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IN VITRO SCREENING FOR BURROWING NEMATODE, *RADOPHOLUS CITROPHILUS*, TOLERANCE AND RESISTANCE IN COMMERCIAL *ANTHURIUM* HYBRIDS

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Summary

A reliable method to screen *Anthurium* for burrowing nematode resistance and tolerance *in vitro* was developed using 17 genetically distinct *Anthurium* cultivars. Based on nonparametric data analysis, tolerance and resistance were found to be independent traits to be evaluated separately. An effective parameter for tolerance evaluation was ranking of relative leaf retention, whereas an effective parameter for resistance evaluation was the ranking of nematode reproduction, log(Rf + 1). A comparison of the ranking of leaf retention with ranking of nematode reproduction clustered the cultivar responses to burrowing nematode infection into four groups: intolerant and resistant, moderately tolerant but susceptible, intolerant and susceptible, and tolerant and susceptible. 'Ozaki' was identified as an intolerant reference, 'Nitta' as a susceptible reference. 'Blushing Bride' was the most tolerant cultivar among those screened, but it may not be an ideal tolerant reference due to its low vigor. Future screening for burrowing nematode-tolerant and -resistant cultivars in *Anthurium* should include 'Ozaki' and 'Nitta' as internal controls. Evaluation of resistance should be based on a resistance index obtained by log(Rf of hybrid tested + 1) divided by log(Rf of 'Nitta' + 1); tolerance should be based on ranking of relative leaf retention.

Key words: breeding selection; nematode control; Fastclus; nonparametric variables; leaf retention; nematode reproduction.

Introduction

Burrowing nematode, *Radopholus citrophilus*, is one of three common pests that reduce productivity of *Anthurium* (Hara et al., 1988), with flower yields reduced by up to 50% (Aragaki and Apt, 1984). Fenamiphos is an effective nematicide to control *R. citrophilus* in *Anthurium* (Hara et al., 1988); however, its allowable application rate has been lowered recently from 15 G to 10 G. There is a need for additional environmentally sound methods of control.

Breeding for resistance is a major component of pest management (Roberts, 1992). For example, nematode resistance offers more effective control of *Globodera rostochiensis* and *G. pallida* on potato than does chemical treatment of infested soils (Trudgill et al., 1978; Gurr, 1992). While current management of nematodes is largely based on chemical control, many nematicides may be banned in the near future. Other methods of management that include biological control, physical control, crop rotation, soil amendment, or field sanitation reduce nematode populations but are unable to eradicate them. Plant host resistance and tolerance can complement other integrated pest management strategies and provide more direct means of control.

Host plant resistance inhibits pathogen invasion, development, or multiplication. Tolerance enables the plant to withstand nematode infection without suffering undue damage (Trudgill, 1985). In nematology, resistance may be independent of tolerance, therefore, plants can be categorized into four groups: intolerant and resistant, moderately tolerant but susceptible, intolerant and susceptible, and tolerant and susceptible (Trudgill, 1985).

No work had been done on screening for burrowing nematode resistance and tolerance in *Anthurium*. For most crops, such screening is done in the field. However, nonuniformity of nematode infestation in the field, seasonal fluctuations of nematode reproduction, and complication due to multispecies nematode communities have lead to the unreliability of field screenings (Boerma and Hussey, 1992). *In vitro* screening, in contrast, can lessen these problems by controlling the inoculum level, shortening the screening period, and avoiding seasonal effects (Boerma and Hussey, 1992). For example, studies on *Meloidogyne incognita* resistance in peach required 3 yr for the field evaluations but only 5 wk for *in vitro* evaluations (Huettel and Hammerschlag, 1993).

The objectives of this experiment were to develop a reproducible method to screen for *R. citrophilus* resistance and tolerance in *Anthurium* and to identify standard susceptible and intolerant cultivars for future screening reference.

Materials and Methods

Seventeen Hawaiian *Anthurium* cultivars (Fig. 1) were grown on an *Anthurium* plant maintenance medium containing half strength macro elements of Murashige and Skoog's inorganic salts, 0.4 mg/l thiamine·HCl, 0.5 mg/l each of nicotinic acid and pyridoxine·HCl, 20 g/l sucrose (Kunisaki, 1980), lacking Benzyladenine (BA) and solidified with 0.7% Difco Bacto agar. 'Ozaki' was cultured on the same medium but with macronutrient concentration reduced by one-eighth. Four 2-cm-tall plantlets per cultivar were transferred into a 2.5 cm × 2.5 cm × 10 cm Magenta GA-7 box (Sigma Chemical Co., St. Louis, MO) containing the same, but firmer medium (0.3% Gelrite), and inoculated with nematodes 2 wk later.

