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The resistance to powdery mildew (*Oidium lycopersicum*) in *Lycopersicon* species is mainly associated with hypersensitive response

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Abstract

The cultivated tomato (*Lycopersicon esculentum*) is susceptible to powdery mildew (*Oidium lycopersicum*). Six accessions of three related *Lycopersicon* species show high levels of resistance (Lindhout et al., 1994b). The present research aimed at describing the development of *O. lycopersicum* on susceptible cv Moneymaker and characterizing the defence response to *O. lycopersicum* in *Lycopersicon* accessions by histological analysis. Spore germination and (primary) haustorium formation in resistant accessions were as frequent as in the susceptible *L. esculentum* cv Moneymaker. A high frequency of necrosis of epidermal cells in which a haustorium was formed appeared to be the major defence response, indicating that resistance to *O. lycopersicum* in the *Lycopersicon* genus was predominantly based on the hypersensitive reaction. However, the resistance in *L. parviflorum* was less associated with hypersensitivity than in other resistant accessions, suggesting the existence of a different but still unknown resistance mechanism. In addition, evidence is provided that the level of resistance could depend on the genetic background and the plant age.

Introduction

Since 1986 the occurrence of powdery mildew caused by *Oidium lycopersicum* Cooke & Massee (Noordeloos & Loerakker, 1989) has been frequently reported in greenhouse tomato crops in Western Europe (Lindhout et al., 1994b). The disease has also spread rapidly to Eastern Europe: in 1989 in Bulgaria (Neshev, 1993) and soon afterwards in Poland (Kozik, 1993). Screening of a large collection of cultivated and wild *Lycopersicon* accessions has shown that the cultivated tomato was susceptible to *O. lycopersicum* (Lindhout et al., 1994b; Kozik, 1993; Teubner et al., 1993; Neshev, 1993 and Burgerjon et al., pers. comm. [1990]). High levels of resistance were found in *L. hirsutum* (PI247087) (Laterrot and Moretti, 1993), (G1.1257, G1.1290, G1.1560 and G1.1606=CPRO742208), in *L. parviflorum* (G1.1601=CPRO731089) and in *L. peruvianum* (LA2172) (Lindhout et al., 1994b). *L. hirsutum* LA1775 and *L. pennellii* LA716 were completely and moderately resistant, respectively (Kozik, 1993). Moreover, after natural infection, immunity was observed in three accessions of *L. hirsutum* (var. *glabratum*, f. *typicum* and one unknown accession), and in one accession each of *L. peruvianum* (var. *glandulosum*), *L. chmielewskii* and *L. minutum* (Neshev, 1993).

The resistance of *L. hirsutum* Gl.1560 to *O. lycopersicum* is controlled by an incompletely dominant gene, *Ol-1*, on chromosome 6 near the RFLP markers TG25 and GP79 (Van der Beek et al., 1994). The resistance in *L. parviflorum* G1.1601 may be controlled by a recessive gene, provisionally designated *ol-2* (Lindhout et al., 1994a), but more recent research suggested a polygenic inheritance of the resistance (unpublished). The inheritance of resistance in other accessions such as in *L. hirsutum* Gl.1290 and *L. peruvianum* LA2172 is under investigation.

Oligogenic and incomplete resistance to *O. lycopersicum* derived from an interspecific hybrid of tomato x *L. hirsutum* PI247087 has been integrated into different varieties (Laterrot and Moretti, 1995). In addition,

a commercial hybrid (DRW 4061) with resistance to *O. lycopersicum* has recently been released (Nunnink, 1996).

