Sudden Wilt of Passionfruit in Southern Florida Caused by *Nectria haematococca*

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ABSTRACT

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A sudden wilt of Possum Purple passionfruit (*Passiflora edulis* × *P. edulis* f. *flavicarpa*) has been recognized for about 5 yr in southern Florida. Results from artificial inoculation studies indicate that a collar rot and canker disease, incited by homothallic strains of *Nectria haematococca* (anamorph: *Fusarium solani*), causes sudden wilt. Monoconidial strains of the fungus always caused cankers on wounded vines but did so inconsistently on nonwounded vines. When vines were not wounded, canker development was greater on younger than on older vines. Artificially infected plants supported large cankers without wilting, defoliation, or growth reduction. Reductions in vine growth and vine mortality occurred only after stems became completely girdled. The pathogen was recovered from necrotic and apparently healthy roots and from symptomless vine tissue, from both rooted cuttings from a commercial nursery and from tissue distal to cankers on naturally and artificially infected vines. Thus, it is possible that cuttings taken from latently infected vines may perpetuate the disease. This is the first detailed report of Nectria canker in the Western Hemisphere.

Passiflora is a diverse genus of mainly vines, most of which are endemic in the neotropics (4,6). About 50–60 species of *Passiflora* bear edible fruits. The juice and pulp of these fruits possess exceptional organoleptic qualities, and they are important throughout the tropics as components of ice cream, sherbert, and various drinks and desserts. A small passionfruit industry in south Florida is based on cultivars of *P. edulis* Sims and *P. edulis* f. *flavicarpa* Degener and hybrids of the two taxa (R. J. Knight, Jr., *personal communication*).

For about 5 yr, a sudden wilt syndrome of unknown etiology has affected production of the most important passionfruit cultivar in southern Florida, Possum Purple (*P. edulis* × *P. e. flavicarpa*). The syndrome affects plantings usually within 1–2 yr of planting. Vines wilt suddenly and, subsequently, do not recover turgor or shed leaves and fruit. Before wilting, vines usually do not exhibit chronic decline symptoms; leaves remain green and exhibit no loss of turgor and plants may continue to set fruit until they terminally wilt. Although cankers are often found on these plants, they are usually inconspicuous, occurring either under vine supports or at and beneath the soil line. Roots on affected plants are often, but not always, necrotic.

Preliminary work implicated *Nectria haematococca* Berk & Broome (anamorph: *Fusarium solani* (Mart.) Sacc.) as the cause of cankers and the wilt syndrome of Possum Purple described earlier (10). *N. haematococca* has been reported twice previously, in Taiwan and Uganda, as the cause of a collar rot of passionfruit (3,5). The objectives of the current study were to investigate the role of *N. haematococca* in the sudden wilt syndrome in southern Florida and certain aspects of the epidemiology of this disease.

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MATERIALS AND METHODS

Isolations. Isolations from symptomatic and nonsymptomatic roots, rooted cuttings, and artificially and naturally infected vines were made either on PCNB medium (8) or on 1.5% water agar (WA) amended with 100 mg of streptomycin sulfate, 10 mg of rifamycin, and 0.1 ml of Danitol 2 HEC miticide (Chevron Chemical Co., San Francisco, CA) per liter. Tissue was surface-disinfested for 20 s in 70% ethanol and blotted dry with sterile paper towels before it was placed directly on PCNB medium or submerged in molten (45 C) amended WA. During experiments in which movement of the pathogen within vines was assessed, tissue was disinfested for an additional 60 s in 0.525% NaClO and rinsed once in sterile deionized water before it was blotted and placed on PCNB medium.

Monoconidial strains of *N. haematococca* were obtained by streaking conidia from colonies that developed on the above media on water agar. Within 20 hr, germinated microconidia were cut from these plates using sterile, sharpened spatulas, and a binocular microscope at ×100 and transferred, individually, to plates of Difco potato-dextrose agar (PDA). Monoconidial strains of interest were stored for future use both on filter paper, as described by Correll et al (2), and in sterile 50% glycerol at −80 C. One of these strains, PF(N)3, has been deposited in the American Type Culture Collection, Rockville, MD.

Artificial inoculations. Five experiments were conducted using various inoculation methods. Vines of Possum Purple, produced from rooted cuttings, were used in each experiment. Before inoculation, plants were either not wounded or were cut three times with incisions 3 mm long and 1 mm apart, perpendicular to the longitudinal axis of the stem. Isolates of *N. haematococca* were grown either on sterile millet seed or PDA, and two to three colonized seed or a 5-mm^2 piece of mycelium, stripped of excess agar, were placed on wounded or nonwounded stems. Inoculum was sealed on stems with either Parafilm or masking tape and was removed after 5–7 days. Uninoculated controls consisted of

wounded or nonwounded sealed stems or wounded stems mock-inoculated with noncolonized millet seed or agar.

