Plant Pathology (2009) **58**, 956–963 Doi: 10.1111/j.1365-3059.2009.02088.x

Role of the *Helix aspersa* snail as a vector of *Phytophthora citrophthora* causing branch cankers on clementine trees in Spain

L. A. Alvarez*, D. Gramaje, P. Abad-Campos and J. García-Jiménez

Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 – Valencia, Spain

This study investigated the suspected role of invertebrate vectors in the transmission of phytophthora branch canker, a severe disease of clementine cultivars in Spain, caused by *Phytophthora citrophthora*. Ants (*Lasius grandis*) and snails (*Helix aspersa* and *Rumina decollata*) were collected in spring and autumn 2005 from 15 commercial citrus fields which were severely affected by the disease. Isolations made from *L. grandis* and *R. decollata* bodies did not yield positive results. However, *P. citrophthora* was isolated from 5·0% of bodies of *H. aspersa* and 4·8% of samples of their faeces. In one assay, after snails were allowed to feed for 5 h on citrus branches which had been artificially infected with *P. citrophthora*, the pathogen was isolated from 79% of their faeces. In another experiment, snails were infested by placing them in contact with a substrate colonized by *P. citrophthora* and then transferred to the base of potted 4-year-old trees of cvs Clemenules, Fortune and Nova in the glasshouse. One day after their release, infested snails were widely distributed throughout the tree canopies and 10 days later bark discoloration and gum exudations were observed on the trees. *Phytophthora citrophthora* was readily isolated from tissues showing symptoms.

Keywords: *Citrus clementina*, inoculum transport, *Lasius grandis*, *Phytophthora palmivora*, *Rumina decollata*, snail faeces

Introduction

Phytophthora branch canker of citrus (PBC) is a severe disease caused by *Phytophthora citrophthora*, which has become a major problem in the production of clementines (*Citrus clementina*) in Spain (Alvarez *et al*., 2008a). The first observations of this disease in the major Spanish clementine-producing areas were made in 2002. To date, its origin is unknown. Symptoms include visible sunken and distended lesions with gum exudations on the scaffold branches of infected trees. From these infection points, lesions progress towards the secondary branches or to the base of the tree. As symptoms develop, individual branches, and eventually the entire tree, may collapse and die. The cankers and dieback caused by the disease have rendered many orchards unproductive within a few years of the first infection being observed.

The reason for the occurrence and increased incidence of this disease in Spain is unclear; however, disease surveys suggest a genetic component to the susceptibility of clementines and their hybrids to PBC (Alvarez *et al*., 2008a). Trees of all ages have been associated with the disease, but the impact is more striking on large, mature trees.

*E-mail: lalvarezb@cip.org.pe

Published online 16 June 2009

Epidemiological studies of this syndrome are limited; nevertheless, there is a strong seasonal pattern to infection, and temperature and crop phenology apparently play a key role in the development of the disease. In the Mediterranean citrus-growing areas of Spain, tree infections generally occur from March to May and from September to October (Alvarez *et al*., 2009). Temperatures in these regions are moderate during these periods of the year, favouring the development of lesions. As a result of the severe tree losses recorded and the limited effectiveness of current strategies for managing the disease (Alvarez *et al*., 2008b), this disease causes considerable concern among citrus growers.

Although Koch's postulates have been completed for *P. citrophthora*, the mechanisms for the dispersal of this pathogen to citrus branches are so far unknown. Ristaino & Gumpertz (2000) considered processes for spatial and temporal dispersal of *Phytophthora* spp. Splash dispersal of *Phytophthora* species from soil to aboveground parts of the tree by rainfall is a major means of spread for a number of species in the genus (Upstone, 1978; Madden & Ellis, 1990; Erwin & Ribeiro, 1996). This mechanism was previously reported for species of *Phytophthora* infecting citrus (Graham *et al*., 1998; Graham & Menge, 1999), species which cause gummosis and brown rot of citrus fruits (Graham *et al*., 1998).

