Sensitivity of *Rhizoctonia solani* isolates to fungicides and evaluation of peanut cultivars to Rhizoctonia limb rot[1]

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ABSTRACT

Twenty-one isolates were made from typical Rhizoctonia limb rot lesions of Florunner peanuts (*Arachis hypogaea* L.) and one each from a peanut pod and cowpea (*Vigna unguiculata* Walp.). Isolates of *Rhizoctonia solani* were characterized for sensitivity to three sterol-inhibiting fungicides, PCNB and chlorothalonil. Diniconazole, cyproconazole, and tebuconazole were the most effective inhibitors of radial growth, with a mean EC_{50} of 0.028, 0.056, and 0.166 µg/µL, respectively. EC_{50}s for PCNB and chlorothalonil were 4.06 and 4.85 µg/mL, respectively. A technique to reproduce the disease in the greenhouse was developed and used to evaluate 18 peanut cultivars for resistance to limb inoculation with *R. solani*. NC 6, NC 7, New Mexico Valencia A, and Florunner were the most susceptible cultivars based on lesion length. Wounding before inoculation resulted in significantly increased lesion length for 15 of 18 cultivars. In a three-year (1986-88) field trial, NC 6, Florigiant, and NC 7 were the most susceptible cultivars, while VA 81B and Toalson appeared to be the most resistant.

Key Words: *Rhizoctonia solani* AG-4, groundnut, soil-borne disease, *Arachis hypogaea* L.

Soilborne diseases severely limit the yield and quality of peanuts (*Arachis hypogaea* L.) in Georgia (8, 9). In 1988 losses from disease were estimated at about 24% (17). *Sclerotium rolfsii* (Sacc.), the causal agent of southern stem rot, was estimated to have caused 7.3% loss in Georgia in 1988, while *Rhizoctonia solani* (Kühn) anastomosis group 4 (AG4), causal agent of Rhizoctonia limb rot, caused an estimated 8.5% loss (17).

Unlike southern stem rot, Rhizoctonia limb rot is a relatively new problem in Georgia, with very little known about the basic epidemiology or control of the disease. Thompson (16) first reported the disease in Georgia after an epidemic of limb rot in 1981. Infection by *R. solani* occurs on the pegs and lower branches in close contact with the soil. By mid-season, reddish-brown to dark brown zonate lesions are observed on branches and pegs. As the disease progresses, branches and pegs are partially or completely girdled and killed, resulting in the loss of pods or immature pods when the crop is dug and inverted. Previous observations indicate that the disease occurs more frequently under cool, moist conditions late in the season (16), especially when branches have been injured (6).

Rhizoctonia solani is also involved in preemergence damping off (19), pod breakdown (10, 19), hypocotyl cankers (AG-4) (3) root cankers (18) and can be a foliar pathogen of peanut (AG-1) (13). However, these disease symptoms are different from Rhizoctonia limb rot and are distinguished from these other diseases based on the symptoms described above.

Control measures for southern stem rot and Rhizoctonia limb rot are only partially effective. Even when the best cultural practices and available fungicides are used, control of southern stem rot and limb rot is poor (8, 9). Several experimental, sterol-biosynthesis-inhibiting (SBI) fungicides have given excellent control in the field and *in-vitro* inhibition of *R. solani* (2, 5, 7, 8).

Resistance to root diseases of peanut caused by *R. solani* (AG type unknown) has been observed in spanish peanuts (1), but this resistance has not been incorporated into presently grown peanut culitvars. Peanut germ plasm introductions screened in controlled environment chambers have exhibited resistance to preemergence and seedling disease caused by *R. solani* (20).

In this study, Koch's Postulates were carried out for *R. solani* on peanut, and a method was developed to quickly and effectively reproduce the symptoms under greenhouse conditions. A second objective was to use this technique to screen peanut cultivars for resistance to limb inoculation with *R. solani*.

Materials and Methods

Laboratory Test

Twenty-one isolates of *R. solani* were collected from a research plot of *Arachis hypogaea* L. cv. Florunner displaying typical limb rot symptoms and one each from peanut pod and cowpea (*Vigna unguiculata* Walp.). The research area was on a Tifton loamy sand (fine-loamy, siliceous, thermic Plinthic Paleudult, pH 5.7) with a rotational history of two years peanuts, one year sorghum, and five years peanuts prior to the test. The research area had never received an application of an SBI fungicide.

Rhizoctonia limb rot isolates were obtained by removing branch lesions from lower lateral branches. Lesions were surface sterilized in 70% ethanol for one minute, rinsed three times in sterile distilled water, and placed on tannic acid-benomyl (TAB) medium for 48 hr at room temperature. Hyphal tips from colonies were transferred to fresh potato dextrose agar (PDA) for identification. To determine the nuclear condition of each isolate, staining was performed according to the procedure by Herr (11). Isolates were maintained on fresh PDA at 25 C in darkness.

