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CITRUS BROWN ROT IN QUEENSLAND

By B. L. OXENHAM, B.Agr.Sc., and YOLANDE N. STONE, B.Sc.

SUMMARY

The occurrence of *Phytophthora nicotianae* var. *parasitica* and *P. citrophthora* and their roles in Queensland citrus orchards are described. It is shown that with the exception of Ellendale mandarin fruit, local strains of *P. nicotianae* var. *parasitica*, the widespread root and trunk pathogen, cannot infect uninjured fruit and leaves. *P. citrophthora*, on the other hand, readily causes a rot of both types of tissue. However, following injury, both organisms will cause typical brown rot symptoms at the same rate.

The restricted occurrence of brown rot and *P. citrophthora* in the State's orchards is considered. The possibility that a fruit-rotting strain of *P. nicotianae* var. *parasitica* may appear is discussed.

I. INTRODUCTION

Brown rot of citrus was first recorded in Queensland by Tryon in 1918 affecting oranges from Cairns, North Queensland (Tryon 1919). The organism concerned was identified by him as *Pythiacystis citriophora* (Smith), and could well have been *Phytophthora citrophthora* (Smith & Smith). From that time until 1936, sporadic outbreaks of the disease resulting in slight to severe losses in lemons, oranges and mandarins were reported from the major citrus-growing areas. Although in most cases the species of *Phytophthora* responsible was not determined, *P. citrophthora* was identified on occasions and in one outbreak *P. nicotianae* B. de Haan var. *parasitica* (Dast.) Waterh. was reported as the causal agent following severe fumigation injury. There were no records of the disease between 1936 and 1963.

After about 1940, *Phytophthora* root and collar rot became a common and often serious citrus disease in Queensland. During investigations carried out from 1953 to 1964, isolations were made from diseased trees in over 30

localities in this State. In every case *P. nicotianae* var. *parasitica* was the pathogen isolated. Despite the prevalence of this pathogen in the orchard soils and its activity as a root-rotting pathogen, brown rot of fruit was not recorded.

Following prolonged rains in the autumn of 1963, severe brown rot outbreaks were reported from a group of orchards in the Beerwah area in south-eastern Queensland. Severe leaf defoliation and twig blight were also recorded. All isolations from infected fruit and leaves yielded *P. citrophthora*. Control measures were applied but the disease has recurred in trace amounts and *P. citrophthora* has been isolated on each occasion.

P. citrophthora is considered to be a major root and trunk pathogen of citrus elsewhere in Australia and overseas. Although it has been reported as causing damping off, tip blight, and root, base and stem rot of seedling stock in Queensland nurseries, no record has been made of it causing collar rot of advanced trees in this State. It has been isolated from the roots and soil under trees where brown rot was severe.

P. nicotianae var. *parasitica* has been recorded as a root and collar rot pathogen in over 20 citrus producing countries, while it has been reported as a causal agent of brown rot in at least 10 countries, including California and Florida in the United States, Puerto Rico, Lesser Antilles, Spain, Palestine, Paraguay (Klotz 1943), Szechuan (Wei 1940), Venezuela (Malaguti 1951), France (Moreau 1954) and Morocco (Moreau and Moreau 1960).

The limited occurrence of brown rot in Queensland citrus orchards prompted investigations into the comparative abilities of the two species of *Phytophthora* occurring on citrus here to cause fruit and leaf disease.

II. MATERIALS AND METHODS

Four isolates of *P. nicotianae* var. *parasitica* and seven of *P. citrophthora* were compared. The sources of the isolates used are given in Table 1. Washington Navel and Late Valencia orange, Lisbon lemon, Marsh grapefruit and Emperor, Glen Retreat and Ellendale mandarin fruit were tested for susceptibility. Tip and leaf infection was tested on Rough lemon and Emperor mandarin.

In early experiments, comparisons were made with mycelial inoculations. In later experiments, zoospore inoculum, the usual source of natural infection, was used exclusively.

The fruit surfaces were sterilized with 1% sodium hypochlorite or 70% alcohol and then washed with sterile distilled water. Each fruit was inoculated at two or three sites. The fruit were maintained at 100% relative humidity for at least the first 48 hr after inoculations.

For inoculations with mycelium, small pieces of agar and mycelium from 5 to 10-day cultures grown on potato dextrose agar (P.D.A.) were used. In injured treatments, the inoculum was inserted in shallow wounds in the rind. In uninjured treatments, it was placed on the rind surface.