In general, growth of vines was assessed every 2–7 days after inoculation as the linear extension of the vine apex; daily growth rates were calculated in cm day^{-1}. Lesion size was assessed at the same intervals after inoculation and was recorded either as canker length or as the percentage of the vine circumference that was girdled. Analyses of variance, mean separations, and regression analyses were conducted with SAS statistical programs for personal computers (12).

RESULTS

N. haematococca was usually recovered from cankers on dead or dying plants from the field, but rates of recovery were generally highest for plants with intact, well-defined cankers and lowest for badly decayed vines. Although other fungi were also recovered from affected vines (e.g., *Alternaria* sp., *Botryodiplodia* sp., *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., *Fusarium oxysporum* Schlechtend.:Fr., *Fusarium* sp., etc.), none of these fungi produced cankers on plants when used in inoculation studies (*data not shown*). All 18 monoconidial strains of *N. haematococca* that were recovered from naturally and artificially infected passionfruit vines formed perithecia on autoclaved vine sections on PDA or on amended WA after 3–4 wk of incubation under fluorescent light and had a distinct cultural morphology on PCNB medium. Only fungi that met these criteria caused cankers on Possum Purple.

Cankers were easily reproduced during each of five artificial inoculation experiments; representative results from three of the experiments are shown in Table 1. Canker development was always greater on wounded than on nonwounded plants and on young vines rather than old vines. The type of inoculum used (infested millet seed or PDA cultures) and the manner in which inoculum was sealed on the stem (Parafilm or masking tape) had no effect on subsequent canker development. Cankers never developed on control plants.

The pathogen was always recovered from cankers on artificially inoculated plants and was also recovered from 5 cm below and as high as 25 cm above the point of inoculation (Fig. 1). The xylem in stem sections from which the pathogen was recovered was usually discolored, although the fungus was also recovered from nondiscolored tissue about 15% of the time (*data not shown*). In addition, the pathogen was recovered from roots and stems of healthy plants (i.e., without cankers) that were obtained from a commercial nursery (Table 2).

Only wounded, inoculated plants died during artificial inoculation studies and then only after vines were completely girdled. In general, plants supported large cankers without becoming chlorotic or wilting and without a reduction in growth (Table 1 and Fig. 2). Plants exhibited conspicuous reductions in growth only shortly before they wilted and died.

Table 1. Effects of homothallic strains of *Nectria haematococca* and different inoculation methods on the development of cankers on vines of *Passiflora edulis* × *P. e.* f. *flavicarpa* 'Possum Purple'

Treatment	Mean canker size[v] (% stem circumference) Experiment[x] 1	2	3	Mean growth rate[w] (cm day^{-1}) Experiment[x] 1	2	3
Nonwounded, uninoculated	0.0 c	...	...	4.6 a	...	...
Wounded, mock inoculation[y]	0.0 c	...	0.0 b	4.6 a	...	2.0 a
Wounded, mock inoculation[z]	0.0 c	0.0 c		4.8 a	4.5 a	...
Nonwounded, inoculated with strain PF(N)3	17.5 b	35.0 b	...	4.8 a	4.2 a	...
Wounded, inoculated with strain PF(N)3	45.0 a	70.0 a	...	4.4 a	2.1 b	...
Wounded, inoculated with strain PF(N)5	43.3 a	...	..	4.2 a	...	...
Wounded, inoculated with strain PF(N)9	45.0 a		75.0 a	4.0 a		1.5 a
Wounded, inoculated with strain PF(N)14	46.7 a		...	4.7 a		...

[v] Percentage of the stem circumference that was necrotic.
[w] Mean growth rate of vines for the week before the measurement of canker size.
[x] All experiments were conducted with rooted cuttings of Possum Purple. Plants in experiments 1–3 were about 6, 2, and 1 mo old, respectively, and treatments were replicated six times in each experiment. Inoculum for experiments 1 and 2 consisted of millet seed colonized or not colonized with a strain of *N. haematococca*, and for experiment 3 consisted of PDA colonized or not colonized with the fungus. Within a column, mean canker sizes and growth rates followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$ (for canker data, 0-values were set at 0.1 and data were arcsine-square root transformed before mean separation).
[y] Plants in treatment 2 were inoculated with sterile millet seed.
[z] Plants in treatment 3 were not inoculated but were still wrapped with Parafilm.