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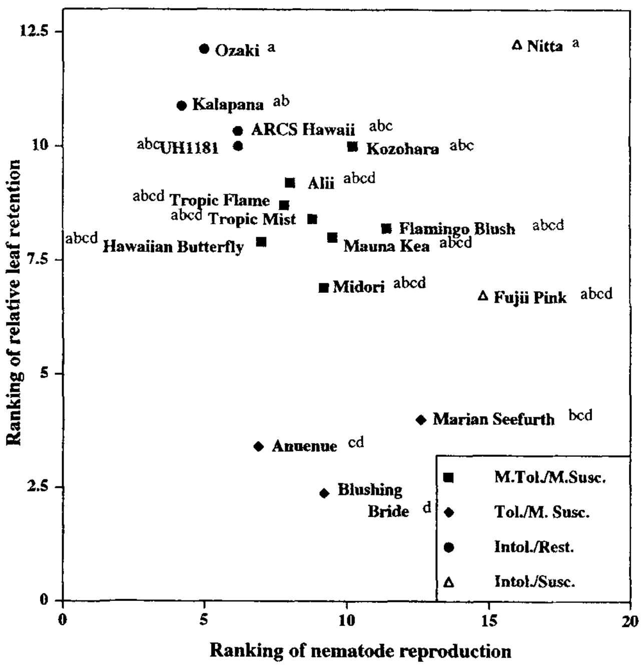

FIG. 1. Plot shows ranking of relative leaf retention in relation to the ranking of nematode reproduction, log(Rf + 1), for 17 *Anthurium* cultivars screened *in vitro* 3 mo. after inoculation with *Radopholus citrophilus*. Rf is the final nematode number in the roots divided by the initial nematode number inoculated. Data points with the same symbol are clustered into four groups according to the Fastclus procedure (SAS Institute, 1985). Tol., M. Tol., Intol., Rest., Susc., and M. Susc. represent tolerant, moderately tolerant, intolerant, resistant, susceptible, and moderately susceptible, respectively. Leaf retention is the percentage of green leaves remaining per total leaves. "Relative" refers to the difference between inoculated and uninoculated plants. Cultivars followed by the same letters do not differ in leaf retention according to the Waller-Duncan k-ratio t-test.

Radopholus citrophilus, population HA11, collected from Paradise Pacific Farm in Kurtistown, Hawaii were maintained on alfalfa root callus (Ko et al., 1996) and later used as sources of inoculum. The numbers of nematodes per unit length of alfalfa callus were estimated by counting the nematodes that emerged from callus placed in a mist chamber. Callus pieces containing approximately 400 nematodes were placed into each GA-7 box. The same length of root callus without nematodes was placed in similar GA-7 boxes to serve as controls.

A randomized complete block design was used with five replicates repeated over 10 wk. Plants were harvested 3 mo. after inoculation and rated for disease symptoms, and the roots and media were placed in a mist chamber for 7 d to extract nematodes. Plant damage was expressed in a symptom index (SI) derived from the summation of percentages of leaf damage levels multiplied by the level of leaf damage. Leaf damage levels were assigned as: Level 1 = brown spots, 2 = chlorotic, 3 = partly yellow, 4 = completely yellow, 5 = partly brown, 6 = completely brown.

Analysis of variance was used to test the parametric variables (relative SI, relative leaf retention, and nematode reproduction in the roots and the media) and their corresponding nonparametric variables (ranking of relative SI, ranking of relative leaf retention, and ranking of nematode reproduction in the roots and those in the media). Relative SI is the difference between inoculated and uninoculated treatments; relative leaf retention is the percentage of green leaves retained on inoculated plants with respect to the uninoculated plants; and nematode reproduction is log(Rf + 1), where Rf is final nematode population in the roots divided by initial nematode population inoculated. Analysis of nonparametric variables was suggested by Eskridge (1995) and Phillips (1984). Waller-Duncan's multiple-range test was used to compare differences among cultivars and treatment means. The means of the ranking of relative SI for each cultivar were plotted against means of ranking of nematode reproduction in the root. The Fastclus procedure of the SAS program (SAS Institute, 1985) was used to cluster data points of the plot into four groups. Analysis of variance of the leaf retention of inoculated and uninoculated plants was also conducted. Correlation analysis was conducted among nematode reproduction in the root, relative SI, and relative leaf retention.