Phytophthora species that infect aboveground portions of plants often spread to other plants by aerial dispersal of

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inoculum from sporulating lesions on leaves, stems or fruits (Ristaino & Gumpertz, 2000). Therefore, it was originally suspected that infected fruits played a role in epidemics of brown rot of citrus as inoculum sources for branch infections. In citrus, *Phytophthora* sporangia are an important inoculum source in brown-rot epidemics (Timmer *et al*., 2000). In tropical regions, the species *P. palmivora*, and, to a lesser extent *P. nicotianae*, produce sporangia on fruit surfaces (Timmer *et al*., 2000). In regions with Mediterranean climates similar to that of Spain, brown rot in citrus is mainly associated with *P. citrophthora* and there are reports of its sporulation on affected fruits on the tree (Tuset, 1983). Taylor & Griffin (1981) observed that rain splash on diseased cocoa pods and leaves created 'aerosol droplets' that moved the inoculum of *P. palmivora* upwards by convection. In addition, Thévenin (1994) demonstrated in field experiments that rain splash transmitted this pathogen between bunches and nuts within the canopy of coconut palms. However, in PBC, there was no association between branch infections on the tree and outbreaks of brown rot in fruits, perhaps because *P. citrophthora* produces noncaducous sporangia (Erwin & Ribeiro, 1996). In Spain, brown rot is prevalent in orange cultivars during late autumn and early winter, when the fruits are ripening. In contrast, branch infections occur in mid-spring, coinciding with shoot growth, flowering or fruit development, mainly on clementine cultivars and their hybrids (Alvarez *et al*., 2008a).

Ristaino & Gumpertz (2000) proposed that humans or invertebrates can also disperse *Phytophthora* species. Some *Phytophthora*-induced diseases are known to be spread by ants, rodents and snails from soil to aerial host sites (Taylor & Griffin, 1981; El-Hamalawi & Menge, 1996; Konam & Guest, 2004). Ants and snails are common in citrus fields and they live in and around both the below- and aboveground parts of the trees. However, the possibility that these invertebrates might be a vector of the observed canker disease in *Citrus* has never been reported.

Knowledge of the relative importance of ants and snails in spreading *P. citrophthora* could lead to new methods for disease management or prevention. Quantitative information could be useful in developing models to predict seasonal risks of PBC associated with the infective potential of invertebrates. Because of the importance of PBC in Spain and the threat to clementine production, research was conducted to test the hypothesis that ants and snails are vectors of *P. citrophthora* and to establish their importance in the transmission of pathogens in nature. Another aim was to determine the capacity of the snails to transmit the pathogen through their faeces.

Materials and methods

Study sites, collection of specimens and sample processing

Ants and snails were sampled at biweekly intervals during April to May and October to November 2005, from 15 citrus orchards with natural infections of PBC (Table 1) in the province of Valencia (eastern Spain). Ants and snails were captured at three heights in each tree: from the trunk, crotch angles and major limbs. At least five affected trees in each orchard were sampled. Ants were captured using an insect aspirator, while the snails were captured using tweezers. Simultaneously, snail faeces were collected from the branches and leaves of the trees.

The captured specimens and the collected faeces were immediately stored in plastic bags, transported to the laboratory and processed later that same day. Killing jars were used as small-scale fumigators to kill collected invertebrates as rapidly as possible using a liquid fumigant (ethyl acetate/nail-polish remover), which produces a toxic atmosphere.

Isolations of *Phytophthora* propagules from the body surfaces of the ants and snails were attempted; the specimens were processed directly without disinfection. The body of each snail was extracted from the shell, dissected into five 4-mm-wide pieces and subsequently placed on modified PARBPH selective medium (cornmeal agar amended with 10 μg mL^{-1} pimaricin, 200 μg mL^{-1} ampicillin, 10 μg mL^{-1} rifampicin, 10 μg mL^{-1} benomyl, 25 μg mL^{-1} pentachloronitrobenzene and 50 μg mL^{-1} hymexazol) (Jeffers & Martin, 1986). Ants and portions of snail faeces were placed at nine points on the agar surface of each plate of this medium. Plates were incubated at 24°C in the dark and examined within 2–3 days. Pure cultures were obtained by transferring hyphal tips onto potato dextrose agar (PDA). Isolates were identified on the basis of colony morphology, mycelial characteristics, cardinal growth temperatures, and the production, morphology and dimensions of sporangia, oogonia and antheridia (Erwin & Ribeiro, 1996). The identification of isolates was supported by sequencing the amplified internal transcribed spacer (ITS) region and the 5·8S rRNA gene, using the conserved primers ITS6 and ITS4 (White *et al*., 1990; Cooke *et al*., 2000). The sequences were aligned with the CLUSTAL X program and compared with available sequences in the EMBL/GenBank database.

