Phytopathol. Mediterr. (2011) 50, 159–165

Host range of *Phytophthora parsiana*: a new high temperature pathogen of woody plants

Somieh HAJEBRAHIMI and Zia BANIHASHEMI

Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, Iran

Summary. Among several *Phytophthora* spp. reported previously from *Pistacia vera* in Iran, a high temperature species recently identified as *P. parsiana* (formerly known as high temperature *P. cryptogea*) is becoming important in woody plants, including *P. vera*. The host range of this newly recognised species, including both annual and perennial plants, is reported here. The pathogen infected 4–5 month-old glasshouse grown seedlings of *P. vera, Ficus carica, Malus pumila* and *Prunus dulcis*, and detached stems of 23 woody plants collected during dormant and growing seasons. Nineteen field and vegetable crops and 17 weed species were not infected by *P. parsiana* in these pathogenicity assays.

Key words: *Pistacia vera,* Iran, *Phytophthora cryptogea,* gummosis, crown rot.

Introduction

Phytophthora species are major soil-borne plant pathogens in various agricultural commodity crops in Iran, causing high losses in annual and perennial plant species. Major economic losses are experienced in *Pistacia vera* (pistachio) under saline and non-saline conditions (Banihashemi, 1995).

Several species of *Phytophthora* have been reported on pistachio in Iran, including *P. citrophthora* (Banihashemi, 1983; Mirabolfathy *et al*.,1989), *P. megasperma* (Mirabolfathy and Ershad, 1987). *P. drechsleri* (Aminaee and Ershad, 1991; Banihashemi, 1995), *P. cryptogea* and *P. nicotianae* (Banihashemi, 1995). In a re-examination of *P. megasperma* and *P. drechsleri* from pistachio in Iran using RFLP and ITS sequence analyses, Mirabolfathy *et al.* (2001) showed that the *P. megasperma* like isolates from pistachio were a newly recognised species, *P. pistaciae*; while *P. drechsleri* from pistachio was considered to be *P. melonis*.

Corresponding author: Z. Banihashemi
Fax: + 98 711 2286087
E-mail:Ziabani@shirazu.ac.ir

The three *Phytophthora* species *P. melonis, P. drechsleri* and *P. cryptogea* are morphologically identical and cannot be separated by conventional methods. Ho and Jong (1986) indicated both *P. drechsleri* and *P. cryptogea* to be identical and indicated that growth at 35°C , or this in combination with other criteria, could not separate these species. The two species were, therefore, merged into *P. cryptogea*, which has priority. Later (Ho and Jong ,1991) growth at 35°C was used to separate the two species.

Controversy remains concerning the morphological identity of *P. melonis*, *P.drechsleri* and *P. cryptogea*. Based on molecular analysis, some isolates of *P. drechsleri* from pistachio from Iran initially identified by morphology, were re-identified as *P. melonis* (Mirabolfathy *et al*., 2001). The high temperature isolates of *P. cryptogea* reported from fig (Banihashemi and Ghaisi, 1993) and pistachio (MacDonald *et al*., 1992; Banihashemi, 1995) could not be separated morphologically from common isolates of the pathogen. Mostowfizadeh-Ghalamfarsa (2005), examining the phylogeny of the taxonomically challenging species *P. drechsleri* and *P.cryptogea* from various sources, found a monophyletic group of isolates distinct from either spe-

cies on the basis of rDNA ITS sequence, which was later reported as *Phytophthora parsiana* sp. nov. a new high temperature tolerant species (Mostowfizadeh-Ghalamfarsa *et al*., 2008). All isolates previously identified morphologically as high temperature *P. cryptogea* isolates had been obtained from woody plants such as *Ficus carica* (Banihashemi and Ghaisi ,1993), and *P. vera* (MacDonald *et al*.,1992; Banihashemi, 1995).

The host range of *P. cryptogea* is mostly reported to be herbaceous plants, on which the pathogen causes root rot and seedling blight, but a few woody plants were included as hosts (Erwin and Ribeiro,1996).

The objective of the present study was to examine the host range of *P. parsiana* (formerly known as high temperature tolerant *P. cryptogea*) under glasshouse conditions. A summary of this study was reported earlier (Hajebrahimi and Banihashemi**,** 2008).