Stock solutions of each fungicide were made be mixing appropriate amounts of each fungicide (diniconazole as Spotless 25 WP (Valent), cyproconazole as technical grade SAN 619 (Sandoz), tebuconazole as Folicur 1.2 EC (Mobay), PCNB as Terraclor 75 WP (UniRoyal), chlorothalonil as Bravo 720F (Fermenta ASC) in sterile distilled water, except for cyproconazole, which was dissolved in 100% ethanol. PDA was amended with fungicide after being cooled to 50C. The amended media was mixed thoroughly be stirring, dispensed into 9 cm petri dishes (approximately 20 mL per dish), and allowed to gel. Diniconazole, cyproconazole, and tebuconazole were tested at 10.0, 1.0, 0.1, 0.01 and 0.001 µg/mL, and PCNB and chlorothalonil at 50.0, 10.0, 1.0, 0.5, and 0.1 µg/mL.

Mycelial plugs (5-mm diameter) were cut from the margins of 4-5 day old cultures of each isolate and inverted in the center of fungicide-amended and unamended PDA plates. Five replicate plates were used for each isolate-fungicide combination. Plates were placed in plastic bags and incubated in darkness at 25 C for 42 hr. Colony diameters were measured twice (largest diameter and right angle) and adjusted for the diameter of the inoculum plug. The EC_{50} values were determined by interpolating probit plots for growth inhibition (9) vs. log of each fungicide concentration.

[1]The use of trade names in this publication does not imply endorsement by the University of Georgia of the products named, nor criticism of similar ones not mentioned.

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Greenhouse Study

Peanut cultivars including eight runner, eight virginia, one spanish, and one valencia-type were evaluated in the greenhouse for resistance to limb inoculation with *R. solani*. Four seed of each cultivar were planted (3-cm deep) in each of 10 (15-cm diameter) pots containing a mixture of pasteurized Tifton loamy sand and Vermiculite (2:1, v:v). Approximately 0.1 g *Rhizobium* inoculant granules were added around each seed before covering with soil. Each plant received 2 cc of 6-12-6 fertilizer at 34 days after planting. At 64 days after planting, plants were thinned to two plants/pot.

Ryegrass (*Lolium perrenne* L.) seed inoculum was prepared by placing 60 g ryegrass seed in 250 mL Erlenmeyer flasks, with 100 mL distilled water, and autoclaved for 30 min on two consecutive days. Two 5 mm mycelial plugs of a 4-5 day PDA culture of *R. solani* (isolate G33) were placed in the flask and incubated for 14 days in darkness at 25 C. Sterile ryegrass seed was used for control inoculations.

Inoculations were performed 64 days after planting. Five pots (10 plants) of each variety served as controls, and five pots were used for *R. solani* inoculations. One lower lateral branch on each plant was wounded, while a second lower lateral branch on the same plant was left unwounded. Wounding was performed by gently pinching the branches with a pair of pliers on the third internode from the main stem producing a 1 cm wound. A single infested ryegrass seed was placed on each *R. solani* inoculation site and wrapped with a 2 x 5 cm double layer of moistened cheesecloth and wrapped with Parafilm. Control sites (wounded or unwounded) were inoculated as stated above, with sterile ryegrass seed.

Pots were placed on greenhouse benches in a randomized complete block design. To reduce light and temperature, benches were covered with black plastic mesh supported by a pvc pipe frame. A hygrothermograph was placed under the mesh to monitor temperature and humidity after inoculation. After 14 days, wrapping was removed, and lesion lengths were measured to the nearest mm.

Cultivar Trial, Tifton, 1986-88

Eighteen peanut cultivars representing three U. S. market types (spanish, runner, and virginia) were evaluated for resistance to Rhizoctonia limb rot in the field. The field site chosen was at the Gibbs research farm near Tifton, GA, which had a history of moderate incidence Rhizoctonia limb rot occurrence. The soil type was a Tifton loamy sand, pH 5.7. A randomized complete block design was used each of the three years, with four, six, and five replications in 1986, 1987, and 1988, respectively. Two row plots were 6.1 m long and 1.8 m wide. Row spacing was 1.0 M between plots, and 0.8 within plots.

Seed of each cultivar were planted at the rate of five seed per 30 cm on May 6, 1986, May 26, 1987, and May 13, 1988. Recommended cultural practices were followed each year (12). Rainfall was supplemented by overhead irrigation as needed to avoid water stress. Other pests, including leafspot, were controlled according to recommendations made by the Cooperative Extension Service (12). Cultivars were individually harvested based on visual maturity estimates.

Rhizoctonia limb rot evaluations were made immediately after each cultivar was dug and inverted. A visual rating of disease incidence was made by randomly selecting six, 30 cm locations within each plot and estimating the percentage of vines (limbs) infected by *R. solani* (8). Areas of row infected by *S. rolfsii* were avoided to prevent confusion of symptoms. Peanuts were combined, dried, hand-cleaned, and weighed.

Data were subjected to analysis of variance, and means were separated by Waller-Duncan's multiple range test (K-ratio = 100). A linear regression program supplied by M. D. Coffey, Univ. of CA, Riverside, was used for analysis of laboratory tests. A paired t-test (P = 0.05) was used to determine differences between wounded and unwounded *R. solani* inoculations.