Various resistance mechanisms to powdery mildews (*Erysiphaceae*) and rusts have been reported, and can be roughly classified as pre- and post-haustorial. Post-haustorial resistance is usually associated with plant cell necrosis (hypersensitive reaction, HR). This is the mechanism typical of the major genic race-specific resistance, e.g. in cereals to powdery mildews (Koga et al., 1990; Tosa and Shishiyama, 1984a) and to rust fungi (Heath, 1981 & 1982; Niks and Dekens, 1991). With prehaustorial resistance formation of haustoria is prevented or reduced by papillae and is not associated with plant cell necrosis. This type of mechanism has been reported in quantitative race-non-specific types of resistance (Heath, 1981 & 1982; Carver and Carr, 1977), and also in plants inoculated with non-pathogenic ('inappropriate') powdery mildew fungi (e.g. Carver and Carr, 1977; Tosa and Shishiyama, 1984b). Though most studies focus on one type of resistance, in nature several resistance mechanisms may exist in the same plant-pathosystem (e.g. Tosa and Shishiyama, 1984b; Koga et al., 1990). Research on the histology of the interactions of powdery mildews with their dicotyledonous host plants is scarce. Neger showed in 1923 that resistance to *Erysiphe cichoracearum* was brought about by enclosure of the haustoria of the fungus in a gummy substance which prevented further fungal development (cited by Lupton, 1956). Hypersensitivity seemed to be the prevailing mechanism of resistance in clover varieties resistant to *E. polygoni* (Smith, 1938). However, resistance in pea to *E. pisi* (*Pisum sativum*) is of the prehaustorial type, similar to that in some non-host and partial resistance interactions (Stumpf and Gay, 1989), while resistance in apple to *Podosphaera leucotricha*, another member of *Erysiphaceae*, appeared to be based on inhibition of spore germination, probably by leaf cuticles (Korban and Riemer, 1990).

Macroscopically, resistance to *O. lycopersicum* in wild tomato species is characterized by a very low infection frequency and a strongly restricted mycelium growth and lack of sporulation (Lindhout et al., 1994b). However, the infection process of the fungus and the nature of the defence reaction in the host plants are still unknown. The present paper describes the development of *O. lycopersicum* on susceptible cv Moneymaker and characterizes the histological reactions of three wild tomato species and several resistant advanced breeding lines (ABLs) with *O. lycopersicum*. We report that the resistance in tomato to *O. lycopersicum* is predominantly associated with the hypersensitive response.

Materials and methods

Plant and fungal materials

Five highly resistant accessions *L. peruvianum* LA2172, *L. hirsutum* G1.1257, G1.1290, G1.1560 and G1.1606 as well as one completely resistant accession *L. parviflorum* G1.1601 (Lindhout et al., 1994b) were obtained from the Centre of Genetic Resources, Wageningen, The Netherlands. Seven indeterminate advanced breeding lines (ABLs), originating from *L. hirsutum* G1.1560 (ABL1560.1, ABL1560.2, ABL1560.3), or *L. hirsutum* G1.1290 (ABL1290.1, ABL1290.2, ABL1290.3, ABL1290.4), were obtained from breeding programmes for resistance to *O. lycopersicum*. Moneymaker, as susceptible control, was maintained at the authors' Department.

The stock of *O. lycopersicum* originated from infected commercial tomato plants (Lindhout *et al*., 1994a), and was maintained on cv Moneymaker plants in a growth chamber at $20 \pm 2°$ C with $70 \pm 5\%$ RH and a 16 h photoperiod.

Disease tests

Disease tests consisted of three experiments. In Experiment 1, six resistant accessions of wild species were investigated in October 1995. In Experiment 2, the seven ABLs were investigated in November 1995. In Experiment 3, six wild accessions and some ABLs were tested again in July 1996. The experiments were set up according to complete randomized block designs with four (in Experiment 2) or six blocks (in Experiments 1 & 3). Each block contained one plant of every genotype as an experimental unit. Each experiment was divided into two sets, each consisting of two blocks in Experiment 2 and three in Experiments 1 and 3 resp.. Two inoculation methods were applied. Tomato seedlings at the four true leaf stage or cutting-derived plants with 4 to 5 leaves (designated 'older plants') in one set, were inoculated by print-inoculation of three leaflets each of two fully expanded leaves per plant. For this *printing* method a direct contact of the sporulating leaves with the healthy ones was established by gently pressing these leaves together. Plants in another set were inoculated by using a second inoculation method, i.e. *spraying* with a spore

suspension (3×10^4 or 12×10^4 conidia.ml^{-1}). The inoculum was prepared by washing conidial spores from freshly sporulating leaves of heavily infected Moneymaker plants in tap water and used immediately. The inoculated plants were grown at $20 \pm 3°$ C with $70 \pm 10\%$ RH under natural light supplemented with artificial light to provide a photoperiod of 16 h per day.