Materials and methods

Sources of isolates and host-range studies

Three representative isolates of *P. parsiana* from different woody hosts and locations were used in this study (Table 1).

Woody plants

In a glasshouse test**,** six woody plant species were tested for susceptibility to *P. parsiana* (Table 2). Seeds of apple (*Malus pumila*), almond (*Prunus dulcis*) pistachio (*Pistacia vera*), walnut (*Juglans regia*) and lemon (*Citrus limon*) were surface sterilized for 5 min in 0.5% NaOCl and planted in 20 cm diameter pots containing steam sterilized soil:sand (2:1 v/v) and raised in a glasshouse at 16–35°C. Stem cuttings of fig were also planted in the same substrate. Three replicate pots each containing three plant species were used in each treatment.

Herbaceous plants

Seeds of 18 species of field and vegetable crops (Table 3) were surface sterilized as described above, and planted in 9 cm diameter pots containing the same soil mix and raised in a growth chamber at 22–24°C with 16:8 light-dark cycle. Three replicate pots each with five seedlings were used.

Weeds

Seeds of 16 common weed species (Table 3) were surface sterilized as described above, and planted in steam-sterilized soil mix and kept in

Table 1. Sources of isolates of *Phytophthora parsiana*.

Isolate code	Host	Location	Year isolated	Source
SUC 7	*Pistacia vera*	USA	1992	Z. Banihashemi
SUC 19	*Pistacia vera*	Iran	1992	Z. Banihashemi
SUC 25	*Ficus carica*	Iran	1991	Z. Banihashemi

Table 2. Pathogenicity of *Phytophthora parsiana* isolates in some species of woody plants.

Scientific name	Common name	Pathogenicity of isolates[a]		
		SUC7	SUC25	SUC19
Ficus carica	Fig	+	+	+
Pistacia vera	Pistachio	+	-	+
Malus pumila	Apple	+	+	+
Prunus dulcis	Almond	+	+	+
Juglans regia	Walnut	-	-	-
Citrus lemon	Lemon	-	-	-

[a] *P. parsiana* re-isolated; - *P. parsiana* not re-isolated.

glasshouse (16–35°C). Three replicate pots each with five seedlings were used.

Detached stems of woody plants

Stems (20×1 cm) of 21 woody plant species were collected during dormant and active growth stages (Table 4). Six replicate cuttings were inoculated for each species

Fruit, tuber and root crop plants

Fresh fruits, tuber and roots of various crops were obtained from local markets and fields and used for inoculation. Eight replicates were used for each treatment (Table 5).

Inoculum production

Inocula of the *P. parsiana* isolates were produced on vermiculite amended with hemp seed extract (Banihashemi and Fatehi 1989). Two hundred ml of vermiculite and 120 mL hemp seed extract (extract of 60 g hemp seed $^{-1}$L distilled water) were autoclaved for 20 min and inoculated with three to four 6mm blocks of fresh culture grown on corn meal agar and incubated at room temperature for 4–6 weeks.

Table 3. Herbaceous plants and weeds inoculated with *Phytophthora parsiana* under glasshouse conditions.

Plant	Scientific name	Common name
Herbaceous	*Cucumis sativus*	Cucumber
	C.melo var. *flexuosus*	Snake melon
	C.melo	Melon
	Cucurbita maxima	Pumpkin
	Citrullus lanatus	Watermelon
	Vicia sativa	Mungbean
	Vigna unguiculata	Cowpea
	Soja hispida	Soja
	Phaseolus coccineus	Scarlet runner bean
	Lens esculenta	Lentil
	Cicer arietinum	Chick pea
	Lycopersicum esculentum	Tomato
	Solanum melongena	Egg plant
	Helianthus annuus	Sunflower
	Daucus carota	Carrot
	Sesamum indicum	Sesame
	Brassica napus	Rape seed
	Spinacia oleracea	Spinach
Weeds	*Prangos uloptera*	'Djashir'
	Echinochloa sp.	Panic grass
	Avena sativa	Oat
	Triticum polonicum	Polish wheat
	Hordeum spontaneum	Spontaneum barley
	Cardaria draba	Cress
	Chenopodium album	Pig weed
	Rumex crispus	Curled dock
	Solanum dulcamara	Night shade
	Malva sylvestris	mallow
	Launaea sp.	Launaea
	Amaranthus sp.	Amaranth
	Plantago major	Way bread
	Portulaca oleraceae	Purslane
	Melitotus alba	Sweet clover
	Glycyrhiza glabra	Liquorice

