Pathogenicity of *Rhizoctonia* Isolates to Papaya in Hawaii

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ABSTRACT

Yamamoto, D. T., and Aragaki, M. 1982. Pathogenicity of *Rhizoctonia* isolates to papaya in Hawaii. Plant Disease 66:1136-1137.

Based on morphological characteristics of vegetative hyphae, nuclear numbers, and hyphal anastomoses, 15 isolates obtained from papaya roots were in anastomosis group 4 of *Rhizoctonia solani*. Twelve of these isolates were pathogenic to papaya, killing 24.4–98.8% of inoculated seedlings. Fourteen binucleate isolates of *Rhizoctonia* were not pathogenic to papaya seedlings.

Additional key words: *Carica papaya, Ceratobasidium, Thanatephorus cucumeris*

Before 1970, identification of *Rhizoctonia solani* (Kühn) was based primarily on morphology of the vegetative hyphae. Unfortunately, because these are not sufficiently distinctive for accurate identification, many *R. solani*-like fungi were erroneously called *R. solani*, resulting in a chaotic grouping of taxonomically unrelated fungi (4,5). More reliable means were needed to characterize *R. solani* adequately.

Studies have demonstrated that *R. solani* (teleomorph: *Thanatephorus cucumeris* (Frank) Donk) has multinucleate vegetative hyphal cells, whereas other *Rhizoctonia* species (teleomorph: *Ceratobasidium*) have binucleate vegetative hyphal cells (6). These findings, verified by Tu and Kimbrough and Tu et al (12–14), provided additional information to facilitate separation of *R. solani* from other species of *Rhizoctonia*.

Hyphal anastomosis groups (AG) in *R. solani*, representing possible genetic isolation, do not represent host specialization, although some tendencies are evident (3,4,9). Earlier workers identified four anastomosis groups (7,8), but a fifth group has recently been added along with subdivisions of AG-1 and AG-2 groups (1,3,9). Vegetative characteristics, such as narrow hyphal diameters (3,9), presence of runner hyphae (9), and colony topography (9), are distinctive for AG-4; other vegetative characteristics have been useful for separating anastomosis groups (9).

Rhizoctonia has been reported to cause damping-off of papaya (*Carica papaya* L.) seedlings (2,10,11), but without documented pathogenicity studies and species identification. To establish the pathogenicity and identity of *Rhizoctonia* sp. causing damping-off of papaya seedlings in Hawaii, the following study was undertaken.

