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Host range of *Oidium lycopersici* occurring in the Netherlands

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Abstract

Nine accessions of three cucurbit species, ten of eight legume species, three of lettuce (*Lactuca sativa*) and 34 of 14 Solanaceae species were inoculated with a Dutch isolate of the tomato powdery mildew fungus (*Oidium lycopersici*) to determine its host range. Macroscopically, no fungal growth was visible on sweet pepper (*Capsicum annuum*), lettuce, petunia (*Petunia* spp.) and most legume species (*Lupinus albus, L. luteus, L. mutabilis, Phaseolus vulgaris, Vicia faba, Vigna radiata, V. unguiculata*). Trace infection was occasionally observed on melon (*Cucumis melo*), cucumber (*Cucumis sativus*), courgette (*Cucurbita pepo*), pea (*Pisum sativum*) and *Solanum dulcamara*. Eggplant (*Solanum melongena*), the cultivated potato (*Solanum tuberosum*) and three wild potato species (*Solanum albicans, S. acaule* and *S. mochiquense*) were more heavily infected in comparison with melon, cucumber, courgette, pea and *S. dulcamara*, but the fungus could not be maintained on these hosts. All seven tobacco (*Nicotiana tabacum*) accessions were as susceptible to *O. lycopersici* as tomato (*Lycopersicon esculentum* cv Moneymaker), suggesting that tobacco is an alternative host. This host range of the tomato powdery mildew differs from that reported in some other countries, which also varied among each other, suggesting that the causal agent of tomato powdery mildew in the Netherlands differ from that in those countries. Histological observations on 36 accessions showed that the defense to *O. lycopersici* was associated with a posthaustorial hypersensitive response.

Introduction

Many species of powdery mildew can grow on more than 100 host plant species. For example, *Erysiphe cichoracearum* has a host range of at least 1753 plant species (Amano, 1986). Such a host range of a powdery mildew species does not necessarily imply that the host range of individual isolates is equally wide. A forma specialis or an isolate of a powdery mildew fungus may be confined to one plant species, such as the isolates of *E. cichoracearum* on tomato (Abiko, 1983) and tobacco (Reddy et al., 1979), and *Sphaerotheca fuliginea* on eggplant (Abiko, 1978; 1982). However, there are examples of wide host ranges of other formae speciales or isolates. For instance, an isolate of an *Erysiphe* sp. from eggplant could also infect tomato, tobacco and, to some extent, cucumber (Whipps and Helyer, 1994). In addition, cucumber, melon and courgette were as susceptible as tomato to an isolate of *S. fuliginea* f. sp. *lycopersicum* (Angelov et al., 1993).

On tomato, several species of powdery mildew have been reported to occur. One of these species, *Leveillula taurica*, is characterized by the development of endophytic mycelium (Palti, 1988). Another species, *Erysiphe orontii* (also known as *E. cichoracearum* and *E. polyphaga* (Braun, 1987)), is exclusively ectophytic, and characterized by the formation of conidia in long chains. Since 1986, outbreaks have been reported of another, morphologically distinct, species of tomato powdery mildew, both in greenhouses and fields around the world (Mieslerová and Lebeda, 1999). This powdery mildew fungus is ectophytic and differs morphologically from *Erysiphe orontii* and *Leveillula taurica* on tomato (Noordeloos and Loerakker, 1989).