Pathogenicity test

Plant inoculation

Ten to 50 ml of inoculum, depending on the size of the pot, was used in each pot containing plants. Inoculum was spread over the soil surface and, following closure of the drainage holes using melted paraffin wax, the pots were flooded with water over night with incubation at the indicated temperatures. The formation and release of zoospores were monitored for 4 weeks, as reported previously (Banihashemi, 2004). After overnight flooding, the drainage hole in each pot was re-opened by removing the paraffin wax plug. The drained water was collected separately from each pot and filtered through a double layer of cheese cloth. Fifty to 100, 6 mm citrus leaf disks were added to each collected sample and incubated at room temperature for 48 h. Subsequently, bait discs were washed gently under running tap water, blotted dry and plated on PARP medium (Jeffers and Martin, 1986). Flooding was repeated every second week. Number of baits colonized by *P. parsiana* was recorded at each time point and percent colonization of baits counted to ensure the presence of the active pathogen in the pots.

Detached stem inoculation

Stems (20×1.5 cm) were washed, blotted dry and the surface was wiped with cotton impregnated with 95% ethanol. The two end cuts of each stem were dipped in warm melted paraffin wax to reduce desiccation during incubation. Three T-shaped cuts (2–3 cm) were made along each stem. A 6 mm corn meal agar (CMA) plug from actively growing hyphae of each isolate was inserted into each cut, the bark replaced, and the wound and inoculum wrapped with Parafilm. Stems were incubated at room tempera-

Table 4. Pathogenicity of isolates of *Phytophthora parsiana* on detached stems of different woody plants..

Scientific name	Common name	Pathogenicity of isolates					
		SUC19		SUC17		SUC25	
		Winter	Growing season	Winter	Growing season	Winter	Growing season
Morus alba	White mulberry	+	-	+	-	+	-
Ulmus campestris	Elm	+	-	-	-	-	-
Cupressus sempervirens	Cypress	-	-	-	-	-	-
Magnolia grandiflora	Magnolia	-	-	-	-	-	-
Ficus carica	Fig	+	-	+	-	+	-
Eucalyptus globus	Eucalyptus	-	-	-	-	-	-
Pistacia vera	Pistachio	+	+	+	+	+	+
Fraxinus rotundifolia	Ash	-	-	-	-	+	-
Pinus eldarica	Pine	-	-	-	-	-	-
Platanus orientalis	Sycamore	+	-	-	-	-	-
Acer monspessulanum	Maple	+	+	+	+	+	+
Juglans regia	Walnut	+	-	+	-	+	-
Ailanthus altissima	Ailanthus	+	-	+	-	+	-
Robinia pseudoacacia	Acacia	-	-	-	-	+	-
Citrus aurantium	Sour orange	-	-	-	-	-	-
Punica granatum	Pomegranate	+	-	+	-	-	-
Malus pumila	Apple	+	+	+	+	+	+
Prunus dulcis	Almond	+	-	+	-	+	-
Cydonia oblonga	Quince	+	-	+	-	+	-
Persica vulgaris	Peach	-	-	-	-	-	-
Rosa canina	Dog rose	+	-	+	-	+	-

Table 5. Percentage infection of various fruits, roots and tubers of different plants by *Phytophthora parsiana* isolates 5 days after inoculation.