Sampling and staining

For description of the infection process on susceptible tomato plants, leaf segments of each Moneymaker plant were harvested from the print-inoculated leaves at 17, 24, 41, 65, 89, 137 and 185 hours post inoculation (HPI). For investigation of the mechanisms of resistance, leaf segments of each plant were sampled at 41, 65, 96 and 137 HPI. The sampled leaf segments, 1×3 cm in size, were fixed in acetic acid-ethanol (1:3, v:v), and stained with 0.03% trypan blue in lactophenol-ethanol (1:2, v:v) as described by Hering and Nicholson (1964).

Micro- and macroscopic evaluations

Conidia were considered to have germinated when they had formed a germ tube with a primary appressorium or a germ tube of at least half the length of the spore. The percentage of germination was microscopically assessed on a sample of 100 conidia per leaf segment. A germinated spore which produced a primary appressorium or a primary haustorium, was considered as an infection-unit (IU). Thirty infection-units per leaf segment were observed. The presence of a primary haustorium (which was formed by the primary appressorium), the number of hyphae (which were at least as long as the spore), secondary appressoria (formed on hyphae) and secondary haustoria (developed from secondary appressoria) per IU were recorded as well as the number of secondary appressoria per hypha. The number of hyphae per IU, and of secondary appressoria per IU and per hypha were considered as growth components of *O. lycopersicum*. Also, the presence of cell necrosis and papilla formation was recorded as components of the resistance mechanism. Sporulation on the print-inoculated plants (leaf segments) was also recorded microscopically, according to scales '-' to '++++'. Here '-' meant no sporulation, and '±' to '++++' indicated the severity of sporulation from very faint to abundant.

Sporulation on the spray-inoculated plants was evaluated macroscopically at 1, 2 and 3 weeks post inoculation (WPI), according to the same scales as for microscopic evaluations.

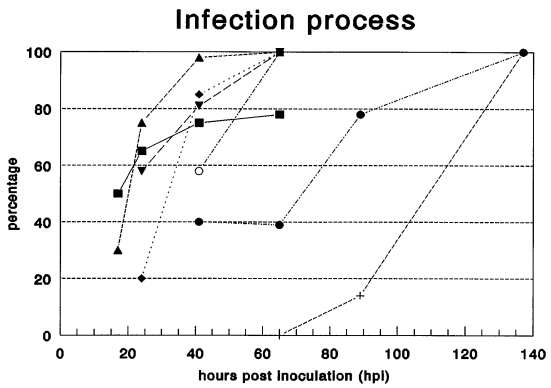

Figure 1. Infection process of *Oidium lycopersicum* on susceptible *L. esculentum* cv Moneymaker ■ percentage germinated spores (17–65 HPI) ▲ percentage germinated spores with primary haustoria (17–65 HPI) ▼ percentage germinated spores with primary hyphae (24–65 HPI) ♦ percentage infection-units with secondary hyphae (24–65 HPI) ○ percentage infection-units with secondary appressoria (41–65 HPI) ● percentage infection-units with secondary haustoria (41–137 HPI) + percentage infection-units with conidiophores (89–137 HPI).

Data analysis

The numeric data were statistically analyzed by using the computer software *SPSS*5.0. Duncan's multiple range test (DMRT) was used to compare the means for all possible pairs of genotypes.

