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RESEARCH PAPERS

Pathogenicity of *Phytophthora nicotianae* isolates to tobacco and tomato cultivars

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Summary. Sixty-one isolates of *Phytophthora nicotianae* were tested for pathogenicity to tobacco and tomato. All the isolates but one from tobacco were pathogenic to tobacco but none of the isolates from tobacco were pathogenic to tomato. Of the 53 isolates from non tobacco hosts, 19 proved pathogenic to tomato. Seven isolates from different non tobacco hosts colonized the tobacco stem and produced necrosis but not black shank. Five isolates colonized the tomato stem without causing disease symptoms. Tobacco cultivars BX 2a and KP 14/a were susceptible to isolate BPIC 1921. Tomato cv. Early Pak was susceptible to isolate BPIC 1932 and the Tondino cv. was susceptible to isolate BPIC 1933. No isolate was virulent on both tobacco and tomato. Our results showed that the black-shank isolates of *P. nicotianae* are a separate group with a specific pathogenicity to tobacco plants only and that prolonged growth in colture may or may not produce loss of virulence - pathogenicity.

Key words: black shank, *Phytophthora nicotianae*, tobacco, tomato.

Introduction

Phytophthora nicotianae Breda de Haan (syn. *Phytophthora parasitica* Dastur) is a destructive pathogen on a wide range of herbaceous and woody plants, which causes black shank on all types of tobacco (*Nicotiana tabacum* L.). This is the most destructive disease of tobacco and has spread to most tobacco growing countries in the world (Sarejianni and Stamatini, 1935; Colas *et al.,* 1998). *P. nicotianae* infects roots, basal parts of the stem (black shank) and leaves of tobacco. Young tobacco seedlings are very susceptible to infection and they damp off (Lucas, 1965), while the older plants are attacked on foot or higher (30 cm or more). The first symptom of the disease is the wilting or dropping of all leaves and severity is generally related to the extent of the local lesion. This wilting is more rapid and severe than that occurring in *Fusarium* wilt of tobacco. Tobacco leaves turn brown, shrivel and they are not marketable. In resistant cultivars, the roots may become infected and rot, but the upper leaves remain green without obvious symptom development (Shew, 1991). The fungus is spread by water, and by infested soil (Lucas,1965)

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which facilitates secondary infections (Shew, 1987). The severity of black shank is increased by concomitant infections with root-knot nematodes. Tobacco cultivars vary significantly in their level of resistance to black shank. Cultivars resistant to black shank lose much of their resistance if infected by root-knot nematodes (Shew, 1991). Black shank is controlled by means of cultural practices, nematode control, fungicides and host resistance.

Tomato (*Lycopersicon esculentum* Miller) has also been reported to be attacked by *P. nicotianae* (buckeye and stem rot). The fungus causes seedling damping off, stem canker near the soil and fruit rot. The affected seedlings often fail to become established and may wilt, especially under unfavourable conditions. In older plants the fungus produces a dark-brown to black canker at soil level, which often extends below ground but may reach 60-120 cm above ground in wet greenhouses. Buckeye rot of fruits is a common disease transmitted mainly when contaminated soil is splashed onto plants. A characteristic symptom of buckeye rot is the formation of brown, concentric rings on a grey-brown lesion with a grey centre (Sherf and Macnab, 1986; Fletcher, 1989; Stevenson, 1991).

Isolates of *P. nicotianae* from tobacco are a separate group, specialized in causing only black shank and only on tobacco (Ho and Jong, 1989; Ricci *et al.,* 1992). Besides pathogenicity, *P. nicotianae* isolates from tobacco can be differentiated because they do not produce parasiticein, a protein elicitor (Ricci *et al.,* 1992).

The purpose of the present study was to determine the pathogenicity of 61 isolates of *Phytophthora nicotianae* from various hosts, including tobacco and tomato, on tobacco and tomato plants, and to evaluate the susceptibility of five tobacco and two tomato cultivars to this pathogen.

Materials and methods

Sixty-one cultures of *P. nicotianae* strains, isolated from a diversity of plant hosts and geographic locations in Greece were used. Four isolates were received from J.L. Apple (USA) and one from the CBS collection (The Netherlands). All strains were included in the Benaki Phytopathological Institute Culture Collection (BPIC) and they were kept on corn meal agar (CMA) slants at 22°C (Table I). Isolates were subcultured on CMA in 9-cm Petri dishes at 22°C for 7 days. Inoculum for infecting the soil was prepared by blending the contents of 14 inoculated dishes in a litre of deionized water (Carlston *et al*., 1977).