Scientific name	Common name	Pathogenicity of isolates		
		SUC25	SUC7	SUC19
Malus pumila	Apple (cv. Golden delicious)	20	15	15
Malus pumila	Apple (cv. Red delicious)	15	10	10
Malus pumila	Apple (cv. Golden delicious, unripe)	10	10	10
Citrus nobilis	Mandarine	40	40	40
Citrus lemon	Lemon	40	40	40
Citrus sinensis	Orange	40	40	40
Citrus aurantium	Sour orange	40	40	40
Musa paradisiaca	Banana	60	40	60
Ficus carica	Fig (ripe)	40	60	50
	Fig (unripe)	30	20	40
Cucumis sativus	Cucumber	90	90	90
Lycopersicon esculentum	Tomato	100	100	100
Solanum melongena	Egg plant	20	10	50
Daucus carota	Carrot	0	0	0
Beta vulgaris	Sugar beet	0	0	0
Solanum tuberosum	Potato	0	0	0

ture and observed every other week for symptom development. Experimental controls comprised of stems inoculated with sterile CMA.

Fruit, tuber and root crop plants

Fruits, tubers and roots were thoroughly washed, left to dry at room temperature and wiped with 95% ethanol. A cork borer was used to make two 6 mm holes on opposing sides of each of these, and an equal diameter plug of an actively growing CMA culture of each isolate was inserted in the hole. The block of plant tissue was replaced in the wound. Wounds were covered with adhesive tape and the fruits, roots and tubers were incubated in plastic bags at room temperature. Sterile CMA plugs were used as negative experimental controls.

Re-isolation of pathogens

Roots, tubers, crowns of the seedlings, stems and fruits showing any discoloration or decay were transferred to PARP medium and incubated at room temperature. Colonies emerging from inoculated tissues were identified morphologically.

Results

Woody plants

Walnut and lime were not infected by *P. parsiana* and the pathogen was not recovered from inoculated plants. Pistachio, fig, apple and almond were infected and the pathogen was re-isolated from infected tissues (Table 2). The first disease symptoms in pistachio appeared 5 months after inoculation. Infected plants showed mild wilting with root and crown necrosis. Apple seedlings showed disease symptoms 4 months after inoculation, including defoliation and extending necrosis in the lower stems. In almond, severe root rot and shoot necrosis occurred 4 months after inoculation. In fig, severe root rot and lower stem necrosis were observed 5 months after inoculation. The pathogen was re-isolated from infected tissues.

Herbaceous plants and weeds

Of the 18 herbaceous plants and 16 weeds (Table 3) inoculated with *P. parsiana* isolates, none were infected by the pathogen. No pathogen was re-isolated from inoculated plants or plant organs.

Detached stems of woody plants

Only stem samples of pistachio, *Malus pumila* and maple collected during active plant growth showed infection by *P. parsiana*. Stem samples of all 23 woody plants collected during dormant stages were infected by *P. parsiana* and the pathogen was re-isolated from infected tissues (Table 4).

Fruit, tuber and root crop plants

Phytophthora parsiana caused severe rot on all fruits but none of the roots or tubers were infected by the pathogen (Table 5).

Discussion

The present study showed that the newly described high temperature tolerant *Phytophthora parsiana* might be a serious threat to pistachio and some important woody plants especially under high temperature conditions. From a limited number of woody plants examined, both fruit and nut crops which are grown under various climatic conditions were hosts susceptible to the pathogen. None of the herbaceous plant species examined, including vegetable and field crop plants and weeds, were immune to the pathogen. At present, it is premature to finally conclude that the pathogen specifically attacks perennial woody plants.

Very few species of *Phytophthora* which are limited to woody plants grow above 35°C. *Phytophthora melonis, P. drechsleri* and *P. nicotianae,* which are high temperature species, infect herbaceous and woody plant species. As a result of recent climate change and global warming in many parts of the world (Garret *et al*., 2006) the new high temperature species may become serious threats to many woody plants.