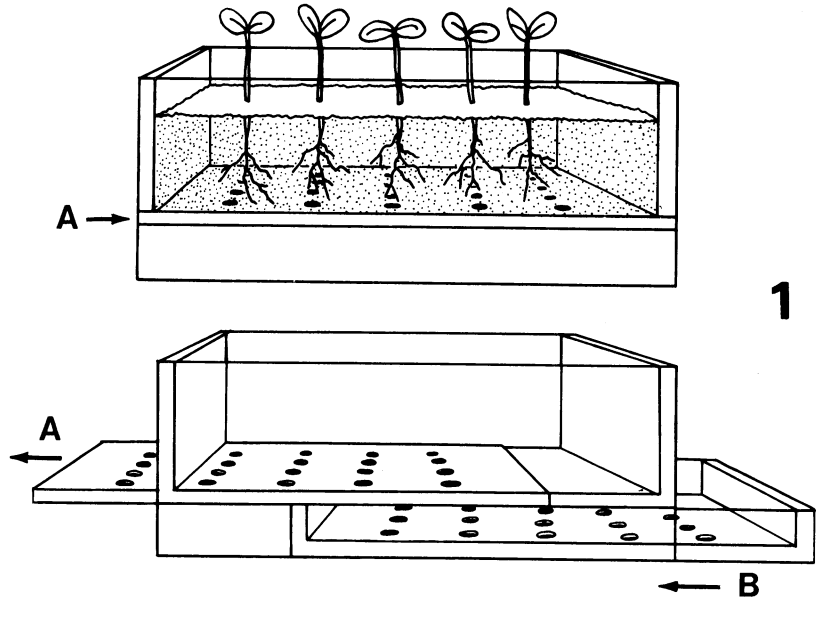
MATERIALS AND METHODS

Twenty-nine isolates of *Rhizoctonia* obtained from papaya root rots on the islands of Kauai, Oahu, Maui, and Hawaii were used. All isolates were maintained on vegetable juice agar (VJA; 10 ml of Campbell's V-8 juice, 0.2 g of calcium carbonate, 1.8 g of agar, and 90 ml of deionized water) at 22 ± 2 C under 2,700 lux of continuous light provided by fluorescent lamps. Fifteen isolates were multinucleate and, based on Parmeter's criteria (with the exception of the formation of the perfect state), were determined to be *R. solani*. The other 14 isolates were binucleate. Determination of the nuclear number of the vegetative hyphal cells was made with acridine orange fluorescence (15). The 15 *R. solani* isolates were tested for anastomosis group with nine AG testers supplied by E. E. Butler, University of California, Davis, using dialyzing membrane strips placed on VJA and patterned after the cellophane strip method of Ogoshi (3).

Approximately 200 seeds of papaya cv. Sunrise Solo were sown in vermiculite contained in Plexiglas planting boxes (Fig. 1). These boxes were kept in a glasshouse with a temperature range of 18 to 29 C. Germination occurred in about 2 wk, and the number of seedlings was recorded.

Inoculum was prepared by growing isolates on glass microfibre filters (Whatman Glass Microfibre filters, 7.0 cm) that had previously been placed on the surfaces of 24 ml of 10% VJA in 9-cm petri plates. Inoculum cultures were allowed to grow under ambient laboratory conditions (about 24 C) for 4 days.

The seedlings were inoculated 1 wk after germination. Ten glass microfibre filter cultures of each isolate were placed on a layer of vermiculite in each inoculum tray (Fig. 2). The inoculum tray was then



Figs. 1 and 2. Planting box **(1)** with sliding base **(A)** in place. Planting box **(2)** with base **(A)** being removed and sliding tray **(B)** for inoculum partially in place.

Journal Series 2649 of Hawaii Institute of Tropical Agriculture and Natural Resources.

Accepted for publication 3 March 1982.

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0191-2917/82/12113602/$03.00/0

Table 1. Pathogenicity of 29 papaya root isolates of *Rhizoctonia* to papaya seedlings[a]

Isolates	No. of isolates	Seedlings inoculated[b]	Percentage of seedlings killed
Rhizoctonia solani			
Virulent	11	2,264	92.2
Moderately virulent	1	258	24.4
Avirulent	3	759	0.1
Rhizoctonia sp.			
(Binucleate)	14	2,813	0.1
Control (uninoculated)	···	496	0.0

[a]Composite of two tests.
[b]Minimum of 150 seedlings were inoculated by a given isolate.

placed under the planting box and the sliding base was removed (Fig. 2). Established seedlings were then in contact with the inoculum with minimal damage to the root system. The number of seedlings that were killed by each isolate during 3 wk following inoculation was recorded. The experiment was repeated.

The fungus was reisolated from killed seedlings by taking a portion of the infected hypocotyl, surface-sterilizing in 0.5% sodium hypochlorite, and then plating on 1.8% water agar. Identification was based on the nuclear number and morphological characteristics of the cells in the vegetative hyphae.

RESULTS

All 15 *R. solani* isolates were determined to be in AG-4, based on hyphal anastomosis studies. These isolates also had narrow hyphal diameters, runner hyphae, and mealy colonies, which are vegetative characteristics usually associated with AG-4 (3,9).

Based on two tests, the average percentage of seedlings killed by the *R. solani* isolates ranged from 0 to 98.8%. Eleven of these isolates were highly pathogenic (>68% mortality); isolate Y-11 was moderately pathogenic; and isolates Y-1, Y-2, and Y-21 were nonpathogenic (Table 1). Reisolation of *R. solani* was accomplished for all 12 pathogenic isolates.

All 14 binucleate isolates were avirulent to the papaya seedlings (Table 1). Two seedlings inoculated with Y-15 died in the first test, but no seedlings were killed by this isolate in the second test. Binucleate *Rhizoctonia* was reisolated from both seedlings. One seedling inoculated with isolate Y-33 died in the second test, but no fungus was recovered from this seedling.