In some countries, the causal agent has been identified as *O. lycopersicum* (Noordeloos and Loerakker, 1989; Whipps et al., 1998), but was provisionally designated as an *Erysiphe* sp. in many other countries. In the present paper, we will refer to this species as *O. lycopersici* as has been recommended by The International Code of Botanical Nomenclature (Mieslerová and Lebeda, 1999). The formation of conidia singly is a key character in distinguishing *O. lycopersici* from *E. cichoracearum* and *Sphaerotheca* species, although pseudo-chains of three to eight conidia were sometimes observed in humid conditions (Noordeloos and Loerakker, 1989). Since in all reports, due to the lack of cleistothecia, no complete description of the morphology of the tomato powdery mildew is presented, it remains an open question whether the powdery mildews referred to as '*Erysiphe* sp.' (Table 1) may belong to *O. lycopersici*. For example, the tomato powdery mildew in UK, that was reported as *E. orontii* Castagne (Cook et al., 1997), was designated as *O. lycopersicum* by Whipps et al. (1998). In other studies (Lindhout et al., 1994; Huang et al., 1998), the Dutch isolates of the recently occurring tomato powdery mildew, which produce conidiospores singly, were very similar to the *O. lycopersici* as described by Noordeloos and Loerakker (1989). We therefore consider this species as the causal agent of the relatively novel powdery mildew disease on tomato.

The origin of the organism(s) causing the recent outbreaks of tomato powdery mildew in the world is unknown. The pathogen may originate from the center of origin of tomato in South America, and be imported inadvertently to the areas of tomato cultivation. Another possibility is that a pathogen 'jumped' from its host species to tomato by the acquisition of pathogenicity to the latter species, as has been documented for other pathogens like *Monilinia* (Sclerotiniaceae) (Holst-Jensen et al., 1997), pitch canker (*Fusarium subglutinans* f. sp. *pini*) (Storer et al., 1994) and rust (Uredinales) (Savile, 1971; Baum and Savile, 1985). Such a jump to tomato may have occurred in one or different powdery mildew species or forms, so that this novel 'tomato powdery mildew' may be of one or various origins.

All authors agree that all tomato cultivars are susceptible to this newly occurring powdery mildew. Reports differ on the host range of the pathogen (Table 1). In some locations the host range includes Solanaceae species (Fletcher et al., 1988), and in other locations cucumber (Ignatova et al., 1997) (Table 1) and melon (Corbaz, 1993). These differences might be due to plant genotypes, environmental conditions or the definition of susceptibility. But these results might also indicate existence of genetic variation of the pathogen(s) responsible for the recent outbreaks. Host range studies may provide clues to the possible origin(s) of the pathogen(s).

The most extensive studies on host range (and morphology) of the recently occurring tomato powdery mildew were conducted by Whipps et al. (1998). In their study, based on morphology, the causal agent of tomato powdery mildew was designated *Oidium lycopersicum* (Table 1, hereafter we refer to it as British *O. lycopersici* isolate). They mainly focused on the early stages of sporulation (two weeks after inoculation) and the morphology of the causal agent on tomato and some alternative hosts. They considered any accession or species that supported sporulation to any extent as an alternative host of tomato powdery mildew. In the present study, the susceptibility of 25 plant species to a Dutch *O. lycopersici* isolate was evaluated to assess whether there is/are alternative host(s) of this pathogen. We consider those plant species as alternative hosts of *O. lycopersici* only if they allow the fungus to successfully reproduce for more than one generation. In order to better understand the interaction between *O. lycopersici* and plant species outside the genus *Lycopersicon*, we investigated histologically the infection process of the fungus on, and the responses of, these plant species.

Materials and methods

Plant and fungal material

Fifty-six accessions of 25 plant species were used in this study (Table 2). Eggplant (*Solanum melongena*), sweet pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*), melon (*Cucumis melo*) and courgette (*Cucurbita pepo*) plants were raised in greenhouses at $24 \pm 2\,^{\circ}$C, lettuce (*Lactuca sativa*) at $18 \pm 2\,^{\circ}$C, tomato and other *Solanaceae* accessions at $20 \pm 2\,^{\circ}$C, and legumes at $20 \pm 1\,^{\circ}$C in a growth chamber with a 16-h day length. The light intensity was 10–40 $W \cdot m^{-2}$ in the greenhouses depending on the weather, and at least 20 $W \cdot m^{-2}$ in the growth chamber.