Transmission of *P. citrophthora* via snail faeces under controlled conditions

Detached citrus branches of clementine cv. Hernandina *c*. 20 cm long and 20 mm in diameter were selected for inoculations with *P. citrophthora* isolate Phy 114, originally recovered from cankered lemon trees. Selected branches were surface-disinfected using 70% ethanol, then a disc of the bark was removed using a 5-mm-diameter cork borer. The exposed cambium was inoculated by placing a 5-mm PDA agar plug cut from a culture of the isolate mycelium-side-downward on the wound. Controls were inoculated with sterile PDA plugs. After inoculation, the wound was covered with moist cotton wool, sealed with a strip of Parafilm® and wrapped with foil to prevent it from drying out. Ten shoots were placed in each of 16 moist chambers (35 × 20 × 15 cm) and incubated at 24°C. The experiment comprised four treatments with four

Table 1 Clementine orchards sampled and records of positive oomycete isolations from invertebrates captured during April–May (spring) and October–November (autumn) of 2005

		Oomycete isolations													
		Ant (*Lasius grandis*) bodies				Snail (*Helix aspersa*) bodies					*H. aspersa* faeces				
		Spring		Autumn		Spring			Autumn		Spring			Autumn	
						Positive[a]					Positive[c]				
Location of citrus orchard	Citrus cultivar	Positive[a]	Total[b]	Positive[a]	Total[b]	*Phytophthora*	*Pythium*	Total[b]	Positive[a]	Negative	*Phytophthora*	*Pythium*	Total	Positive[c]	Negative
Montesa	Fortune	1	22	0	18	0	2	12	0	0	0	0	4	0	0
Montesa	Clemenules	0	18	0	20	0	1	16	0	0	0	0	4	0	0
Canals	Fortune	3	27	0	18	2	2	15	0	0	0	1	5	0	2
Tabernes	Hernandina	0	18	0	16	0	2	12	0	0	0	0	5	0	3
Montserrat	Clemenules	2	27	0	18	1	0	16	0	0	1	2	4	0	2
Montserrat	Fortune	1	16	0	18	2	3	14	0	0	0	1	6	0	2
Montserrat	Arrufatina	0	18	0	16	0	1	12	0	0	1	2	6	0	0
Guadassuar	Fortune	1	27	0	20	1	2	16	0	0	0	0	4	0	2
Pedreguer	Hernandina	2	18	0	20	0	1	17	0	0	2	1	4	0	0
Ribaroja	Clemenules	2	18	0	18	1	2	15	0	0	0	0	5	0	3
Ribaroja	Fortune	1	18	0	18	1	1	14	0	0	0	3	5	0	0
Ribaroja	Clemenules	0	17	0	22	0	1	16	0	0	0	0	4	0	2
Bétera	Hernandina	3	20	0	18	1	3	14	0	0	0	0	3	0	2
Lliria	Arrufatina	3	24	0	22	2	2	15	0	0	0	0	3	0	0
Picassent	Hernandina	0	18	0	18	0	1	16	0	0	0	0	4	0	0
Percentage[d]		3·2	0	0	0	5·0	10·9	0	0	0	4·8	11·9	0	0	0

[a]Each positive value represents a specimen (ant or snail) from which an oomycete colony was isolated on PARBPH selective medium. All positive isolations from ant bodies were identified as *Pythium* species.
[b]Total number of captured specimens.
[c]Each positive value represents a snail faeces portion from which a colony of *Phytophthora* or *Pythium* was isolated on PARBPH selective medium.
[d]Percentage of oomycete colonies isolated from the total number of specimens captured.

replicates (moist chambers) each. Treatments 1 and 2 contained detached branches inoculated with *P. citrophthora*, whilst treatments 3 and 4 had branches inoculated with sterile PDA as controls.