Plant material

Young plants of tobacco (cv. BX 2a Oriental aromatic) and tomato (cv. Early Pak No. 7) were used. Tobacco seeds were sown in pots with compost and grown at 25°C and a 15-h photoperiod. Plants were watered with tap water. When seedlings were 40 days old (two-leaf stage), they were transplanted to 9-cm diameter plastic pots (one seedling per pot) filled with the same compost. Tomato seedlings were grown under the same conditions as tobacco and they were transplanted at 10 days from germination.

For inoculation, plants in pots were watered to saturation and two holes were made in the soil around the stem of each plant. Then 10 ml of inoculum was poured into each hole (20 ml per pot). Control plants received the same amount of blended CMA medium. Seven plants of tobacco and an equal number of tomato were inoculated with each *Phytophthora* strain 5 days after transplantation and seven uninoculated plants served as controls. The number of diseased plants was recorded every 3 days from day 3 to day 15 after inoculation.

Five tobacco cultivars, BX 2a (Oriental aromatic), B 21E (Burley), S 53 (Oriental flavour), VE 9 (Greek Virginia) and KP 14/a (Oriental neutral), obtained from the Tobacco Institute, were assessed for their susceptibility to two pathogenic isolates of *P. nicotianae,* BPIC 1142 and BPIC 1921. These cultivars are the most commonly grown in Greece. Similarly, two tomato commercial cultivars, Early Pak and Tondino, were assessed for their susceptibility to *P. nicotianae* isolates BPIC 1932 and BPIC 1933. Tobacco and tomato cultivars were grown and inoculated as described above for the pathogenicity tests. Disease reactions were scored on a 0-5 scale, where 0 indicated no symptoms on the aerial parts and roots, and 5 indicated a plant killed by the pathogen 15 days after inoculation (Carlson *et al*, 1997). Seven plants per cultivar were inoculated and placed in a randomized block design. Data were analysed by one-way analysis of variance. Treatment means were compared with Duncan's multiple range test.

Experiments were repeated two times for the

pathogenicity tests, and once to determine the susceptibility of tobacco and tomato cultivars.

Results and discussion

The symptoms on inoculated tobacco and tomato plants consisted of necrosis of stem tissue starting 3 to 6 days after inoculation. *P. nicotianae* isolates from tobacco infected the collar region and stems of tobacco plants giving rise to characteristic rot which spread up to the leaves. The lower stem tissues were always colonized by the pathogen. The roots showed a high level of infection but plants that did not exhibit root necrosis did not exhibit aboveground symptoms either. Seven isolates from different non-tobacco hosts colonized the tobacco stem producing some necrosis but not black shank, and the plants survived the infection. On tomato, pathogenic isolates from tomato caused stem shrivelling, the leaves wilted and plants died. Data in Table 1

Table 1. Response of tobacco and tomato to inoculation with *Phytophthora nicotianae* isolates collected from 1929 to 1997.

Strain	Host	Year of isolation	Response to inoculation	
			Tobacco cv. BX 2a	Tomato cv. Early Pak
BPIC 1142[a]	*Nicotiana tabacum*	before 1970	+[c]	-
BPIC 1143	*Pistacia vera*	1979	-[d]	+
BPIC 1144	*Citrus deliciosa*	1982	-	-
BPIC 1206[a]	*Nicotiana tabacum*	before 1970	+	-
BPIC 1207[a]	*Nicotiana tabacum*	before 1970	-	-
BPIC 1208[a]	*Nicotiana tabacum*	before 1970	+	-
BPIC 1209[b]	*Nicotiana tabacum*	1929	+	-
BPIC 1210	*Dianthus caryophyllus*	1972	-	-
BPIC 1211	*Lycopersicon esculentum*	1964	-	-
BPIC 1212	*Lycopersicon esculentum*	1964	-	-
BPIC 1238	*Dianthus caryophyllus*	1975	-	-
BPIC 1239	*Dianthus caryophyllus*	1974	-	-
BPIC 1241	*Dianthus caryophyllus*	1976	-	+
BPIC 1242	*Lycopersicon esculentum*	before 1980	-	-
BPIC 1243	*Lycopersicon esculentum*	1966	-	-
BPIC 1244	*Lycopersicon esculentum*	1966	-	+
BPIC 1245	*Lycopersicon esculentum*	1966	-	-
BPIC 1246	*Lycopersicon esculentum*	1976	-	+
BPIC 1247	*Lycopersicon esculentum*	before 1980	-	-
BPIC 1248	*Lycopersicon esculentum*	1976	-	+
BPIC 1249	*Lycopersicon esculentum*	1974	-	+
BPIC 1250	*Lycopersicon esculentum*	1975	-	+
BPIC 1251	*Lycopersicon esculentum*	1974	-	-
BPIC 1252	*Lycopersicon esculentum*	1978	-	+
BPIC 1253	*Lycopersicon esculentum*	1978	-	+
BPIC 1254	*Cyclamen* sp.	1962	-	-
BPIC 1255	*Cyclamen* sp.	1974	±[e]	-
BPIC 1256	*Cyclamen* sp.	before 1980	-	-
BPIC 1258	*Pistacia vera*	1975	-	-
BPIC 1259	*Pistacia vera*	1973	-	-
BPIC 1261	*Citrus limon*	1979	-	-
BPIC 1262	*Citrus sinensis*	1971	-	-
BPIC 1263	*Citrus limon*	before 1980	-	-
BPIC 1264	*Citrus sinensis*	1976	-	-
BPIC 1265	*Rosmarinus officinalis*	1973	-	-
BPIC 1266	*Rosmarinus officinalis*	1974	-	-