Three field isolates of *O. lycopersici* were collected from infected commercial tomato plants at

Table 1. Conidiospore arrangement and host range of the novel tomato powdery mildew

Causal agent[1]	Origin	Conidia borne in chain	Susceptibility					Reference
			Tobacco (*N. tabacum*)	Cucumber (*C. sativus*)	Potato (*S. tuberosum*)	Eggplant (*S. melongena*)	Sweet pepper (*C. annuum*)	
Oidium sp.	USA – California	Yes	+	−	nd	nd	−	Arredondo et al., 1996
Erysiphe sp.	USA – Connecticut	No	+	nd	nd	+	nd	Smith et al., 1997
O. lycopersici	Czech Rep.	Yes/no*	−	+	nd	nd	nd	Mieslerová and Lebeda, 1999
O. lycopersicum	Russia	nd	+	+	+	nd	−	Ignatova et al., 1997
Erysiphe sp.	Canada	nd**	+	nd	nd	nd	nd	Cerkauskas, 1997 (pers. comm.)
Erysiphe sp.	Hungary	Yes	−	−	nd	nd	nd	Kiss, 1996
Erysiphe sp.	UK	Yes/no	+	−	+	+	−	Fletcher et al., 1988
Erysiphe sp	UK	Yes/no	+?	+	nd	+	−?	Whipps and Helyer, 1994
O. lycopersicum	UK	No	(+)	+	−/+	+	−	Whipps et al., 1998
O. lycopersici	NL	No	+	−	±	±	−	Present study

Notes: +: Susceptible, −: resistant; ?: uncertain, because the pathogen originated from eggplant, and was pathogenic on tomato; nd: not determined; (): other *Nicotiana* species; ±: not consistently susceptible in all replications; −/+ 4 out of 44 tested cultivars were susceptible.

*: Sometimes in chains but sometimes singly.

**: Mixture of conidiospores in chains and singly, observed by the present authors.

[1]Designation as used by the respective authors.

Table 2. Degree of susceptibility of different plant species/accessions tested against a Dutch isolate of *Oidium lycopersici*

Immune[1]		Slightly susceptible		Moderately susceptible		Susceptible	
Capsicum annuum (DR)[2]	PI123469	*C. melo* (DR)	PI125956	*S. melongena*	PI286107	*Nicotiana*	904750309
	PI183922		PI136223	(CPRO, De Ruiter &	PI175917	*tabacum*	904750310
Cucumis melo (DR)	PI187331	*Cucumis sativus* (DR)	PI222782	PGRCU)	PI358232	(BGUN)	904750318
Lactuca sativa (CPRO)	PI179895		PI206953	(Bruinsma)	169328		904750304
	CGN14653		PI204692		169329		904750305
	CGN05237	*Cucurbita pepo*	Sardanz		169331		904750160
	CGN04884	(CPRO)	Albina	(Rijk Zwaan)	10-73		944750092
Petunia hybrida (BGUN)	804750083		Marba		10-90	*Lycopersicon*	Moneymaker
	914750153	*Pisum sativum* (LPB)	Finale		10-15	*esculentum*	
P. nyctaginiflora (BGUN)	954750063		Gastro	(Enza Zaden)	Bonica	(LPB)	
	954750067		Paloma	*S. tuberosum* (LPB)[3]	breeding line		
Solanum nigrum (BGUN)	944750095	*Solanum dulcamara*	914750008	*S. albicans* (BGUN,	PI365376		
Lupinus albus (LA)	00TPA0079	(BGUN)	914750046	LT)			
L. luteus (LA)	00TPA0077		924750023	*S.acaule* ssp. *punae*	BGRC7958		
L. mutabilis (LA)	84TPE0649	*S. villosum* ssp.	814750090	(BGUN, LT)			
Phaseolus vulgaris (LA)	00TPA0097	*puniceum* (BGUN)		*S.mochiquense*	BGRC32672		
Vicia faba (LA)	00TPA0096			(BGUN, LT)			
Vigna radiata (LA)	71TPE0042			*S.mammosum*	924750111		
V. unguiculata (LA)	72TPE0532			(BGUN)			