Six days after inoculation, each inoculated branch was uncovered and the developing lesions exposed. The parts of the branches that were not colonized by expanding lesions were cut off and discarded. In treatments 1 and 3, 25 brown garden snails (European brown snail), *Helix aspersa*, and in treatments 2 and 4, 25 decollate snails, *Rumina decollata*, were placed in each chamber. Prior to this experiment, the snails were captured from sweet-orange orchards free from PBC and maintained for 3 weeks on grape leaves. Ten days before being released onto the infected branches, the snails were kept on a starvation diet.

The snails were allowed to feed for 5 h on infected branches. They were then collected using sterile tweezers, rinsed twice in sterile distilled water and immediately placed on sterile glass dishes for 12 h. Secreted fresh faeces were collected and placed on PARBPH selective medium, incubated in the dark at 24°C and the formation of colonies was observed within 2–3 days. Portions of the faecal material were also examined microscopically. Snails fed on branches inoculated with sterile PDA plugs served as controls.

Transport of *Phytophthora* spp. by means of the snail body

This experiment was carried out on 4-year-old citrus trees, 1·20 m high, grown in plastic pots (30 cm diameter × 40 cm deep) and belonging to the species: clementine cv. Clemenules and the hybrid mandarin cvs Fortune (*C. clementina* × cv. Dancy) and Nova [(*Citrus reticulata* × (*C. paradisi* × *C. reticulata*)], all grafted on Carrizo citrange (*Poncirus trifoliata* × *C. sinensis*). Eight days before the beginning of the experiment, the watering of the trees was stopped. Two isolates of *Phytophthora*: Phy 033 (*P. citrophthora*) and PS-89 (*P. palmivora*), originally recovered from badly affected citrus trees, and snails of the species *H. aspersa* collected from citrus orchards free from PBC and reared on grapes leaves, were used in all experiments.

A substrate (75% peat, 25% sand, v/v) was sterilized twice and distributed between three plastic trays (50 × 30 × 20 cm). The substrate in each plastic tray was immediately inoculated with 400 g V8-oat medium (200 g oat seeds and 120 mL V8 juice to 1 L distilled water). This medium had previously been inoculated by placing five plugs of agar colonized with each *Phytophthora* species on its surface and incubated in the dark at 24°C for 4 weeks. The third plastic tray was inoculated with 400 g sterile medium as a control. After inoculation, the trays were flooded with approximately 1·5 L sterile distilled water. Three days later, 100 snails, which had been starved for 10 days, were placed on the infested substrate and each tray covered with a net to avoid the snails escaping. All treatments were maintained under greenhouse conditions at 24 ± 2°C and 100% relative humidity. A day later, the snails were captured using sterile tweezers and placed at the base of each citrus tree, which had been well watered a day earlier. A tree was considered 'treated' when 10 snails had climbed on it. Snails confined on sterile substrate were used as controls.

The experiment consisted of three treatments (inoculation with Phy 033, Ps-89 and control) and four replications for each treatment–cultivar combination. Two experiments of the same design were conducted: the first from May to June (spring) 2006 and the second from August to September (summer) 2006. Treatments were maintained under glasshouse conditions at 24 ± 2°C. Assessments of individual trees were made 10 days after the release of the snails, on the basis of the symptoms: surface bark discolorations, cankers and gum exudations. Each of these symptoms was considered to represent an infection site. Additionally, these infection sites were categorized according to their distribution on the tree: lower, middle or upper third of the tree. At the end of the experiment, isolations onto PARBPH selective medium were made from all infection sites to confirm infection by the pathogen.

The number of plants infected and the number of lesions per plant were recorded. The average number of lesions per plant for each treatment from both experiments was analysed by Kruskal-Wallis analysis of variance (ANOVA) by ranks ($P < 0{\cdot}05$) and differences between treatments were identified using pairwise multiple comparisons by Dunn's method (SigmaStat, SPSS, Inc.). These methods were used because the data were not normally distributed and there was no appropriate transformation (Hoshmand, 1988).