Several *Phytophthora* species have been reported to cause pistachio gummosis resulting in crown and root rot. *Phytophthora citrophthora* was the most aggressive species isolated from pistachio growing areas of southern Iran (Banihashemi, 1984), due to the low tolerance of most local Iranian rootstocks used in pistachio plantations (Banihashemi, 1998). Other *Phytophthora* species were also reported to cause pistachio gummosis in Rafsenjan a major pistachio growing area in Iran. These include *P. megasperma,* a high temperature *P. cryptogea* (McDonald *et al*., 1992; Banihashemi, 1995), *P. drechsleri* (Aminaee and Ershad, 1991; Banihashemi, 1995) and *P. nicotianae* (Banihashemi, 1995). Mis-identification of isolates when using conventional morphological features resulted in the introduction of hitherto unrecognised species of *Phytophthora* associated with pistachio gummosis. Mirabolfathy *et al*. (2001), using RFLP and ITS analyses, reported that isolates of species previously identified as *P. drechsleri* were actually *P. melonis*, and that *P. megasperma* was actually the new species *P. pistacia*, which could only be differentiated by molecular analysis. No detailed morphological features could be used to separate these species. A high temperature *P. cryptogea*, reported by Banihashemi (1995) as the causal agent of pistachio gummosis in Iran was also isolated from fig in southern Iran (Banihashemi and Ghaisi, 1992) and pistachio in California USA (MacDonald *et al*., 1992).

Based on morphological criteria, the taxonomic position of these isolates was not defined. Mostowfizadeh-Ghalamfarsa *et al*. (2008), using analysis of ITS sequences of various isolates of *P. drechsleri* and *P.cryptogea* (including high temperature isolates) found that, although the isolates were morphologically identical, the species represented distinct monophyletic groups. High temperature *P. cryptogea* from various hosts and geographical regions were distinct from *P. drechsleri* and *P. cryptogea* (low-maximum temperature) and all members of *Phytophthora* ITS clades 1-8. This lead to the proposal for the new species *P. parsiana* (Mostowfizadeh-Ghalamfarsa *et al*., 2008), which had been isolated only from woody hosts. Information on the host range of this new species is very limited.

The host range of *P. cryptogea* isolates identified on the basis of morphological criteria mostly included herbaceous plant species. Very few woody plants were considered hosts (Erwin and Ribeiro, 1996). Re-examination of the identity of the species and host range is required to determine if different species were involved.

The present study explored the host range of *P. parsiana* for disease management. None of the herbaceous plants (field and vegetable crops, weeds) were hosts for the pathogen under high inoculum po-

tential. Although maximum temperature for growth is the present criterion for separating *P. cryptogea* from *P. parsiana*, morphological or physiological features other than molecular analysis should be available for identification. Some isolates of *P. parsiana* caused mild infections in safflower (Hajebrahimi, 2008), although *P. cryptogea* is highly pathogenic to safflower cultivars (Mirtalebi and Banihashemi, 2006; Banihashemi and Mirtalebi, 2007).

Recently, several isolates of *P. parsiana* with high maximum growth temperatures have been recovered from old pistachio trees in Kerman Province (A.H. Mohammadi, personal communication); identity of the isolates was confirmed by molecular analysis (C.X. Hon g, personal communication). The presence of the pathogen from various parts of the world requires confirmation by stringent identification techniques. Distribution of the pathogen, especially under high temperature climatic conditions, also requires investigation.

Literature cited

Aminaee M.M. and D. Ershad, 1991. Isolation of *Phytophthora* cf. *drechsleri* from infected pistachio trees. In: *Proceeding of 10th Plant Protection Congress of Iran*, 1–5 September 1991, Bahonar University, Kerman, Iran, 106 (abstract).

Banihashemi Z., 1983. Phytophthora disease of pistachio in southern Iran. *Phytophthora Newsletter* 12, 3.

Banihashemi Z., 1995. Identification of *Phytophthora* species associated with pistachio gummosis in Iran. *Acta Horticulture* 419, 349–352.

Banihashemi Z., 1998. Assessment of pistachio rootstocks to *Phytophthora* spp. the cause of pistachio gummosis. *Iranian Journal of Plant Pathology* 34, 213–224 (in Farsi with English summary).

Banihashemi Z., 2004. A method to monitor the activity of *Phytophthora* spp. in the root zone of *Pistacia* spp. *Phytopathologia Mediterranea*. 43, 411–414.

Banihashemi Z. and J. Fatehi, 1989. Reaction of cucurbit cultivars to *Phytophthora drechsleri* and *P. capsici* in greenhouse. *Proceeding of 9th Iranian Plant Protection Congress,* 9–14 September 1989, Ferdosi University, Mashhad, Iran, 89 (abstract).

