MENON & SCHACHINGER (1957) no evidence was obtained for a clear-cut correlation between resistance against tomato wilt disease and the amount of phenolic acids, present as acid-hydrolyzable phenolics.

The results are in agreement with DIMOND's statement in which he holds, among others, vascular discolouration as a result of oxydative polymerization of free phenolics, responsible for the wilting of *Fusarium* infected tomato plants.

SAMENVATTING

Infectie van een voor *Fusarium* vatbare tomatevariëteit leidde tot een vermeerdering van in zuur hydrolyseerbare fenolzuren. Deze vermeerdering ondersteunt de mening niet, dat juist de fenolzuren verantwoordelijk zijn voor de resistentie tegen de verwelkingsziekte.

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