Results

Association of ants, snails and snail faeces in the transmission of *Phytophthora* propagules

The ants captured were almost entirely of the species *Lasius grandis* (98%). During the study, 586 ants were processed, and *Pythium* spp. were isolated from only 3·2% of the samples, exclusively from ants captured in the spring months (Table 1).

A total of 335 snails were examined, of which 220 were identified as *H. aspersa* and 115 as *R. decollata*. Oomycetes were isolated only from *H. aspersa*: 10·9% of the isolates were identified as *Pythium* spp. and 5% as *Phytophthora* spp. (Table 1). On the basis of the morphological, physiological and molecular profiles, 91% of these *Phytophthora* isolates were identified as *P. citrophthora* and 9% as *P. palmivora*. During the study, 84 portions of snail faeces were processed: *Pythium* spp. were isolated from 11·9% of the samples and *P. citrophthora* from 4·8% (Table 1). *Phytophthora* species were isolated from snail bodies and faeces only during the spring months.

Transmission of *P. citrophthora* by *H. aspersa* under controlled conditions

Helix aspersa snails secreted 67 faecal portions and *P. citrophthora* was isolated from 79% (Table 2). *Rumina*

Table 2 Recovery of *Phytophthora citrophthora* colonies from faeces secreted by *Helix aspersa* and *Rumina decollata* snails fed on infected and healthy citrus branches

Infected branches				Healthy branches			
H. aspersa		*R. decollata*		*H. aspersa*		*R. decollata*	
Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
53	14	0	8	0	71	0	4

Each positive value represents a snail faeces portion from which a colony of *P. citrophthora* was isolated.

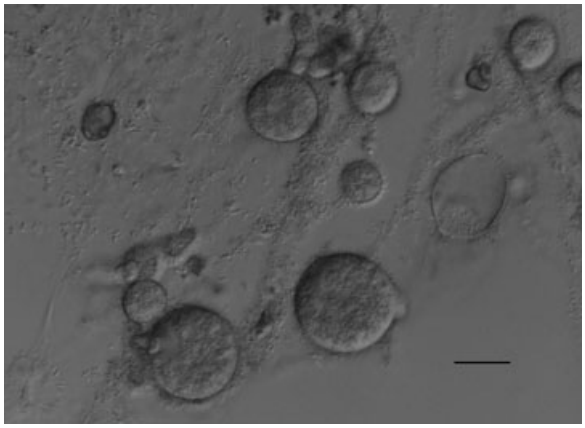

Figure 1 Microscopical observation of *Phytophthora citrophthora* chlamydospores on snail faeces. Bar represents 20 μm.

decollata snails produced eight faeces portions, but *P. citrophthora* was not identified. *Helix aspersa* and *R. decollata* snails fed on healthy branches inoculated with sterile PDA secreted 71 and 14 faeces portions, respectively. Isolation of *Phytophthora* was negative from these samples. Chlamydospores of *P. citrophthora* were microscopically detected in the faeces of snails fed on detached cankered citrus branches (Fig. 1).

Symptoms of gummosis, discoloration of the bark surface and canker formation were observed on citrus trees treated with snails previously fed on substrate containing *Phytophthora* (Fig. 2). These symptoms were similar to those observed in trees affected by PBC in the field. Incubation of the affected tissues on PARBPH selective medium confirmed that the *Phytophthora* spp. isolated from the infected bark were the species used as inoculum.

Differences among the citrus cultivars in their susceptibility to infection by *Phytophthora* and in the aggressiveness of the *Phytophthora* species to these cultivars were detected. Cultivars on which snails infested with *P. citrophthora* were released had the highest number of infection sites. Conversely, in both experiments, low incidence of disease was observed in citrus regardless of cultivar, with snails infested with *P. palmivora*. In the control treatments, no infection sites were detected on the trees.