Results

Inoculation method

The spray-inoculation with spore suspensions containing either 3×10^4 or 12×10^4 conidia.ml^{-1}, resulted in an even distribution of only a few spores per square centimetre of leaf segment. However, the density of spores was far too low for a reliable microscopic evaluation of the infection process. The print-inoculation resulted in a sufficiently high spore density but a very heterogeneous spore distribution. Even though many spores were clustered and could not be scored, this inoculation method allowed thousands of spores to be scored on each leaf segment. Mock-inoculation by *printing* with healthy leaves did not cause visible damage. Hence, the *printing* method was applied in further experiments.

Infection process of O. lycopersicum *on susceptible cv. Moneymaker*

Typically, a spore produced a short germ tube, ending in a primary appressorium, from which a primary haustorium was formed. From the primary appressorium or from another pole of the spore, a first hyphae (primary hyphae) arose, that formed small opposite or spread, lobed-shaped (secondary) appressoria from which secondary haustoria arose. Later, the primary hyphae branched to secondary hyphae. We refer to all haustoria and hypha of higher order than primary as secondary. The progress of the infection process of *O. lycopersicum* on the susceptible cv. Moneymaker is presented in Figure 1. Spore germination and primary appressorium formation started before 17 HPI, but the percentage of germinated spores continued to increase to about 80% at 65 HPI. Per time point there was (great) variation in the percentage of germinated spores between blocks. For example, it varied between blocks from 20% to 75% at 41 HPI in Experiment 1 and from 26% to 81% at 65 HPI in Experiment 3. Within 41 HPI, nearly all primary appressoria had formed a primary haustorium. Primary and secondary hyphae were observed from 24 HPI. On these hyphae appressoria and haustoria were formed first between 24 and 41 HPI. At 65 HPI, branching of secondary hyphae were observed. At 89 HPI, each infection-unit on average had produced 9.8 hyphae which frequently interlaced each other. At that time also the first conidiophores were observed. At 137 HPI, mycelia interlaced extensively, and fully developed conidiophores were observed as erect mycelial structures. At 185 HPI, conidiophores matured and the top cell (the new generation of spores) became swollen like a normal spore and thus might be ready to be released. One vegetative generation cycle of the pathogen was then completed. No further sampling of leaves was carried out.

The compatible infection process elicited plant cell reactions to only a limited extent. At 41 and 65 HPI, 2% to 12% of the (primary or secondary) haustorium-invaded cells became necrotic. Up to 137 HPI, one percent of the appressoria (including primary appressoria) had induced papilla formation. However, even if papillae had been induced, haustorium formation succeeded in 50% of the cells with a papilla.

Infection process of O. lycopersicum *in resistant accessions*

Based on the development of *O. lycopersicum* on Moneymaker, further microscopic observations were focused on the samples harvested at 41 or 65 HPI.

Germination

The percentage of spore germination in some accessions varied considerably between blocks. For example, it varied from 10% to 80% at 41 HPI in *L. peruvianum* LA2172, and from 16% to 81% at 65 HPI in *L. hirsutum* G1.1560. However, the percentage of germination was not significantly different between resistant accessions and Moneymaker, and among resistant accessions (Tables 1 & 2). Thus, spore germination was not affected on resistant accessions, indicating that resistance became effective only after spore germination.

Primary haustorium formation

At 41 and 65 HPI, at least 70% of the infection-units had formed a primary haustorium. There was no significant difference in the frequency of primary haustorium formation between resistant accessions and Moneymaker (Tables 1 & 2), indicating that resistance to fungal infection did not take place before primary haustorium formation.

Hypha and appressorium formation

Compared to susceptible cv Moneymaker, significant reductions in the fungal growth components (i.e. number of hyphae per IU, and of secondary appressoria per IU and per hypha) were observed in *L. peruvianum* LA2172. The reductions in *L. hirsutum* G1.1560 were also great but not always significant (Tables 1 & 2). This indicated a strong reduction in the growth and development of the fungus after primary haustorium formation in these two resistant accessions. No significant difference in appressorium formation between the other four accessions and Moneymaker was observed. This illustrated that resistance in these accessions did not significantly influence the early development of the fungus. Furthermore, similar hypha production on the resistant accessions and Moneymaker revealed that the resistance acted at a later stage of fungal development.