Table 2. Recovery of *Nectria haematococca* from apparently healthy Possum Purple vines from a commercial nursery[w]

Location[x]	Mean recovery[y] (%)	Production of perithecia[z]	Production of cankers[z]
Roots	44	Yes	Yes
+ 5 cm	83	Yes	Yes
+ 10 cm	33	Yes	Yes
+ 15 cm	0	...	...
+ 20 cm	0	...	...

[w] *N. haematococca* was recovered from surface-disinfested tissue from vines without cankers; tissue was plated on PCNB medium (8). These are results from one of two recovery experiments. Although the magnitudes of recovery varied between experiments, trends for both were similar.
[x] Tissue was recovered from roots or from various distances above the stem end.
[y] Recovery of *N. haematococca* is expressed as means for six vines; six 1-cm-long root segments and a single disk of tissue from each of four locations above stem ends (vines were produced from rooted cuttings) were assayed for each plant.
[z] Representative strains of *N. haematococca* that were recovered from vines were tested for production of perithecia on sterile vine sections on PDA and for the production of cankers on Possum Purple.

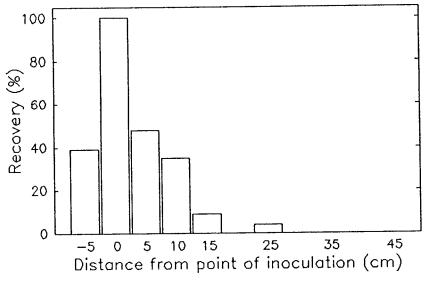

Fig. 1. Relative recovery of *Nectria haematococca* from various locations on artificially inoculated vines of Possum Purple; locations are relative to the point of inoculation (0 cm). Each bar represents the percentage of 24 disks, one from each of 24 plants, from which the pathogen was recovered on PCNB medium (8). Data are from one of two experiments conducted on pathogen movement within vines.

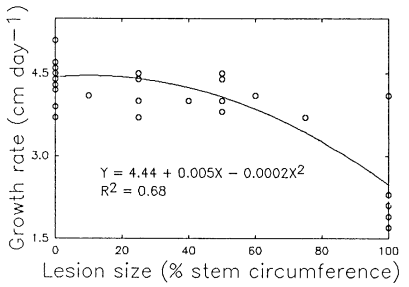

Fig. 2. Influence of size of cankers caused by *Nectria haematococca* on growth of Possum Purple vines. Lesion size is the percentage of the stem circumference that was necrotic (completely girdled stems = 100%), and growth is the daily growth rate of the vine for the week before the data on which lesion size was evaluated. Data are from a single experiment and represent three consecutive weekly disease assessments on a total of six noninoculated and 12 inoculated Possum Purple vines. Trends from two other growth rate studies, which are not shown, were similar.

DISCUSSION

Results from the current work provide evidence for *N. haematococca* as the cause of sudden wilt of passionfruit in southern Florida and corroborate the insidious nature of this disease. Artificially inoculated plants exhibited conspicuous symptoms (i.e., acute wilting) without prior evidence of chronic distress and only after cankers had completely girdled stems. In addition, recovery of the pathogen from apparently healthy nursery plants and from points distal to cankers formed on artificially and naturally infected vines indicates that Nectria canker may be perpetuated when latently infected plants are used to produce rooted cuttings (rooted cuttings are the primary propagation material used in commercial production of Possum Purple).

This is the first detailed report on Nectria canker of passionfruit from the Western Hemisphere. Previously, Nectria canker was reported from Taiwan and Uganda (3,5). Other reports on nonspecified wilts, collar rots, and diebacks of passionfruit, however, suggest that Nectria canker may be more widespread than is currently recognized. A base rot of passionfruit with symptoms similar to Nectria canker, but with an unknown etiology, was recognized by Simmonds in Australia (13). Fusarium collar rots (species of *Fusarium* not reported) have also been reported as production constraints on Reunion and in South Africa (1,14), and *F. solani* has been associated with a dieback and enhanced development of anthracnose, caused by *C. gloeosporioides*, of passionfruit in Surinam (11). Although authors of the latter report were able to experimentally produce a root rot and girdle stems of *P. e. flavicarpa* with *F. solani*, they indicated that sudden death of vines, as reported in Uganda, did not occur in Surinam. Interestingly, these authors also reported the formation of perithecia of *N. haematococca* on infected plants in Surinam.

In 1973, Matuo and Snyder (7) reported that all formae speciales and races of *Hypomyces solani* (*N. haematococca*) from the United States and Japan that they had tested were heterothallic. They also indicated that some homothallic strains of this fungus had been collected in the United States and Japan but that they were all saprophytic. Subsequent to the publication of Matuo and Snyder (7), a limited number of reports have indicated that plant-pathogenic, homothallic strains of this fungus exist but that they are relatively uncommon (3,9,10,15).

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