With *P. citrophthora*, plants in experiments conducted during May–June had a greater incidence of infections

Figure 2 Gum and lesions of phytophthora branch canker on clementine bark surface 10 days after the release of snails (*Helix aspersa*) previously fed on substrate containing *Phytophthora citrophthora*.

than the plants in experiments conducted in August–September (Table 3). Among the cultivars, the incidence of infection was significantly greatest in cv. Fortune, followed by cv. Clemenules, and lowest in cv. Nova in both experiments (Table 3). The middle third of the tree had the most infection sites in all cultivars and in both experiments.

Discussion

The data obtained in this study strongly support the hypothesis that *P. citrophthora*, causal agent of phytophthora branch canker of citrus trees, can be transmitted by the brown garden snail, *H. aspersa*. This conclusion is based on the isolation of the pathogen from the bodies and faeces of naturally infested snails. Two glasshouse

Table 3 Incidence of branch canker infection sites on citrus cultivars 10 days after snails artificially infested with *Phytophthora citrophthora* (isolate Phy 033) were released on citrus trees under greenhouse conditions

	Number of lesions by tree[a]															
	Lower third					Medium third					Upper third					
	Tree					Tree					Tree					
Cultivars	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total	Total
May–June																
Clemenules	1	0	0	0	1	1	0	2	0	3	0	0	2	0	2	6b
Fortune	2	0	0	2	4	1	3	2	4	10	3	1	1	1	6	20a
Nova	1	0	0	0	1	0	2	0	0	2	0	0	0	0	0	3c
Total	4	0	0	2	6b[b]	2	5	4	4	15a	3	1	3	1	8b	
August–September																
Clemenules	0	0	0	0	0	2	1	0	0	3	0	0	0	0	0	3b
Fortune	0	2	1	0	3	0	0	2	3	5	0	0	0	2	2	10a
Nova	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0c
Total	0	2	1	0	3b	2	1	2	3	8a	0	0	0	2	2b	

[a]Gummosis, discoloration of the bark surface and cankers were considered as lesions 10 days after snail release onto the trees.
[b]Statistical analysis using Kruskal-Wallis analysis of variance by ranks and pairwise multiple comparison Dunn's method. Values for both test were significant at $P < 0{\cdot}05$.

procedures were used in this study; one demonstrated the snail's ability to transport propagules of *P. citrophthora* through the faeces in its digestive tract, and the other showed that lesions from which *Phytophthora* could be isolated developed on trees on which infested *H. aspersa* snails had been allowed to feed.

Snails have been associated with the dispersal of a number of plant pathogenic *Phytophthora* species (Turner, 1964; El-Hamalawi & Menge, 1996). In citrus, Magnano di San Lio & Pennisi (1984) showed that the unarmored snail *Agriolimax agrestis* transmitted, either directly or indirectly, propagules of *P. citrophthora* associated with brown rot of fallen citrus fruits on the orchard floor. Hardy (2004) demonstrated that snails can carry propagules of *P citrophthora* from infected fruits to healthy ones in the tree canopy.

Contaminated snails were only found during the May–June period. This implies that there is a temporal variability in the importance of *H. aspersa* as a natural vector of *P. citrophthora*. This could be related to a greater concentration of *P. citrophthora* in the soil during these months. In Mediterranean citrus plantations, *P. citrophthora* is active during the cooler seasons of spring, autumn and winter, but not during the summer (Erwin & Ribeiro, 1996; Alvarez *et al*., 2008a). In Spain, the greatest susceptibility of citrus to infection by *P. citrophthora* is during May–June, which is related to crop phenology (Alvarez *et al*., 2009). Greenhouse experiments with citrus cultivars in the current work using snails artificially infested with *Phytophthora* support this observation. In the first assay (May–June), a greater disease incidence in infection sites on the trees than in the second experiment (August–September) was observed, suggesting a possible effect of temporal host susceptibility to the infections of *Phytophthora*.

Coincidently, under natural conditions, the peak abundance of *H. aspersa* has been shown to occur in the spring months. In this period, the brown garden snail is able to ascend into the trees and cause damage within the aerial parts by feeding on the ripe and ripening fruits or the leaves of young trees (Melero, 2004). This species is nocturnal; however, following rain they may come out of their hiding places during the day (Dekle & Fasulo, 2002) and feed on organic matter in the soil, tree bark and, in particular, vegetation (Capinera, 2001).