Cell necrosis

In all resistant accessions, many epidermal cells in which a primary haustorium was formed became

Table 1. Development of *O. lycopersicum* on wild *Lycopersicon* accessions, expressed as percentage of germination, formation of primary and secondary haustorium, induction of necrosis by primary and secondary haustorium, number of hyphae per infection-unit (IU) and number of appressorium per IU and per hypha (at 65 HPI unless otherwise indicated)[1]

Accession	Germination (%)	Prim. haust. (%)	Sec. haust. (%)		Necrosis 1st haust. (%)		Necrosis 2nd haust (%)	# Hyphae per IU	# Appres. per hypha	# Appres. per IU	Sporulation[2]	
			41 HPI	65 HPI	41 HPI	65 HPI					Print	Spray
L. esculentum cv. MM	15*a*[3]	94*a*	45*b*	82*b*	12*a*	12*a*	3*a*	3.2*c*	1.7*b*	5.5*b*	+++++	++++
L. peruvianum LA2172	23*ab*	83*a*	20*ab*	39*a*	45*bc*	62*d*	32*b*	2.1*ab*	0.9*a*	1.9*a*	+	±
L. parviflorum G1.1601	43*b*	90*a*	18*ab*	66*ab*	24*ab*	27*ab*	4*a*	2.7*bc*	1.3*ab*	3.5*ab*	+++	+
L. hirsutum G1.1257	17*a*	91*a*	22*ab*	58*ab*	31(28)[4]*ab*	53(29)*cd*	20(37)*ab*	2.5*abc*	1.4*ab*	3.2*ab*	±	−
L. hirsutum G1.1290	25*ab*	96*a*	35*b*	64*ab*	31(29)*ab*	50(42)*bcd*	30(40)*b*	2.6*bc*	1.3*ab*	3.4*ab*	±	−
L. hirsutum G1.1560	13*a*	92*a*	9*a*	37*a*	65*c*	66*d*	32*b*	1.9*a*	0.8*a*	1.5*a*	+	−
L. hirsutum G1.1606	17*ab*	97*a*	42*b*	62*ab*	24*ab*	33*abc*	19*ab*	2.5*abc*	1.1*ab*	2.9*a*	++	+

[1] Means over three blocks, *Experiment 1*.
[2] Sporulation was evaluated microscopically for the print-inoculated plants (leaf segments) and macroscopically (2 WPI) for the spray-inoculated ones. −: no sporulation, ± to ++++ indicated the severity of sporulation from very faint to abundant.
[3] Means followed by a different letter in each column are significantly different at the 5% level, determined by *DMRT* after *arcsine* (for percentages, if necessary) or *square root* (for whole numbers) *transformation*.
[4] The figures in parenthesis indicate percentages of spreading necrosis over single cell necrosis.

necrotic, indicating a hypersensitive response (HR). Compared to Moneymaker, the percentage of haustoria which induced (single cell) necrosis was significantly higher in *L. peruvianum* LA2172 and in *L. hirsutum* G1.1560 at 41 HPI, and in most resistant accessions except for *L. parviflorum* G1.1601 and *L. hirsutum* G1.1606 at 65 HPI (Table 1). In another experiment (Table 2), HR was observed much more frequently in all resistant accessions than in Moneymaker, though the level of hypersensitivity differed among resistant accessions. In both experiments, the frequency of HR was much lower in *L. parviflorum* G1.1601 and in *L. hirsutum* G1.1606 than in the other four resistant accessions at any stage after haustorium formation. In G1.1257 and G1.1290, necrosis was also observed in the cells adjacent to the haustorium-invaded cells (spreading necrosis). However, this variation of spreading necrosis between resistant accessions was not repeatable (Tables 1 & 2).