TABLE 1

SOURCE OF ISOLATES OF *Phytophthora citrophthora* AND *P. nicotianae* VAR. *parasitica*

Isolate	Disease	Location
P. citrophthora		
Isolate A	Brown rot of orange	Beerwah, S.Qd.
B	Brown rot of grapefruit	Brisbane, S.Qd.
C	Brown rot of orange	Beerwah, S.Qd.
D	Roots and soil under tree with history of brown rot	Beerwah, S.Qd.
E	Roots and soil under tree with history of brown rot	Beerwah, S.Qd.
F	Tip blight of Rough lemon seedlings ..	Eight Mile Plains, S.Qd.
G	Stem rot of orange seedlings	Eight Mile Plains, S.Qd.
P. nicotianae var. *parasitica*		
Isolate A	Root rot	Helidon, S.Qd.
B	Collar rot	Mundubbera, S.Qd.
C	Root rot	Bundaberg, Cent. Qd.
D	Root rot	Brisbane, S.Qd.

Zoospore inoculum was prepared in the following manner: Five to 9-day-old cultures of *P. nicotianae* var. *parasitica* grown on frozen bean agar (F.B.A.) (Carpenter and Furr 1962) at 25°C and *P. citrophthora* grown on F.B.A. or P.D.A. at the same temperature were flooded with 1-2 mm of sterile distilled water and incubated a further 2-3 days. The water was then changed and the temperature dropped for 20-25 min to 20°C for *P. nicotianae* var. *parasitica* and 16-18°C for *P. citrophthora.* After having been returned to air temperatures for 15 min, the zoospore suspensions were poured off and used immediately. The fruit were inoculated by placing drops of the suspensions of the test isolate on the surface of the rind. The drops were held in position by rings of white petroleum jelly. In a preliminary experiment it was found that a concentration equivalent to at least 10 zoospores per low power microscope field was necessary to obtain consistent infection. Suspensions of at least this concentration were therefore used in subsequent experiments.

The apical shoot tips and young leaves of seedlings grown in pots were inoculated with drops of zoospore suspension of both test fungi. The plants were then held at 100% relative humidity for 48 hr.

Fruit and seedlings treated in a similar manner to the inoculated materials but inoculated with sterile agar or distilled water were maintained as controls. Final ratings were taken 6-10 days after inoculation in all experiments.

III. RESULTS

(i) *Inoculation of orange fruit with mycelium.*—With wound-inoculated fruit, both species of *Phytophthora* produced typical brown rot symptoms at

approximately the same rate (Table 2). When inoculum was placed on uninjured fruit, infection readily occurred with all isolates of *P. citrophthora* but not with *P. nicotianae* var. *parasitica* (Figures 1 and 2).

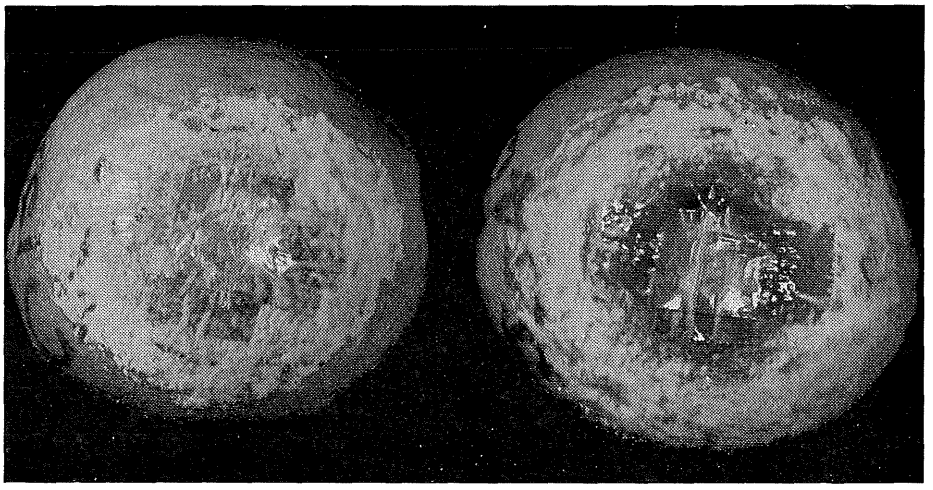

Fig. 1.—Inoculation following injury of Washington Navel orange fruit with mycelium of *P. citrophthora* (left) and *P. nicotianae* var. *parasitica* (right).