RESULTS

Tolerance among *Anthurium* cultivars was differentiated by use of one of the nonparametric variables, that is, ranking of relative leaf retention ($P = 0.02$), but not by ranking of the symptom index ($P = 0.11$). Evaluation of tolerance with the parametric variables, SI difference and relative leaf retention, showed high variation within cultivars ($P = 0.0005$ and $P = 0.0065$, respectively). A lower ranking of leaf retention resulted from a smaller difference between inoculated and noninoculated treatments, and thus represented a relatively tolerant cultivar. 'Blushing Bride' was the most tolerant cultivar, having the lowest rank in relative leaf retention (Fig. 1).

When actual leaf retention among inoculated plants was compared, 'Midori' rated the highest ($P = 0.015$; Fig. 2), but variation within cultivars was high ($P < 0.01$). Both the inoculated and uninoculated leaf retention for 'Midori' was greater than those cultivars in the tolerant and susceptible group (i.e., 'Anuenue,' 'Blushing Bride,' or 'Marian Seefurth') (Fig. 2). This showed that 'Midori' was more vigorous. The performance of 'Midori' was considered to be more stable in different environments as interpreted by its lower standard deviation (Fig. 2).

Resistance among *Anthurium* cultivars was differentiated better by using the nonparametric variable of the ranking of nematode reproduction in the roots ($P = 0.0015$). Differences were less significant when analyzed by the parametric variable, nematode reproduction ($P = 0.1$). 'Nitta' had the highest ranking in nematode reproduction in the roots and thus represented the most susceptible of the cultivars (Fig. 3). Nematode reproduction in the medium was not different among the cultivars ($P > 0.1$).

Relative leaf retention was correlated with relative symptom index ($r = 0.88$, $P = 0.0001$). However, there was no correlation between ranking of Rf and relative leaf retention ($r = 0.02$, $P = 0.85$) or relative SI ($r = -0.06$, $P = 0.62$).

Clustering the 17 *Anthurium* cultivars into one of four groups placed 'Ozaki,' 'Kalapana,' 'ARCS Hawaii,' and UH1181 together. These cultivars had a relatively high ranking in relative leaf retention and a low ranking in nematode reproduction, thus representing an intolerant but resistant group.

'Kozohara,' 'Alii,' 'Tropic Flame,' 'Tropic Mist,' 'Flamingo Blush,' 'Mauna Kea,' 'Hawaiian Butterfly,' and 'Midori' were grouped together because of their moderate rankings of relative leaf retention and of nematode reproduction. Although the tolerance measurement of these cultivars was not different from the previous group, according to a Waller-Duncan k-ratio t-test, their tolerance might be higher because the nematode population was greater. Therefore, this group was considered moderately tolerant and moderately susceptible.

'Nitta' and 'Fujii Pink' formed a third group that includes high relative leaf retention and high nematode reproduction (Fig. 1). These two cultivars had higher nematode reproduction than any other cultivar ($P < 0.02$; Fig. 3) and, therefore, should be considered as relatively susceptible and intolerant.

'Anuenue,' 'Marian Seefurth,' and 'Blushing Bride' comprised the fourth group with low rankings in relative leaf retention and a mod-