Banihashemi Z. and K. Ghaisi, 1992. Identification of Phytophthora disease of fig in Bushehr province. *Proceeding of 11th Iranian Plant Protection Congress*, 28 August–2 September 1993, Rasht, Iran, 218 (abstract).

Banihashemi Z. and M. Mirtalebi, 2008. Safflower seedling a selective host to discriminate *Phytophthora melonis* from *Phytophthora drechsleri*. *Journal of Phytopathology* 156, 499–501.

Ershad D., 1971. Beitrag zur Kenntnis der *Phytophthora*-Arten in Iran und ihrer phytopathologischen Bedeutung. Mitteilungen aus der Biologischen Bundesanstalt fur Land- and Forstwirtschaft, Berlin-Dahlem 140, 80 pp.

Erwin D.C. and O.K. Ribeiro, 1996. *Phytophthora Diseases Worldwide*. APS Press, St. Paul, MN, USA, 562 pp.

Gallegly M.E. and C. Hong, 2008. *Phytophthora Identifying Species by Morphology and DNA Fingerprent*. APS Press, St. Paul, MN, USA, 158 pp.

Garret K.A., S.P. Dendy, E.E. Frank, M.N. Rouse and S.E. Travers, 2006. Climate change effects on plant diseases: Genomic to ecosystems. *Annual Review of Phytopathology* 44, 489–509.

Hajebrahimi S., 2008. *Host range and production of survival units* in vitro *in Phytophthora parsiana*. MSc. Thesis, Shiraz University, Shiraz, Iran, 87 p. (in Farsi with English summary).

Hajebrahimi S. and Z. Banihashemi, 2008. Host range of *Phytophthora parsiana* sp. nov. *Proceeding of 18th Iranian Plant Protection Congress*, 24–27 August 2008, Buali University, Hamadan, Iran, 99 (abstract).

Ho H.H. and S.C. Jong, 1986. A comparison between *Phytophthora cryptogea* and *P. drechsleri*. *Mycotaxon* 27, 289–319.

Ho H.H. and S.C. Jong, 1990. Species concept of *Phytophthora cryptogea* and *P. drechsleri*. *Mycotaxon* 40, 35–39.

Jeffers S.N. and S.B. Martin, 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Dis*ease 70 , 1038–1043.

Khosrowfar F. and Z. Banihashemi, 2003. Role of alternate hosts on survival of *Phytophthora drechsleri* the cause of cucurbit root and crown rot in Fars province. *Iranian Journal of Plant Pathology* 40, 105-126 (In Farsi with English summary).

MacDonald J.D., Z. Banihashemi, S.M. Mircetich, G. Browne and L. Bolkan. 1992. Trunk and branch canker of pistachio caused by *Phytophthora* spp. *Phytopathology* 82, 1089 (abstract).

Mirabolfathy M., D.F.L. Cooke, J.M. Duncan. N.A. Williams, D. Ershad and A. Alizadeh, 2001. *Phytophthora pistacia* sp. nov. and *P. melonis* the principal causes of pistachio gummosis in Iran. *Mycological Research* 105, 1166–1175.

Mirabolfathy M., D. Ershad and G. Hedjaroud, 1989. Isolation of *Phytophthora citrophthora* from root and crown of pistachio in Damghan. *Iranian Journal of Plant Pathology* 25, 73–80.

Mirtalebi M., and Z. Banihashemi, 2006. Reaction of safflower cultivars to *Phytophthora drechsleri* and *P. melonis*. *Proceeding of 17th Iranian Plant Protection Congress*. Volume 2, Plant Diseases, 2–5 September, Tehran University, Karaj, Iran, 262 (abstract).

Mostowfizadeh-Ghalamfarsa R., 2005. Phylogeny, Taxonomy and Genetic Diversity of *Phytophthora cryptogea* and *P. drechsleri*. PhD Thesis, Shiraz University, Shiraz, Iran (in English with Farsi summary).

Mostowfizadeh-Ghalamfarsa R., D.E.L. Cooke and Z. Banihashemi, 2008. *Phytophthora parsiana* sp. nov. a new high temperature species. *Mycological Research* 112, 83–794.

Accepted for publication: February 8, 2011