(continued)

Table 1. *(Continued)*

Strain	Host	Year of isolation	Response to inoculation	
			Tobacco cv. BX 2a	Tomato cv. Early Pak
BPIC 1267	*Primula* sp.	before 1980	-	+
BPIC 1268	Cactaceae	1973	-	-
BPIC 1269	Suculent plant	1975	±	+
BPIC 1270	*Gerbera* sp.	1976	-	-
BPIC 1271	*Lavandula* sp.	1973	-	-
BPIC 1272	*Lavandula* sp.	1974	-	+
BPIC 1273	*Hedera helix*	before 1980	-	+
BPIC 1274	*Gloxinia* sp.	1974	±	-
BPIC 1275	*Aralia elegantissima*	1974	±	+
BPIC 1276	*Saintpaulia ionata*	1977	±	-
BPIC 1277	*Saintpaulia ionata*	1976	-	-
BPIC 1278	*Solanum melongena*	n.a.	-	+
BPIC 1279	*Cyclamen* sp.	1977	-	-
BPIC 1280	*Salvia* sp.	1978	-	±
BPIC 1281	*Anthurium* sp.	1980	-	±
BPIC 1282	*Musa carendishii*	1977	-	-
BPIC 1919	*Dianthus caryophyllus*	1996	-	-
BPIC 1920	*Lycopersicon esculentum*	1996	-	+
BPIC 1921	*Nicotiana tabacum*	1996	+	-
BPIC 1922	*Lycopersicon esculentum*	1996	-	+
BPIC 1932	*Lycopersicon esculentum*	1997	±	+
BPIC 1933	*Lycopersicon esculentum*	1997	-	+
BPIC 1926	*Nicotiana tabacum*	1996	+	±
BPIC 1937	*Nicotiana tabacum*	1997	+	±
BPIC 1938	*Saintpaulia ionata*	1997	±	±
Control			-	-

[a] From J.L. Apple, USA.
[b] From Centraalbureau voor Schimmelcultures, Baarn, The Netherlands (CBS 303.29) isolated by L.H. Leonian.
[c] Pathogenic.
[d] Non pathogenic.
[e] The fungus colonizes the plants without disease symptoms.
n.a., not available

indicate that all isolates of *P. nicotianae* from tobacco except one were pathogenic to tobacco, but none of these was also pathogenic to tomato. Nineteen out of the 61 *P. nicotianae* isolates proved highly virulent to tomato. These tomato-virulent isolates were mainly from tomato. Five other isolates not from tomato colonized the tomato stem but without causing disease symptoms. No isolate was pathogenic to both tobacco and tomato alike (Table 1). Mortality in tobacco plants occurred somewhat later than in infected tomato plants, and some isolates caused disease symptoms more quickly than others. No disease symptoms were noted in the control plants. The incidence of wilting plants was recorded and isolations were made from selected diseased plants.

The five tobacco cultivars tested varied in their reaction to *P. nicotianae* isolate BPIC 1921, but they were equally susceptible to BPIC 1142 (Table 2). Tobacco cultivars BX 2a and KP 14/a were more susceptible than cultivars S 53, VE 9 and B 21E to isolate BPIC 1921. The cultivar BX 2a is also susceptible in field conditions in Greece. Results of inoculation of tomato cultivars indicated a change in ranking of susceptibility depending upon the isolates inoculated. The cv. Early Pak was more susceptible than cv. Tondino to isolate BPIC 1932, but less susceptible than

Table 2. Disease severity recorded on five tobacco cultivars inoculated with *Phytophthora nicotianae* isolates.