Secondary haustorium formation

The frequency of secondary haustorium formation in *L. peruvianum* LA2172 and in *L. hirsutum* G1.1560 at 65 HPI in one experiment, was significantly lower than that in Moneymaker (Table 1). But no significant difference in secondary haustorium formation between Moneymaker and any resistant accession was observed in another experiment (Table 2), indicating that the fungal growth and development was significantly retarded by resistance mainly after secondary haustorium formation.

Necrosis was also induced by secondary haustoria. As with primary haustoria, the frequency of cells which became necrotic was much higher in resistant accessions than in Moneymaker. The percentage of secondary haustoria which induced necrosis was, again, much lower in G1.1601 and G1.1606 than in the other four accessions (Tables 1 & 2).

Papilla formation

Papillae beneath some appressoria were observed at very low frequencies in all accessions including the susceptible Moneymaker. On average, only 0–9% of the appressoria induced papillae. Haustoria were present in at least 50% of the cells where a papilla was induced. Therefore, papilla formation seemed not to be an effective nor a common mechanism of resistance to *O. lycopersicum*.

Sporulation

The development of infection-units was not always stopped when epidermal cells, in which primary haustoria were formed, had become necrotic. One or more new hyphae were usually formed on the other side of the germinating spore, when the growth of the primary hypha was blocked in association with necrosis. The secondary hyphae produced new appressoria and subsequently new haustoria. Eventually, all haustoria could be associated with cell necrosis, and the infection-units may have been arrested completely. Therefore, sporulation on print-inoculated plants was considerably poorer in the resistant accessions than in

Table 2. Development of *O. lycopersicum* on wild *Lycopersicon* accessions and in advanced breeding lines, expressed as percentage of germination, formation of primary and secondary haustorium, induction of necrosis by primary and secondary haustorium, number of hyphae per infection-unit (IU) and number of appressorium per IU and per hypha at 65 HPI[1]

Accession	Germination (%)	Prim haust (%)	Sec haust (%)	Necrosis 1st haust (%)	Necrosis 2nd haust (%)[3]	# Hyphae per IU	# Appres per hypha	# Appres per IU	Sporulation[2]
L. esculentum cv. MM	61*c*	99*a*	39*a*	1*a*	4	4.0*d*	0.7*a*	2.90*b*	++++[4]
L. peruvianum LA2172	44*abc*	91*a*	15*a*	39(8)*cde*	88	2.0*a*	0.7*a*	1.40*a*	±
L. parviflorum G1.1601	40*abc*	76*a*	4*a*	32(21)*bcd*	33	3.0*bcd*	0.7*a*	2.07*ab*	–
L. hirsutum G1.1257	64*c*	97*a*	18*a*	58(17)*e*	72	3.3*bcd*	0.7*a*	2.50*ab*	–
L. hirsutum G1.1290	52*abc*	93*a*	18*a*	39(7)*cde*	59	3.7*cd*	0.6*a*	2.13*ab*	–
L. hirsutum G1.1560	51*abc*	99*a*	18*a*	47(14)*de*	58	3.2*bcd*	0.8*a*	2.57*ab*	–
L. hirsutum G1.1606	51*abc*	94*a*	17*a*	21(9)*bc*	27	3.8*d*	0.7*a*	2.87*b*	–
ABL1560.1 (s)	54*abc*	91*a*	12*a*	13*b*	36	3.2*bcd*	0.7*a*	2.10*ab*	+
ABL1560.1 (o)[5]	23*a*	97*a*	14*a*	55(22)*de*	84(12)	2.4*abc*	0.7*a*	1.73*ab*	±
ABL1560.2 (s)	47*abc*	92*a*	8*a*	19(2)*bc*	50	3.4*bcd*	0.7*a*	2.47*ab*	+
ABL1560.2 (o)	25*ab*	98*a*	13*a*	27(12)*bcd*	64	3.2*bcd*	0.7*a*	2.27*ab*	±
ABL1560.2 (o)	39*abc*	88*a*	22*a*	36(12)*bcde*	54	2.8*abc*	0.7*a*	1.77*ab*	–
ABL1560.3 (o)	39*abc*	99*a*	4*a*	47(17)*de*	67	1.9*a*	0.8*a*	1.53*a*	±
ABL1290.4 (o)	45*abc*	88*a*	35*a*	48(28)*de*	91	2.4*abc*	0.7*a*	1.70*ab*	+