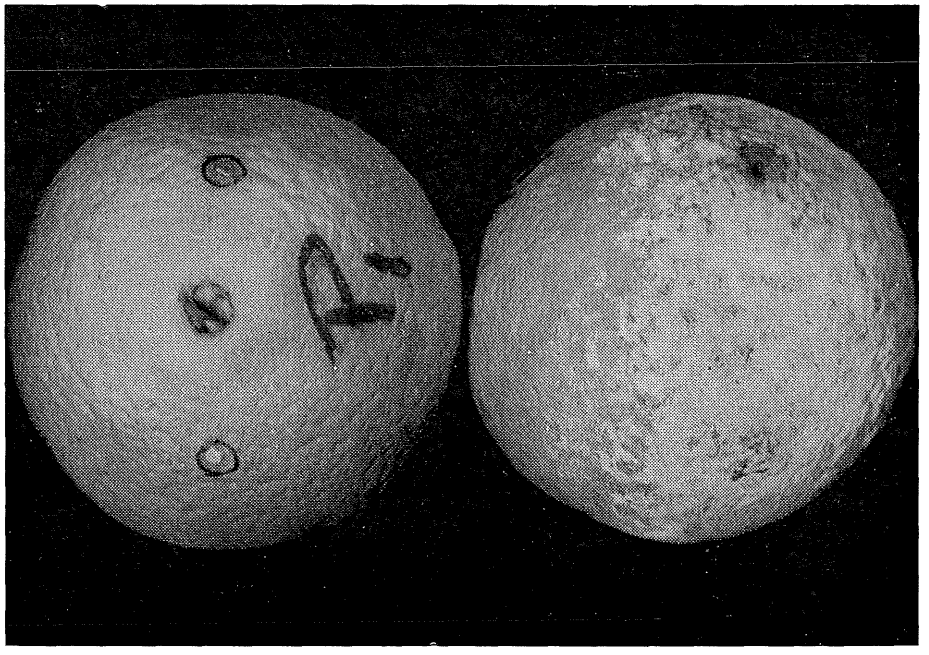

Fig. 2.—Inoculation without injury of Washington Navel orange fruit with mycelium of *P. citrophthora* (left) and *P. nicotianae* var. *parasitica* (right).

TABLE 2

INOCULATION OF WASHINGTON NAVEL ORANGE FRUIT WITH WITH *Phytophthora* MYCELIUM

Fungus	Isolate	Injured		Uninjured	
		Sites	No. Infected	Sites	No. Infected
P. nicotianae var. *parasitica*	A	..	..	4	0
	B	..	..	8	0
	C	12	12	8	0
	D	..	..	6	0
	Total ..	12	12	26	0
P. citrophthora	A	12	11	10	10
	B	..	..	6	5
	C	..	..	6	6
	Total ..	12	11	22	21

(ii) *Inoculation of orange fruit with zoospores.*—On uninjured fruit with *P. citrophthora* zoospores, 75 out of 101 inoculations produced lesions but only 2 of the 127 *P. nicotianae* var. *parasitica* inoculations were successful (Table 3). These two infections occurred in separate experiments. On injured fruit inoculated with zoospores of either one species or the other, disease symptoms were readily produced.

TABLE 3

INOCULATION OF ORANGE FRUIT WITH *Phytophthora* ZOOSPORES

Fungus	Isolate	Washington Navel		Late Valencia	
		Sites	No. Infected	Sites	No. Infected
P. nicotianae var. *parasitica*	A	32	0	30	0
	B	20	2	9	0
	C	12	0	12	0
	D	12	0	..	..
	Total ..	76	2	51	0
P. citrophthora	A	8	8	..	..
	B	12	9	18	17
	C	30	21	9	9
	D	12	4	..	..
	E	12	7	..	..
	Total ..	74	49	27	26

(iii) *Other fruit inoculations with zoospores.*—Results of inoculations of lemon, grapefruit and mandarin fruit are given in Table 4. Fruit were readily and consistently infected by *P. citrophthora* but, with the exception of Ellendale mandarin, not by *P. nicotianae* var. *parasitica.* In the case of Ellendale mandarin inoculated with *P. nicotianae* var. *parasitica,* the experiment was repeated with three batches of fruit, two of which were gathered directly from the tree to avoid surface injuries. Even with these precautions, 22% of the sites inoculated became infected.

