Rhizoctonia solani Anastomosis Groupings in Carnation Fields and Their Pathogenicity to Carnation

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ABSTRACT

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Isolations from carnation stem rot and from soil in carnation problem fields yielded high numbers of *Rhizoctonia solani*-like fungi. Approximately 70% of soil isolates were *R. solani*, the remainder were binucleated fungi. Approximately 70% of *R. solani* from soil were AG-4, 20% were AG-2-2, and 10% were AG-2-1. AG-2-2 was pathogenic to carnation, AG-4 was weakly pathogenic, and AG-2-1 was avirulent. Among carnation cultivars, Atlantis and Red Diamond were the most resistant of nine cultivars tested. *R. solani* AG-2-2 was recovered from soil of two farms out of 11 surveyed.

Additional keyword: multinucleate

The primary carnation production area of Hawaii is in Kula on the island of Maui. A stem and root rot has been one of the important recurring problems experienced by carnation growers (6). Isolations from diseased specimens have primarily yielded *Rhizoctonia solani*-like fungi. Isolations from soil, using an ethanol-potassium nitrate medium (7) have also yielded significant numbers of *R. solani*-like fungi. Because of the continuing and regular losses incurred by carnation growers in Kula, present studies were undertaken to elucidate the etiology of the disease and to develop control methods.

Rhizoctonia stem rot is not considered to be an important disease in carnation culture, and crown rot is not mentioned as a problem in a discussion of carnation diseases (1). The high incidence of *R. solani*-like fungi in isolations from diseased tissue (8) and soil suggested that the pathogenicity and identification of these organisms should be determined so that epidemiological and control studies can be conducted with a substantive understanding of the disease.

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MATERIALS AND METHODS

Populations of *Rhizoctonia solani* Kühn and binucleate *R. solani*-like fungi (BRS) were determined in 1985–1986 at 11 carnation farms from Kula, Maui using an ethanol-potassium nitrate (EKN) agar, selective for *R. solani*-like organisms (7), and a safranin O nuclear staining method (9). Soil samples collected at random from various locations within each carnation field were combined and a 10-g aliquot of soil was placed in 250-ml Erlenmeyer flasks with 100 ml of tap water and shaken vigorously for 1 min. The soil suspension was poured through a 200-mesh screen and the soil fraction collected on the screen was backwashed into a beaker. The new suspension was stirred and allowed to settle. After silt and other soil

particles had settled, the liquid portion was decanted through a 200-mesh screen to collect the organic matter that was washed into two or more plates of EKN agar. The soil fraction in the beaker was plated on several dishes of the selective medium. The soil assay dishes were incubated for 3 days at room temperature (26 ± 2 C) and *R. solani*-like fungi (RSF) were enumerated. Hyphal tip isolations were made from each RSF colony that was enumerated, and were grown on water agar in order to select bacteria-free hyphal tips that were grown on 2% V-8 agar (20 ml of V-8 juice, 0.4 g of $CaCO_3$, 18 g of agar, 980 ml of water). Seven-day-old cultures on 2% V-8 agar were used for nuclear determinations. The nuclear number in young vegetative cells was determined after staining with 0.5% safranin O and 3% KOH solutions (9). Isolates with multinucleate cells were recognized as *R. solani*, and isolates with binucleate cells were BRS fungi (5). Isolates of *R. solani* were grown on 10% V-8 agar (100 ml of V-8 juice, 2.0 g of $CaCO_3$, 18 g of agar, 900 ml of water). Cubes from these cultures were subcultured on sterile dialysis membranes over 2% V-8 agar to determine their anastomosis grouping (2,3).

R. solani isolated from diseased plants with typical symptoms of basal stem and crown rot, and *R. solani* and BRS fungi isolates from field soil were used for pathogenicity tests on different carnation cultivars. Binucleate *R. solani*-like fungi and *R. solani* isolates were initially tested for pathogenicity on two carnation cultivars, Peterson Red Sim and Improved White Sim. Three isolates of BRS fungi, two isolates of *R. solani* AG-4, and one isolate of *R. solani* AG-2-2 obtained from different farms were used in this test.