[1]Immune: no infection; Slightly susceptible: infection only occasionally observed; Moderately susceptible: early sporulation similar to that on Moneymaker but nearly disappearing within two to three weeks; Susceptible: sporulation consistently similar to that on Moneymaker, even several weeks after inoculation.
[2]Letters in bracket after each plant species indicate the donor(s) of the accession(s). BGUN – Botanical Garden of the University of Nijmegen, Nijmegen, The Netherlands. CPRO – Centre for Plant Breeding and Reproduction Research, Wageningen, The Netherlands. LA, LPB and LT – Laboratory of Agronomy, Laboratory of Plant Breeding and Laboratory of Taxonomy, Wageningen University, Wageningen, The Netherlands. PGRCU – Plant Genetic Resources Conservation Unit, University of Georgia, 1109 Experiment Street, Griffin, GA 30223-1797, USA. Bruinsma, Enza Zaden, De Ruiter and Rijk Zwaan are Dutch seed companies.
[3]Potato cultivars and breeding lines tested in another project also consistently showed moderate susceptibility.

three locations of The Netherlands (Lindhout et al., 1994). The stocks of these isolates were maintained on tomato cv Moneymaker in separate growthchambers at $20 \pm 1\,^{\circ}C$ with $70 \pm 3\%$ RH and 16-h day length with the same light intensity as described above.

Inoculation tests

Two inoculation tests (IT) were conducted, according to a complete randomized block design with four blocks for IT1 and six blocks for IT2, to evaluate the susceptibility of these accessions. Each block contained one plant per genotype as an experimental unit. *L. esculentum* cv Moneymaker served as susceptible control. In each test, one to three additional plant(s) of each accession were mock-inoculated with tap water, and added randomly to the blocks of inoculated plants. In these experiments, all plants were inoculated at the four true leaf stage. Plants in two blocks of IT1 and in three blocks of IT2 were inoculated by spraying with a conidiospore suspension ($3–4 \times 10^4$ conidia $\cdot$ ml^{-1}). The inoculum was prepared by washing heavily infected tomato leaves in tap water and used immediately. Because of their smooth and waxy leaf surface on which inoculum drops easily fell off, all the legume plants were inoculated by shaking the sporulating tomato leaves above them. To ensure a high density of conidiospores on leaf segments for histological studies, at least three leaves per plant (except for legumes) in other blocks of each test were print-inoculated by gently pressing *Oidium*-infected tomato leaves onto the healthy leaves (Huang et al., 1998). The inoculated plants were grown in a well-isolated greenhouse at $20 \pm 2\,^{\circ}C$ with $70 \pm 10\%$ RH under natural light supplemented with artificial light to 16 h per day. The light density was 10–40 $W \cdot m^{-2}$ depending on the weather.

Sampling and staining

For microscopical study on the infection process of *O. lycopersici*, leaf samples of 1×3 cm^2 were cut at 41 and 65 h after inoculation (hai) from the print-inoculated leaves of all accessions or some representatives of each species, excluding legumes. They were fixed in acetic acid/ethanol (1 : 3, v/v), stained in 0.03% trypan blue in lactophenol/ethanol (1 : 2, v/v), and cleared in a nearly saturated aqueous solution of chloral hydrate (Huang et al., 1998).

Macro- and microscopic observations

To determine the susceptibility of each accession (Table 2), sporulation and plant tissue necrosis were evaluated macroscopically at 7, 10, 14, 21 and 28 days after inoculation (dai). Leaf samples were analyzed using a phase-contrast light microscope. Fungal growth parameters were recorded, including percentage of conidiospore germination, percentage of infection units which formed secondary hyphae, number of hyphae per infection unit, percentage of infection units which produced secondary haustoria and number of secondary haustoria per infection unit (Table 3) as described previously (Huang et al., 1998). Thirty infection units per leaf sample were observed. An infection unit refers to a germinated conidiospore that produced at least one primary appressorium.