Damping-off occurred within a week and continued through the second week, when papaya seedlings were inoculated with the highly pathogenic isolates. Few seedlings were affected after the second week.

Water-soaked regions developed at the transition zone of the hypocotyl of most seedlings. Brown, sunken lesions (10 mm) also occurred in this area on some seedlings. Reisolation was successful from these infected hypocotyl regions but not from the roots, which remained healthy.

DISCUSSION

R. solani is a well-known soilborne organism causing seedling damping-off and root rots. The importance of making accurate identifications of *R. solani*-like fungi was borne out by the demonstrated avirulence to papaya of binucleate isolates. The isolation of avirulent binucleate *Rhizoctonia* sp. from some of the papaya root lesions indicates that the causal organism was missed in these cases. We suggest that *R. solani* may have been present but was overlooked in a random selection of hyphal tips. Rapid and simple methods for determining nuclear numbers have recently been developed (15), reducing the tedium of identifying *R. solani*. The nuclear condition of the vegetative hyphal cells is a key characteristic and should be included in any study of *Rhizoctonia*. Accurate identification of *R. solani* and other fungi in the *Rhizoctonia* complex is essential in order not to confound further the knowledge and literature on this important group of plant-pathogenic fungi.

LITERATURE CITED

1. Adams, G. C., Jr., and Butler, E. E. 1979. Serological relationships among anastomosis groups of *Rhizoctonia solani*. Phytopathology 69:629-633.
2. Hine, R. B., Holtzmann, O. V., and Raabe, R. D. 1965. Diseases of papaya (*Carica papaya* L.) in Hawaii. Hawaii Agric. Exp. Stn. Bull. 136. 25 pp.
3. Ogoshi, A. 1975. Studies on the anastomosis groups of *Rhizoctonia solani* Kühn. JARQ 9:198-203.
4. Parmeter, J. R., Jr., Sherwood, R. T., and Platt, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59:1270-1278.
5. Parmeter, J. R., Jr., and Whitney, H. S. 1970. Taxonomy and nomenclature of the imperfect state. Pages 7-19 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
6. Parmeter, J. R., Jr., Whitney, H. S., and Platt, W. D. 1967. Affinities of some *Rhizoctonia* species that resemble mycelium of *Thanatephorus cucumeris*. Phytopathology 57:218-223.
7. Richter, H., and Schneider, R. 1953. Untersuchungen zur morphologischen und biologischen Differenzierung von *Rhizoctonia solani* K. Phytopathol. Z. 20:297-307.
8. Schultz, H. 1936. Vergleichende Untersuchungen zur Oekologie, Morphologie, und Systematic der "Vermehrungspilzes." Arb. Biol. Reichanst. Land Forstwirtsch. Berlin-Dahlem 22:1-41.
9. Sherwood, R. T. 1969. Morphology and physiology in four anastomosis groups of *Thanatephorus cucumeris*. Phytopathology 59:1924-1929.
10. Traub, H. P., Robinson, T. R., and Stevens, H. E. 1942. Papaya production in the United States. U.S. Dep. Agric. Circ. 633. 36 pp.
11. Tsai, W. H. 1970. Studies on the papaya damping-off disease. J. Taiwan Agric. Res. 19:51-56.
12. Tu, C. C., and Kimbrough, J. W. 1975. Morphology, development, and cytochemistry of hyphae and sclerotia of species in the *Rhizoctonia* complex. Can. J. Bot. 53:2282-2296.
13. Tu, C. C., Kimbrough, J. W., and Aldrich, H. C. 1977. Cytology and ultrastructure of *Thanatephorus cucumeris* and related taxa of the *Rhizoctonia* complex. Can. J. Bot. 55:2419-2436.
14. Tu, C. C., Roberts, D. A., and Kimbrough, J. W. 1969. Hyphal fusion, nuclear condition, and perfect stages of three species of *Rhizoctonia*. Mycologia 61:775-783.
15. Yamamoto, D. T., and Uchida, J. Y. 1982. Rapid nuclear staining of *Rhizoctonia solani*-like fungi with acridine orange or safranin O. Mycologia 74:146-149.