Inoculum for testing pathogenicity was prepared by inoculating a sterilized vermiculite/V-8 juice mix (4 g of vermiculite, 4 ml of 20% clarified V-8 juice, and 10 ml of deionized water per 9-cm petri dish) with four 5-mm-diameter disks of 7-day-old cultures growing on 10% V-8 agar. The inoculated mix was incubated for 7 days at 26 C under fluorescent lights. Six-inch azalea pots filled with potting soil (Lilly Miller) were infested with 4 g of the inoculum and mixed to a depth of 5 cm. Five pots were infested with each isolate and five uninoculated control pots were planted with two rooted cuttings of each cultivar. This test was done in a greenhouse in Manoa, Oahu with average day and night temperatures of 30 and 25 C, respectively.

Subsequent pathogenicity tests were carried out at the Kula Experimental Station on the island of Maui with average day and night temperatures of 25 and 12 C, respectively. All three isolates of *R. solani* AG-2-1, AG-2-2, and AG-4 used in the study were obtained from carnation fields in Kula, Maui. *R. solani* AG-2-2 and AG-4 isolates were obtained from lesions of diseased carnation plants while the AG-2-1 isolate was obtained from soil. Seven standard cultivars (Peterson Red Sim, Improved White Sim, Scania 3C, Atlantis, Portrait, Red Diamond, and S. Arthur Sim) and two miniature cultivars (Elegance and Exquisite Select) were used in the initial trial.

Inoculum for the tests at Kula, Maui was prepared as described above, except that the vermiculite/V-8 juice mix was slightly altered (7 g of vermiculite, 7 ml of 20% clarified V-8 juice, and 18 ml of deionized water). One-gallon azalea pots were filled with potting soil (Pro-mix) and mixed with 4 g/pot of vermiculite-fungus inoculum to a depth of 5 cm. Five pots were infested with each isolate and later planted with rooted cuttings of each cultivar that same day. The rooted carnation cuttings for these tests were obtained from Yoder Brothers, Barberton, OH 44203, a week before planting.

Cuttings from surviving plants from the first Kula test were propagated and reinoculated to determine the levels of disease resistance to *R. solani* AG-2-2. This was repeated three times with surviving plants from cultivars Red Diamond, Atlantis, Peterson Red Sim, Scania 3C, Improved White Sim, and Portrait.

RESULTS AND DISCUSSION

R. solani-like fungi were isolated from soils of nine out of 11 carnation farms on EKN agar (7). A total of 588 colony-forming units (cfu) were isolated on 10% V-8 agar. Of this number, 308 cfu were determined to be *R. solani*, and 280 cfu were determined to be BRS fungi by staining nucleoli with safranin O (9). Anastomosis group determinations showed 207 of 308 isolates were AG-4, 49 were AG-2-2, and 52 were AG-2-1. AG-4 was found on five farms, AG-2-2 on two farms, and AG-2-1 on two farms. AG-2-2 and AG-4 were also isolated from diseased plants. A few diseased plants

Fig. 1. Cuttings of cultivar Improved White Sim carnation 12 wk after inoculation with *Rhizoctonia solani* AG-2-2. Susceptible cutting on left showing basal stem rot and wilt, characteristic symptoms of the disease, while the plant cutting on the right is healthy.

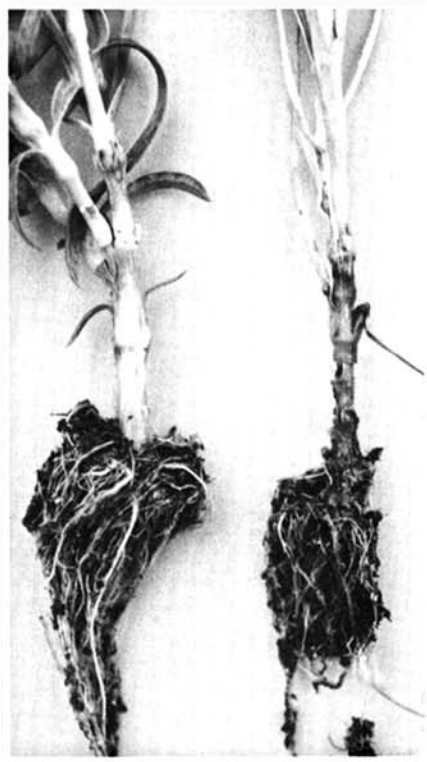

Fig. 2. Same cuttings as in Figure 1, removed from pot to show root system. Resistant healthy cutting on left, susceptible diseased cutting on right.