Cultivar	Disease severity [a]	
	BPIC 1142	BPIC 1921
BX 2a	2.4 a[b]	4.3 a
KP 14/a	1.7 a	2.7 a
S 53	1.6 a	0.8 b
VE 9	1.4 a	0.7 b
B 21E	3.3 a	0.4 b

[a] Disease severity was assessed on a 0-5 scale where 0=healthy plant and 5=plant killed.
[b] Average of seven plants. Means were compare according to Duncan's multiple range test. Numbers followed by different letters differ significantly at $P<0.05$.

Table 3. Disease severity recorded on two tomato cultivars inoculated with *Phytophthora nicotianae* isolates.

Cultivar	Disease severity [a]	
	BPIC 1932	BPIC 1933
Early Pak	4.71 a[b]	2.14 b
Tondino	2.57 b	5 a

Footnotes see Table 2.

this cv. to opposite occurred with isolate BPIC 1933 (Table 3).

Our results showed that isolates of *P. nicotianae* from tobacco caused typical black-shank disease symptoms only on tobacco. This agrees with the concept generally held that the black-shank isolates of *P. nicotianae* are a separate group with specific pathogenicity to tobacco plants only. Only one isolate from tobacco failed to cause disease on tobacco in the pathogenicity tests.

In our study, only 19 of 61 isolates from tomato and non-tobacco hosts were pathogenic to tomato. Isolates from tobacco did not cause infection on tomato but two of them colonized the tomato stem. Five isolates from tomato failed to cause disease on tomato hosts. According to Hall (1993) the loss of pathogenic properties is probably due to repeated subculture for storage, but our results indicated that the strain BPIC 1244 was pathogenic to tomato after 32 years, and strain BPIC 1209, isolated by Leonian (CBS 303.29) was pathogenic to tobacco after 70 years, as reported by Hall (1993).

Literature cited

Carlson S.R., M.F. Wolff, H.D. Shew and E. Wernsman, 1997. Inheritance of resistance to race 0 of *Phytophthora parasitica* var. *nicotianae* from the flue-cured tobacco cultivar Coker 371-Gold. *Plant Disease,* 81*,* 1269-1274.

Colas V., I. Lacourt, P. Ricci, F. Vanlerberghe-Masutti, P. Venard, A. Poupet and F. Panabieres, 1998. Diversity of virulence in *Phytophthora parasitica* on tobacco, as reflected by nuclear RFLPs. *Phytopathology,* 88*,* 205-212.

Fletcher J.T., 1989. Diseases of greenhouse plants. Longman Scientific and Technical, New York, NY, USA, 351 pp.

Hall G., 1993. An integrated approach to the analysis of variation in *Phytophthora nicotianae* and a redescription of the species. *Mycological Research,* 97*,* 559-574.

Ho H.H. and S.C. Jong, 1989. *Phytophthora nicotianae* (*P. parasitica*). *Mycotaxon,* 35(2), 243-276.

Lucas G.B., 1965. Diseases of tobacco. Second edition. The Scarecrow Press, Inc., New York, NY, USA, 778 pp.

Ricci P., F. Trentin, P. Bonnet, P. Venard, F. Mouton-Perronnet and M. Bruneteau, 1992. Differential production of parasiticein, an elicitor of necrosis and resistance in tobacco, by isolates of *Phytophthora parasitica*. *Plant Pathology,* 41*,* 298-307.

Sarejanni J.A. et N. Stamatini, 1935. Le *Phytophthora* du tabac en Grèce. *Annales Institut Phytopathologique Benaki, Année* 1(2*),* 51-56.

Sherf A.F. and A.A. Macnab, 1986. Vegetable diseases and their control. John Wiley and Sons, New York, NY, USA, 728 pp.

Shew H.D., 1987. Effect of host resistance on spread of *Phytophthora parasitica* var. *nicotianae* and subsequent development of tobacco black shank under field conditions. *Phytopathology,* 77*,* 1090-1093.

Shew H.D., 1991. Black shank. *In*: Compendium of Tobacco Diseases (H.D. Shew, G.B. Lucas, eds), APS, St. Paul, MN, USA, 17-20.

Stevenson W.R., 1991. Buckeye rot and *Phytophthora* root rot. *In*: Compendium of Tomato Diseases (Jones *et al*., eds), APS, St. Paul, MN, USA, 11-12.

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