TABLE 4

INOCULATION OF VARIOUS CITRUS VARIETIES WITH ZOOSPORES OF *Phytophthora nicotianae* VAR. *parasitica* AND *Phytophthora citrophthora*

Fungus	Isolate	Lisbon Lemon		Grapefruit		Emperor Mandarin		Glen Retreat Mandarin		Ellendale Mandarin	
		Sites	Sites Infected	Sites	Sites Infected	Sites	Sites Infected	Sites	Sites Infected	Sites	Sites Infected
P. nicotianae var. *parasitica*	A	3	0	2	0	3	0	9	0	27	5
	B	3	0	2	0	3	0	9	0	9	0
	C	..	..	1	0	..	..	9	1	27	9
	Total	6	0	5	0	6	0	27	1	63	14
P. citrophthora ..	D	3	3	2	1	3	3	6	6	12	12
	E	3	0	2	2	3	3	3	3	3	3
	F	3	2	2	2	3	3	6	5	12	10
	G	..	..	1	1	..	..	6	5	6	6
	Total	9	5	7	6	9	9	21	19	33	31

(iv) *Seedling tip inoculations with zoospores.*—On both Emperor mandarin and Rough lemon foliage inoculated with P. *citrophthora,* minute tan necrotic spots were observed on the youngest leaves within 48 hr. These developed rapidly, spreading throughout the leaf and adjoining stem tissue, resulting in the collapse of the whole of the young shoot within a few days. Four of the six mandarin and all of the six lemon seedlings inoculated were infected (Figure 3).

No such infection developed on the seedlings inoculated with *P. nicotianae* var. *parasitica.* It was observed that the zoospores on the surface of the leaves encysted, produced germ-tubes and occasionally appressoria-like structures, but did not penetrate the epidermal cells. On a few occasions where penetration was successful through apparently damaged cells or stomata, death of the surrounding layers of cells resulted. The resulting minute necrotic spot was observed within 48 hr. However, the fungus did not progress beyond this stage.

Fig. 3.—Tip blight of Rough lemon (left) and Emperor mandarin (right) caused by inoculation with *P. citrophthora* zoospores.

IV. DISCUSSION

These experiments have shown that once entry has been gained through an injury, *P. nicotianae* var. *parasitica* will cause a typical brown rot disease of citrus fruit at the same rate as *P. citrophthora,* the organism normally responsible in this State. However, it would appear that this strain of *P. nicotianae* var. *parasitica* cannot itself normally penetrate the rind and cause infection except in the case of Ellendale mandarin, which exhibits a not insignificant susceptibility. The slight infection recorded on Washington Navel orange and Glen Retreat mandarin suggests that small, unobserved injuries may have been present at the sites of inoculation. On the other hand, all species and varieties tested proved very susceptible to *P. citrophthora.*

It is apparent also that this strain of *P. nicotianae* var. *parasitica* will not infect citrus leaves or cause tip blight. A necrotic flecking was observed similar to the reaction described by Pristou and Gallegly (1954) on leaflets of the resistant potato variety Kennebec inoculated with *P. infestans,* potato race A. *P. citrophthora,* however, was again very effective and produced typical symptoms of leaf and tip infection.

The limited distribution of *P. citrophthora* restricts the brown rot and seedling disease problems to a few isolated areas in the State. It is not understood why the organism is not widespread as is the case with *P. nicotianae* var. *parasitica.* It is felt that temperature may not entirely be the limiting factor, as other species of *Phytophthora* with very similar cardinal temperatures occur commonly even in the far northern coastal regions. Also, it is not understood why *P. citrophthora* is not a serious problem as a trunk and root pathogen in those areas where it does occur.

There are a number of other citrus-growing areas where *P. nicotianae* var. *parasitica* is present only as a trunk and root pathogen and not as a causal agent of brown rot. This was the case in Florida until 1951, when brown rot of fruit on the tree first appeared. Rotting of fruit on the ground, however, had been previously reported. Knorr (1956) suggested that this relatively recent occurrence may be explained by the introduction of a fruit-rotting strain of the fungus or by the prevalence of unusually favourable weather conditions. Variations in the pathogenicity of citrus isolates of *P. nicotianae* var. *parasitica* in causing root rot in Florida have been shown by Grimm and Whidden (1963). It appears highly probable that a similar variation could exist within the species in its fruit-rotting abilities. The possibility that a fruit-rotting strain of *P. nicotianae* var. *parasitica* capable of infection without prior injury could also appear in Queensland should not be overlooked.

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B. L. Oxenham is an officer of the Queensland Department of Primary Industries. Mrs. Stone (resigned) was formerly a Departmental officer.