yielding AG-2-2 were collected at farms where *R. solani* could not be detected in the soil, as demonstrated in previous soil samplings using EKN agar. This suggested that the carnation cuttings in these plantings may have been diseased before transplanting in the field. AG-4 apparently was well distributed throughout the Kula area while AG-2-2 and AG-2-1 were more localized. The wider host range of AG-4 may account for the differences in distribution of the different groups (4). The majority of *R. solani* isolates from soil were from organic debris, 289 cfu originated from decomposing plant material, while 19 were from microsclerotia in the soil extract. These data indicated the important role of infested organic matter in the survival of *R. solani*.

The initial pathogenicity trials conducted at Manoa resulted in 80% mortality of Peterson Red Sim and 20% mortality of Improved White Sim 5 wk after inoculation with AG-2-2, and 20% mortality of Peterson Red Sim and 0% mortality of Improved White Sim inoculated with AG-4. The results indicated a possible difference in the resistance of these cultivars to *R. solani* and high virulence of AG-2-2 to carnation. The BRS isolates were not pathogenic to carnation. Subsequent trials carried out at Kula, Maui, showed that *R. solani* AG-2-2 was highly pathogenic to carnation, causing 60–100% mortality to different cultivars tested, while AG-4 and AG-2-1 were not pathogenic and produced 0% mortality in all trials. Of the nine cultivars of carnation tested, Elegance was the most susceptible with 100% mortality 17 wk after inoculation. Atlantis and Red Diamond were the most resistant with 60% mortality. S. Arthur Sim showed 90% mortality while the other five cultivars (Peterson Red Sim, Improved White Sim, Scania 3C, Portrait, and Exquisite Select) had 80% mortality. The difference between these results and the initial results obtained at a Manoa greenhouse, Oahu, was attributed to the warmer temperature at Manoa that favored fungal activity, but was unfavorable to carnation. A pathogenicity test involving the same carnation cultivars inoculated with three isolates of AG-4 and two isolates of AG-2-2 obtained from different farms, showed AG-2-2 to be the carnation pathogen while AG-4 was not. We concluded that *R. solani* AG-2-2 was pathogenic to carnations at Kula, Maui, but AG-4 or AG-2-1 were not.

Surviving healthy plants from cultivars Red Diamond, Portrait, Improved White Sim, Atlantis, Scania 3C, and Peterson Red Sim (Figs. 1,2) that were propagated by cuttings and inoculated 4 wk later with AG-2-2 isolates, resulted in 80% mortality of Scania 3C, Improved White Sim, and Portrait and 60% mortality of Red Diamond, Atlantis, and Peterson Red Sim, 11 wk after inoculation. Cuttings taken from surviving healthy plants from the previous test, and inoculated with AG-2-2 isolates at planting, resulted in 40% mortality of Peterson Red Sim, 25% of Portrait and Scania 3C, 23.5% of Red Diamond, 22% of Atlantis, and 20% of Improved White Sim, 13 wk after inoculation. The fifth selection generation of these cultivars, when compared with the original cultivars from Yoders, showed increased resistance to *R. solani* AG-2-2 by 50% in Improved White Sim and 80% in Portrait, while the original cultivars were 100% susceptible. The trials thus far indicate that the level of resistance in the six carnation cultivars tested increased with subsequent selection of surviving healthy plants. The decrease from 80% mortality to 20%, we feel, is due to selection for resistance in these cultivars that are heterogeneous for this character. We are continuing our trials to test the stability of increased resistance to *R. solani* AG-2-2.

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