Reproduction of Oidium lycopersici

To check conidium production of *O. lycopersici* on different plant species, three infected plants of eggplant and tobacco were separately transferred from the greenhouse to two growth chambers. Infected leaves of eggplant and tobacco plants were used as inoculum sources to inoculate tomato cv Moneymaker plants. Transfers of tomato–tobacco–tomato were cycled over a five-month period. Conidiospore production on tomato, eggplant and tobacco was measured by applying a drop of 15 µl of 0.5% Tween solution to a sporulating leaf area of about 0.2 cm^2. This drop of solution was re-collected, and the conidiospore concentration in this drop was measured by using a haemocytometer. Conidiospore shape and size as well as conidiospore arrangement (i.e., singly or in chains) were also observed microscopically.

Statistical analysis

All data were statistically processed by ONEWAY model using a computer software SSPS5.0. Duncan's Multiple Range Test (DMRT) was applied to compare means.

Results

Macroscopical evaluations of susceptibility

In total, 25 plant species were evaluated in two inoculation tests for their susceptibility to a Dutch

Table 3. Development of *O. lycopersici* on different plant species and accessions, expressed as percentage of germination, induction of leaf cell necrosis by primary haustorium, percentage of IUs forming secondary hyphae, number of hyphae per IU, formation of secondary haustorium and number of haustoria per IU at 65 hai (means over 30 IUs)