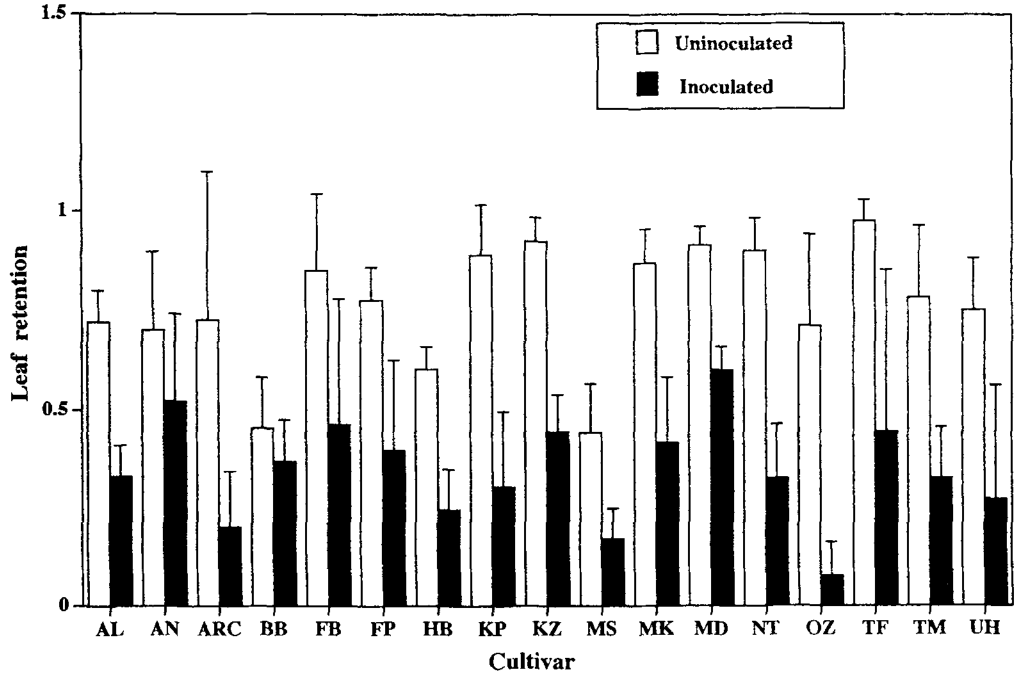

FIG. 2. Leaf retention of inoculated and uninoculated plants of *Anthurium* cultivars. AL = 'Alii'; AN = 'Anuenue'; ARC = 'ARCS Hawaii'; BB = 'Blushing Bride'; FB = 'Flamingo Blush'; FP = 'Fujii Pink'; HB = 'Hawaiian Butterfly'; KP = 'Kalapana'; KZ = 'Kozohara'; MS = 'Marian Seefurth'; MK = 'Mauna Kea'; MD = 'Midori'; NT = 'Nitta'; OZ = 'Ozaki'; TF = 'Tropic Flame'; TM = 'Tropic Mist'; UH = UH1181. *Vertical bars* represent the standard deviation. Cultivars are not significantly different ($P > 0.05$).

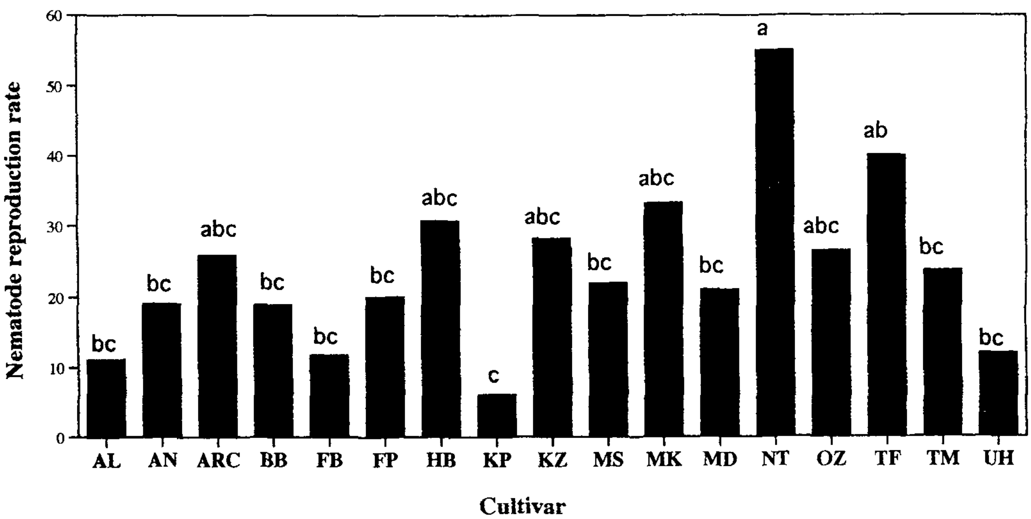

FIG. 3. Ranking of nematode reproduction, log(Rf + 1) in *Anthurium* root tissue 3 mo. after *in vitro* screening. Rf is the final nematode number in the roots divided by the initial nematode number inoculated. AL = 'Alii'; AN = 'Anuenue'; ARC = 'ARCS Hawaii'; BB = 'Blushing Bride'; FB = 'Flamingo Blush'; FP = 'Fujii Pink'; HB = 'Hawaiian Butterfly'; KP = 'Kalapana'; KZ = 'Kozohara'; MS = 'Marian Seefurth'; MK = 'Mauna Kea'; MD = 'Midori'; NT = 'Nitta'; OZ = 'Ozaki'; TF = 'Tropic Flame'; TM = 'Tropic Mist'; UH = UH1181. Cultivars followed by the same letters do not differ in Rf according to Waller-Duncan k-ratio t-test ($P > 0.05$).

erate nematode reproduction. This group should be considered as tolerant and moderately susceptible.