[1] Means over 3 blocks, *Experiment 3*; other notes are identical to those in Table 1.
[2] Sporulation was evaluated macroscopically at 2 WPI by spraying.
[3] The data were not statistically analyzed, because secondary haustoria were often not present, causing many missing values.
[4] No difference in sporulation was observed between seedlings and older plants of Moneymaker.
[5] Older plants (o) derived by cutting from the corresponding ABL seedlings (s).

susceptible cv Moneymaker. Only slight sporulation was sometimes observed, micro- and macroscopically, in *L. parviflorum* G1.1601 and in *L. hirsutum* G1.1606. Sporulation on spray-inoculated plants was almost absent in all resistant accessions (Tables 1 & 2).

Infection process of O. lycopersicum *in ABLs*

During disease tests, the inoculated leaves of the resistant accessions died 1–2 weeks after inoculation, probably due to lack of adaptation of these wild species to greenhouse conditions. In order to minimize the effect of leaf senescence on the accuracy of disease evaluation, and to study the resistance response in an *L. esculentum* genetic background, ABLs derived from *L. hirsutum* G1.1560 and 1290 were evaluated in two experiments. The results of Experiments 2 & 3 were similar and only those of Experiment 3 are presented (Table 2). In this experiment the levels of resistance were also evaluated in seedlings and in older plants of some ABLs to study the effect of plant age.

Compared to Moneymaker, no significant reduction was observed in spore germination on the ABL seedlings and on older plants. As in the wild accessions, appressorium formation in ABLs was almost as good as in Moneymaker, irrespective of plant age except for the older plant of ABL1560.3. Primary and secondary haustorium formation during the first 65 hours post inoculation was not reduced in ABLs, regardless of plant age. Less hypha formation was only observed in most of the older plants (Table 2). These observations indicated that the resistance in ABLs also did not significantly affect the early fungal development.

Necrosis of epidermal cells in which primary or secondary haustoria had been formed, was also commonly observed in ABLs. So was spreading necrosis except in ABL1560.1 seedlings (Table 2). Papilla formation was as low as in wild accessions, and again not an important component of resistance. Undoubtedly, the resistance to *O. lycopersicum*, introgressed into cultivated tomato, was also mainly associated with HR. Eventually, almost no sporulation was visible on the ABLs.

The levels of resistance in older plants and seedlings were also compared (Table 2). The percentage of germination tended to be lower in the older plants than in the seedlings. The frequency of primary (and secondary) haustoria inducing necrosis was much higher in the older plants than in seedlings. Also,

the print-inoculated leaves of all older plants (and ABL1560.2 seedlings) became seriously necrotic and died even one week after inoculation, demonstrating a possible effect of plant age on the level of resistance. Moreover, the frequency of cell necrosis was significantly higher in *L. hirsutum* G1.1560 than in its deriving ABL at the seedling stage. This demonstrated that the levels of resistance might also depend on genetic background.

Discussion

The resistance to *O. lycopersicum* in tomato is clearly not based on the inhibition of spore germination. This is in accordance with reports that the rate of spore germination of *Erysiphe polygoni* (Cirulli, 1976) and *E. pisi* (Singh and Singh, 1983) on resistant pea cultivars was the same as on susceptible ones, but in contrast to the observation that the germination of *E. pisi* conidia was inhibited on resistant pea plants (Reeser et al., 1983). As in pea to *E. pisi* (Singh and Singh, 1983) and in barley to *E. graminis* f.sp. *hordei* (Andersen and Torp, 1986), the resistance to *O. lycopersicum* also does not rely on the inhibition of appressorium formation. Also, papilla formation was rare and ineffective and hence not an important defence mechanism to *O. lycopersicum* infection in tomato. This is in contrast to the occurrence and effectiveness of papilla formation in barley to *E. graminis* f.sp. *hordei* (Koga et al., 1990; Clark et al., 1995) and to an inappropriate *forma specialis E. graminis* f.sp. *tritici* (Tosa and Shishiyama, 1984b), in oat (*Avena* sp.) to *E. graminis* f.sp. *avenae* (Carver and Carr, 1977) and in pea to *E. pisi* (Stumpf and Gay, 1989). Apparently, the importance of papilla formation to the resistance mechanism is pathosystem dependant.