Susceptibility class[1]	Plant species and accessions		Germination (%)	Secondary hyphae (%)	No. hyphae per infection unit (IU)	Secodary haustoria (%)	No. haustoria per infection unit	Leaf cell Necrosis (%)[2]
1	*Capsicum annuum*	PI123469	63*bc*[3]	52*efghi*	1.60*defghi*	68*fghijk*	1.8*efghi*	58*hijkl*
		PI183922	47*ab*	3*abc*	0.40*abc*	10*abc*	0.1*abc*	31*efghij*
		PI187331	64*bc*	29*defg*	1.13*de*	36*cdef*	0.6*bcde*	30*efghij*
1	*Cucumis melo*	PI179895	64*bc*	71*hijklm*	2.40*ghijk*	82*ijkl*	3.0*ijk*	30*efghij*
1	*Lactuca sativa*	CGN14653	70*bc*	2*ab*	0.13*a*	6*ab*	0.1*abc*	74*kl*
		CGN05237	57*abc*	1*ab*	0.10*a*	0*a*	0.0*a*	58*ijkl*
		CGN04884	33*a*	0*a*	0.20*ab*	4*ab*	0.1*ab*	64*jkl*
1	*Petunia hybrida*	914750153	87*c*	8*abcd*	0.37*abc*	11*bcd*	0.2*abc*	28*defghij*
1	*P. nyctaginiflora*	954750063	87*c*	23*cdef*	0.70*bcd*	27*cde*	0.4*bcd*	23*cdefgh*
		954750067	88*c*	32*defg*	1.07*def*	37*defg*	0.6*cdef*	21*cdefg*
2	*C. melo*	PI125956	72*bc*	74*hijklm*	2.37*ghij*	88*jklm*	2.7*hij*	10*abcde*
		PI136223	56*ab*	71*hijklm*	2.63*hijkl*	72*ijk*	3.4*ijkl*	18*cdefg*
2	*Cucumis sativus*	PI222782	68*bc*	50*efgh*	1.43*defg*	66*hijk*	2.0*ghi*	40*fghijk*
		PI206953	64*bc*	81*hijklmn*	2.40*ghijk*	91*klm*	3.2*ijkl*	28*defghij*
		PI204692	75*bc*	59*ghijkl*	1.80*efghi*	86*ijkl*	2.5*hij*	7*abcd*
2	*Cucurbita pepo*	Sardanz	55*ab*	52*fghij*	1.77*efghi*	57*efghi*	1.9*ghi*	23*cdefg*
		Albina	56*ab*	35*defg*	1.07*cde*	38*defg*	0.7*cdef*	4*abcd*
		Marba	53*ab*	50*efgh*	1.60*fgh*	61*fghij*	1.4*defgh*	32*efghij*
2	*Solanum dulcamara*	914750008	71*bc*	54*fghijk*	2.23*fghi*	67*ijk*	2.1*ghi*	46*ghijkl*
		914750046	33*a*	34*defg*	1.13*def*	38*cdefgh*	1.3*defg*	22*cdefgh*
		924750023	29*a*	31*defg*	0.90*cde*	32*cdef*	0.6*cdef*	18*bcdef*
2	*S. villosum* ssp. *puniceum*	814750090	63*bc*	15*bcde*	1.05*cdef*	37*cdefgh*	0.5*bcde*	78*l*
3	*S. melongena*	PI286107	88*c*	83*ijklmn*	3.60*jklmn*	89*jklm*	5.4*lm*	24*cdefgh*
		PI175917	89*c*	84*iklmn*	3.60*jklmn*	86*ijkl*	4.8*klm*	17*cdefg*
		PI358232	89*c*	88*lmn*	3.77*klmn*	91*klm*	5.4*lm*	13*bcdef*
3	*S. albicans*	PI365376	88*c*	93*mn*	3.93*lmn*	93*klm*	4.5*jklm*	14*bcdef*
3	*S. mochiquense*	BGRC32672	84*bc*	77*hijklmn*	2.95*ijklm*	82*ijkl*	2.9*hijk*	23*cdefghi*
3	*S. mammosum*	924750111	81*bc*	82*hijklmn*	2.73*hijkl*	81*ijkl*	2.3*ghi*	24*cdefgh*
4	*Nicotiana tabacum*	904750309	81*bc*	96*mn*	3.90*lmn*	92*klm*	3.5*ijkl*	0*a*
		904750310	72*bc*	97*n*	4.27*mn*	94*lm*	4.3*jklm*	4*abc*
		904750318	82*bc*	98*n*	5.20*mn*	97*lm*	5.9*m*	0*a*
4	*Lycopersicon esculentum*	Moneymaker	85*c*	98*n*	4.57*n*	100*m*	5.9*m*	1*a*

[1]Class 1: Immune; class 2: Slightly susceptible; class 3: Moderately susceptible; class 4: Susceptible. (see Table 2).
[2]Except for tobacco and tomato, almost all infected leaf areas of other plant species became necrotic after 14 dai.
[3]Means followed by a different letter (combination) in each column are significantly different at 5% level, determined by *Duncan's multiple range test* after *arcsine* (for percentages) and *square root* (for whole numbers) transformation

O. lycopersici isolate. The choice of plant species was mainly based on the earlier studies listed in Table 1. During these tests, special care such as using over-pressure growth chambers or greenhouse compartments with spore proof ventilation system, was taken to avoid inadvertent cross-contamination. Since the mock-inoculated plants never showed any sign of infection, the observed infections were due to the inoculum applied. In general, there was no variation in susceptibility within species, except for melon (*Cucumis melo*). However, a large variation occurred between species. Based on the susceptibility, compared with Moneymaker, the 25 plant species could be grouped into four classes (Table 2): (1) immune: no infection observed; (2) slightly susceptible: infection only occasionally observed;

(3) moderately susceptible: early sporulation similar to that on Moneymaker but nearly disappearing within two to three weeks, (within this class, sporulation on eggplant was most abundant compared to that on other species); (4) susceptible: sporulation consistently similar to that on Moneymaker, even several weeks after inoculation.