Phytophthora citrophthora can survive as resistant sporangia and possibly chlamydospores in the soil or tree roots. Under well-aerated, moist conditions, chlamydospores can germinate immediately to form a sporangium (Graham & Menge, 1999). Snails move with a gliding motion by means of a long, flat muscular organ called a foot. Mucus, constantly secreted by glands in the foot, facilitates their movement and leaves a silvery, slimy trail (Dekle & Fasulo, 2002). Chlamydospores or hyphal fragments may stick to the snail mucus and be transported on the snail's body from the soil to aerial parts of the tree. Sprinkler and low-volume irrigation equipment often allow the development of high concentrations of both snail and *Phytophthora* species populations. In California, Fisher *et al*. (1980) observed that *H. aspersa* prospered in cultivated habitats with frequent irrigation. Moist soil conditions and temperature regimens thus increase the opportunities for the snails to disperse the pathogen. This association specifically in the spring months coincides with the greatest susceptibility of the host to PBC events on the tree.

In addition to its role in the transport of infectious propagules on its body, *H. aspersa* can indirectly transmit these propagules through its faeces. Collected faeces contained viable propagules of *P. citrophthora*, which

demonstrated the pathogen's resistance to the digestive secretion of the snail. This phenomenon has been observed previously (Kueh & Khew, 1982; El-Hamalawi & Menge, 1996). The giant African snail *Achatina fulica* is a major agricultural pest (Raut & Barker, 2002), and is considered one of the worst snail pests in the tropics and subtropics. This snail may also increase the spread of plant diseases by transporting *Phytophthora* propagules in its faeces (Muniappan *et al*., 1986).

In greenhouse experiments on citrus cultivars using snails artificially infested with *Phytophthora*, the propagules possibly infected the trees through microwounds generated by the hydration of the cells after the trees were watered, or by wounds created by snail feeding. In nature, *Phytophthora* infections occur through wounds and growth cracks on suberized tissues (Graham & Menge, 1999). Disease incidence among cultivars could be related to the extent of microwounds generated in each citrus cultivar. These studies also showed that the trees were more affected by infections of *P. citrophthora* than of *P. palmivora*, suggesting that *P. citrophthora* is more able to infect the bark via microwounds.

Attempts to isolate *P. citrophthora* from the body and faeces of *R. decollata* were unsuccessful. Therefore, its importance as a vector of *P. citrophthora* in the field is likely to be minimal. This snail was long considered a minor plant pest (Fisher, 1974), although it was recognized as being omnivorous. This species preys and feeds upon the eggs and flesh of small to medium sized *H. aspersa* snails (Fisher, 1974). Occasionally, it climbs trees, although it is primarily a ground dweller, lives among fallen leaves and sometimes burrows in the soil (Fisher *et al*., 1980).

In a previous study, El-Hamalawi & Menge (1996) elucidated the role of ants (*Iridomyrmex humilis*) in the transmission of infectious propagules of *P. citricola* from the sugary exudates around cankers to wounds on avocado stems and to the soil. In the present study, ants of the species *L. grandis* were able to transport *Pythium* spp. from the soil to the trees, but no *Phytophthora* species were isolated from their bodies. It was concluded that these ants do not play a role in transporting *Phytophthora* propagules from cankered lesions on the tree to healthy zones in the canopy.

Pythium species were isolated from the bodies of ants or snails captured in field. This gives evidence of the possible role of these invertebrates in the dissemination of potential pathogens. *Pythium* spp. can be found in greater proportions than *Phytophthora* species because of their saprophytic habits and greater competitive abilities. However, *Pythium* species are weakly pathogenic on citrus and have little relevance in tree infections.

In conclusion, there is an association between *P. citrophthora* and *H. aspersa*. A low number of snails per plant were sufficient to spread the pathogen, so the average level of infectivity of the vector was very high. Disease prevention strategies including vector control will be important in work to reduce spread of the disease.

Acknowledgements

The authors wish to thank Dr Owen Munday for reviewing the manuscript and the anonymous referees for their useful suggestions and comments.

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