As in many other pathosystems (e.g. Greenberg, 1997; Moerschbacher and Reisener, 1997), the resistance to *O. lycopersicum* in the wild resistant accessions and in the advanced breeding lines is posthaustorial. This resistance is clearly associated with a hypersensitive response. This HR is often not confined to the cells in which the haustorium is formed, but may also spread to the adjacent cells. This spreading necrosis was also observed in the resistance of clover to *E. polygoni* (Smith, 1938), barley to powdery mildews (e.g. Toyoda et al., 1978; Koga et al., 1990; Aist and Bushnell, 1991) and several crop species to rust (Heath, 1981).

Posthaustorial resistance associated with hypersensitivity usually indicates a race specific major genic resistance (Heath 1981 & 1982). The posthaustorial resistance to *O. lycopersicum* in tomato might be race-specific, like in many other pathosystems. The resistance originating from *L. hirsutum* G1.1560 has been proven to be monogenic (Van der Beek et al., 1994), and may also be race specific and based on a gene-for-gene interaction. However, this remains to be demonstrated. Till now, there is no evidence that *O. lycopersicum* consists of races that differ in their ability to infect tomato lines with the various *Ol* genes.

There is evidence that the level of resistance to *O. lycopersicum* is affected by the genetic background and by plant development stage. For instance, the frequency of necrosis of epidermal cells, in which the haustorium was formed, tended to be lower in *L. esculentum* background (ABLs) than in their wild species, and in seedlings than in older plants. This is in agreement with the observation that the level of resistance to *E. graminis* f.sp. *avenae*, originating from resistant wild species, was reduced in an oat cultivar (Carver and Carr, 1977). The observation that cell necrosis was less frequent in ABL seedlings compared to older plants may indicate an influence of plant development stage on the level of resistance to *O. lycopersicum*. This is in agreement with the observation that resistance to *E. graminis* f.sp. *avenae* in oat was expressed more strongly in the fifth than in the first formed leaves (Carver and Carr, 1977). In addition, increase in resistance with age has also been reported in soybean against *Phytophthora megasperma* var. *sojae* (Paxton and Chamberlain, 1969; Ward et al., 1981), *Ph. megasperma* f. sp. *glycinea* (Bhattacharyya and Ward, 1986) and soybean rust *Phakopsora pachyrhizi* (Melching et al., 1988), and in North American cultivars of cowpea (*Vigna unguiculata*) to the cowpea rust fungus *Uromyces vignae* (Heath, 1994). Genes for complete resistance in cowpea to cowpea rust (Heath, 1994) and QTLs for partial resistance in barley to leaf rust *Puccinia hordei* (Qi et al., 1998) at different plant development stages have been identified. Whether the different levels of resistance observed between young and older tomato plants in the present study is due to activation of different genes still needs to be verified.

Quantitative differences in the level of resistance observed between the wild *Lycopersicon* accessions may be due to the functioning of different genes. For example, the resistance in *L. parviflorum* G1.1601 on which the rate of HR was low, seems to be different from that in other wild accessions, because microscop-

ically, the growth of the fungus during the first 65 HPI was similar to that on Moneymaker, but macroscopically, the resistance was almost complete (also Lindhout et al., 1994b). This resistance which may be polygenic or quantitatively inherited, is supposed to be more durable. In case the resistance of the various origins are governed by different genes, pyramiding these genes in one tomato cultivar may increase the durability of the resistance in this pathosystem.

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