Infection process of Oidium lycopersici

The infection process of *O. lycopersici* on different accessions was investigated from the print-inoculated leaf samples collected at 65 hai, unless indicated otherwise. Because the trend of variation for the fungal growth parameters between the two tests was similar and there were only two replicates in IT1, only data obtained in IT2 is presented.

Germination of conidia

Significant variation in the percentage of conidiospore germination within plant species was only observed in lettuce and *S. dulcamara* (Table 3). Except for one accession each of melon, sweet pepper, lettuce, two of *S. dulcamara* and all the three of courgette, conidiospores germinated equally well on the remaining plant species or accessions as on the susceptible control Moneymaker. Thus, conidiospore germination was not affected on most of the nonhost plants.

Fungal growth

Typically, each conidiospore produced a short germ tube, ending in a primary appressorium. This structure is referred to as an infection unit, from which a primary haustorium was formed. From the primary appressorium or from the other pole of the conidiospore, a first hypha (primary hypha) arose, that formed small opposite, lobe-shaped secondary appressoria from which secondary haustoria arose. Later, the primary hyphae branched to secondary hyphae. All haustoria and hypha of higher order than primary are referred to as secondary (Huang et al., 1998). At 41 hai, 62–100% of infection units had formed a primary haustorium. No significant difference was found in haustorium formation within or between plant species at that time. This indicates that there was no effective prehaustorial resistance. At 65 hai, in general, the highest values of other growth parameters were obtained from tomato (cv Moneymaker), tobacco, eggplant, four wild potato species (*Solanum albicans, S. acaule, S. mochiquense* and *S. mammosum*), melon and cucumber. The development of the fungus on lettuce, sweet pepper and petunia was very poor (Table 3). These observations corresponded well with the macroscopic evaluations (Table 2).

Defense mechanism

Papilla formation induced by primary appressoria was only observed on *Petunia hybrida* 804750083 in IT1, which was not tested in IT2 because of lack of seeds. This indicated that papilla formation might be important in the resistance in this petunia accession. In the other resistant accessions, cell necrosis was the predominant response of the epidermal cells to the fungal infection. The percentage of primary haustoria which induced cell necrosis (hypersensitive reaction, HR), varied considerably between accessions (Table 3). Except for tobacco, and one accession each of cucumber and courgette, the percentage of cell necrosis was significantly higher than in tomato. The highest percentage of cell necrosis were recorded in *S. villosum* ssp. *puniceum*, lettuce, sweet pepper, and one accession each of cucumber and *S. dulcamara*. This indicated that the resistance in these accessions was associated with HR. However, since the percentage of cell necrosis in courgette cv Albina, cucumber PI204692 and other petunia accessions was relatively low, non-host resistance of these accessions to *O.lycopersici* may be based on a non-hypersensitive type of resistance.

Asexual reproduction of Oidium lycopersici

Considering the early abundant sporulation on eggplant and tobacco, cross-inoculation was carried out to confirm the possible adaptation of *O. lycopersici* from tomato to these species. Conidiospores from eggplant and tobacco were used to inoculate tomato plants by print-inoculation. Sporulation on tomato, inoculated with conidiospores from tobacco, occurred as rapidly as from tomato to tomato, enabling a continuous host switch between tomato and tobacco over a five-month period of experiments. In cross-inoculation from eggplant to tomato, sporulation was retarded seven days as compared with that from tomato to tomato. Conidiospores were still produced singly on eggplant and tobacco; the shape and size of conidiospores produced on eggplant and tobacco were similar to that on tomato.

The number of conidiospores produced on tomato, tobacco and eggplant at two to three weeks after inoculation, was 6.0×10^4, 4.5×10^4 and 1.1×10^4

conidiospores · cm^{-2} respectively, when these plant species were inoculated with conidiospores produced on tomato. Statistically, the number of conidiospores produced on eggplant was significantly lower than that on tomato and tobacco, but the difference between the number of conidiospores produced on tomato and tobacco was not significant.