DISCUSSION

Screening methods. Results showed that we developed a reproducible method to screen *Anthurium* cultivars for resistance and tolerance to the burrowing nematode. Lack of correlation between nematode reproduction and relative leaf retention or between nematode reproduction and SI confirmed that tolerance and resistance are independent traits as concluded by Trudgill (1985). Results also showed that none of the cultivars had relative leaf retention values of 0 or less, or nematode reproduction values equal to or less than 1 (i.e., none were similar to, or better than, the noninoculated plants). Thus, no absolute tolerance and resistance was present among the 17 cultivars that were evaluated.

A bias in assessment of resistance may arise from using a 3-mo. trial because nematode populations may crash if plant tissue of some genotypes is severely damaged during this period. Consequently, late measurements of resistance parameters may show lower populations than would otherwise be observed at an earlier evaluation date. It is recommended that future trials to screen for *R. citrophilus* resistance in *Anthurium* be shortened from 3 mo. to a period before the test materials are severely damaged. Such was the case with *in vitro* screening for *Meloidogyne incognita* resistance in peach scion cultivars, in which trials were conducted for only 5 wk (Huettel and Hammerschlag, 1993).

Use of nonparametric variables proved to be a reliable method for nematode tolerance and resistance evaluation in this experiment. Ranking of variables was previously recommended by Trudgill (1985) for resistance evaluation. In our study, ranking of relative leaf retention is recommended for tolerance evaluation. Nevertheless, although relative leaf retention represents plant tolerance with respect to the uninoculated control, it does not represent plant vigor. For example, 'Midori' is less tolerant than 'Blushing Bride' in terms of relative leaf retention, but it is actually more vigorous than 'Blushing Bride' as indicated by its higher leaf retention under inoculated and uninoculated conditions. Therefore, leaf retention value of the inoculated plants should not be ignored in selection for tolerance as it reflects the actual plant vigor that, in turn, may affect overall plant productivity.

Reference index for breeding tolerant and resistant varieties. Tolerant and resistant reference cultivars provide standards for selection. Based on this study, several cultivars can serve as references in future evaluations. 'Ozaki,' a commonly grown red cultivar, reacted poorly to *R. citrophilus* infection and represents an intolerant reference. 'Nitta,' a common orange cultivar, supported the highest nematode population densities and represents a susceptible reference. No resistant cultivar could be identified among those tested under these screening conditions. Although 'Blushing Bride' was identified as the most tolerant cultivar, it may not be an ideal tolerant reference due to its low vigor.

The susceptible or intolerant reference cultivar can also serve as an internal control and check for effectiveness of inoculum. In soybean cyst nematode resistance breeding programs, *Glycine max* 'Lee' is commonly used as the standard susceptible cultivar and an index of nematode parasitism is based on the average number of females on a differential divided by the average number of females on 'Lee' (Schmitt and Shannon, 1992). Expressing the number of nematodes developing in partially resistant genotypes as a percentage of nematodes on a susceptible control can also be used to quantify resistance (Trudgill, 1985). In testing unknown genotypes, cultivars of known resistance must be used as internal standards to delineate categories of resistance (Phillips and Trudgill, 1983).

Accordingly, future screening for burrowing nematode tolerance and resistance should include 'Ozaki' and 'Nitta' as internal controls for the effectiveness of the inoculum. Secondly, a resistance index, obtained by the log(Rf of the hybrid tested + 1) divided by the log(Rf of 'Nitta' + 1), would be useful for the evaluation of resistance.

The mechanism of burrowing nematode tolerance in *Anthurium* was not studied. However, studies on the distribution of nematodes in the roots of 'Midori,' and 'Alii' showed that 'Midori' restricted nematode reproduction to the root tissue. 'Alii' had greater frequency of nematode invasion in the leaf petiole. These findings might explain the higher tolerance of 'Midori' than 'Alii' in being able to maintain higher leaf retention (Wang, 1996).

Acknowledgments

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