Discussion

In the present study, tobacco may be the only species besides tomato that can be considered as host of *O. lycopersici* because the fungus could successfully maintain a polycyclic infection. Though eggplant and some other *Solanaceae* species also supported early infection to some degree, they could not be considered hosts because the fungus could not be maintained on these species. The Dutch isolate was not pathogenic on sweet pepper nor on cucurbits. This was in accordance with the host range reported for a British (Fletcher et al., 1988) and a Californian isolate (Arredondo et al., 1996). Like the Californian isolate (Arredondo et al., 1996), the Dutch isolate was not pathogenic on cowpea (*Vigna unguiculata*). The other studies on host ranges either included fewer plant species or gave different results, such as pathogenicity on cucurbits and/or eggplant (Table 1). The differences in host range may be due to different genotypes, environmental conditions and particularly due to the definition of susceptibility (Whipps et al., 1998 and references therein). In the present study, pea appeared, initially, to be susceptible to *O. lycopersici*. Even though most of the inoculation tests were conducted in isolated growth chambers or greenhouse compartments with spore-proof filters, detailed cross-infection experiments showed that this result was due to contamination with pea powdery mildew. This stresses the need for demonstrating host status of a plant species by repeated cross-infection cycles of the fungus between its original host and the other plant species. In our study only tobacco met these criteria and thus only tobacco is considered as an alternative host for *O. lycopersici*.

The size and shape of conidiospores of the Dutch *O. lycopersici* isolate formed on tobacco and eggplant were identical to those that formed on tomato. This is in agreement with the constant size of a British *O. lycopersici* isolate on alternative hosts (Whipps et al., 1998). In addition, conidiospores of the Dutch isolate were produced singly on tobacco and eggplant. This is in contrast to other studies in which conidiospore arrangement varied on different hosts (Fletcher et al., 1988; Whipps and Helyer, 1994). Such a morphological change and the lack of the sexual stage make the present taxonomic identification of this powdery mildew fungus ambiguous. Indirect approaches such as host range tests may be helpful to determine the relationship of the tomato powdery mildew with other powdery mildew species (Cook et al., 1997).

The defense response to *O. lycopersici* in most plant species and accessions tested in this study does not seem to be based on the inhibition of conidiospore germination. This is in agreement with the resistance in pea cultivars to pea powdery mildew (Cirulli, 1976; Singh and Singh, 1983) and in barley to its nonpathogenic powdery mildew *E. cichoracearum*, but differs from the resistance mechanism in cucumber to *E. graminis* (Staub et al., 1974). Apparently, leaf properties of the plants are unlikely to be critical to the germination process of *O. lycopersici* and, therefore, are probably not an important factor in determining the host range. The resistance to *O. lycopersici* in the non-hosts is also not based on the inhibition of the formation of a primary haustorium. This resembles the resistance in cucumber to *E. graminis*, but differs from that in barley to *E. cichoracearum* (Staub et al., 1974). The defense response was associated with a posthaustorial hypersensitive response, and maybe other, non-hypersensitive, defense mechanism(s). This is in agreement with the resistance in cucumber to *E. graminis* (Staub et al., 1974), but in contrast to the common observation that the growth and development of rust and powdery mildew fungi ceases in non-host plant species before or during formation of the first haustorium (Johnson et al., 1982; Elmhirst and Heath, 1989).

O. lycopersici has been only occasionally mentioned in the literature since its first description in Australia one century ago (Blumer, 1967). Apparently, it did not cause heavy damage in tomato until 1986. It is an intriguing question whether the *O. lycopersici* causing the recent outbreaks in Europe belongs to the same species as the one found in Australia, or whether it originates from powdery mildews of other plant species. Currently, we are investigating the differences in DNA composition between powdery mildew isolates including tobacco powdery mildew. Such DNA fingerprints should provide information about the origin of the causal agent(s) of the recently occurring tomato powdery mildew disease.

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