Results

Laboratory Test

All isolations from lesions yielded multinucleate *R. solani* AG4 except two from which binucleate *Rhizoctonia*-like fungi were isolated. The mean EC_{50} values for diniconazole, cyproconazole, and tebuconazole were 0.028, 0.056, and 0.166 mg/mL, respectively. The mean EC_{50} values for PCNB and chlorothalonil were 4.06 and 4.85 ppm, respectively. The ranges in fungicide sensitivity for *R. solani* isolates were 0.106, 0.090, 0.743, 11.58, and 11.18 mg/mL for diniconazole, cyproconazole, tebuconazole, PCNB and chlorothalonil, respectively.

Greenhouse Study

Reddish-brown lesions, characteristic of Rhizoctonia limb rot, developed to some degree on all cultivars screened. No lesions resulted from either wounded or unwounded control inoculations with sterile ryegrass seed. Wounding branches before inoculation with *R. solani* significantly increased lesion lengths on all cultivars, except VA 81B, Pronto, and New Mexico Valencia A (NMVA) (Fig 1.). Lesions which developed without previous wounding were limited to 6 mm or less. Lesions which developed on NC 6, the most susceptible cultivar, were significantly longer than all cultivars except Florunner, NMVA, and NC 7. In some cases, infection and lesion development resulted in girdling, wilting, chlorosis, and death of branches. Lesions on susceptible cultivars frequently appeared shredded, encircling the entire branch. Lesions which developed on the resistant cultivars were sunken and generally limited to one side of the branch. No girdling or wilting was observed on the resistant cultivars VA 81B, Florigiant, Okrun, GK3 and NC 9.

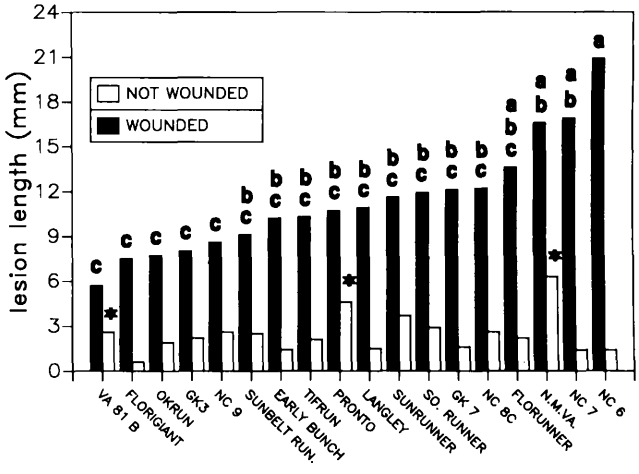

Fig. 1. Evaluation of 18 peanut cultivars for resistance to limb inoculation with *R. solani* AG4 (isolate G33). Lesions were measured to the nearest 1mm, 14 days after inoculation. Closed bars represent lesion length on wounded limbs, open bars represent lesion length on limbs that were not wounded. Bars with same letters indicate no significant difference according to Waller Duncan K ratio t-test, (P=0.05). Lesion lengths for limbs that were not wounded were not significantly different (P=0.05). Lesion lengths for wounded limbs were significantly greater than for limbs that were not wounded, except for cultivars indicated with*, paired t-test (P=0.05).

Cultivar Trial, Tifton, 1986-88

In 1986, disease, ranged from 3.8 to 63.5% branches infected by *R. solani* (Table 1). The spanish market type, Toalson, was found to be the most resistant cultivar, with an incidence rating of 3.8%. Florunner, the most common cultivar grown in Georgia, did not differ significantly from the most susceptible cultivar in 1986.

In 1987, environmental conditions were hot and dry, and thus not as suitable for Rhizoctonia limb rot. However, *Aspergillus niger* crown rot did occur in many of the plots and severely damaged or killed several plants within each row. Although that data is not presented here, Southern Runner was observed to have the best resistance to the Aspergillus crown rot disease under these test conditions.

Due to frequent rains and cool temperatures late in the season, Rhizoctonia limb rot incidence in 1988 was again

Table 1. Evaluation of 18 peanut cultivars for resistance to *R. solani* AG4, causal agent of Rhizoctonia limb rot, Tifton, 1986-88.

Cultivar/ Breeding Line	Market type[a]	Maturity[b]	Rhizoctonia rating (0-100% scale)[c] 1986	1987	1988
Toalson	Sp	E+	3.8 f[d]	-----	-----
VA 81B	Va	ME	19.0 ef	26.9 a	25.7 h
VP 8140	Ru	M	28.8 de	-----	-----
NC 9	Va	ME	35.0 cde	23.9 ab	39.0 fg
GK-3	Va	M	32.5 cde	19.3 abc	47.0 b-f
Langley	Ru	ME	48.0 abc	16.1 bc	35.7 gh
Southern Runner	Ru	ML	42.5 bcd	20.6 abc	44.7 d-g
Early Bunch	Va	ME	47.5 abc	21.8 abc	41.6 efg
Okrun	Ru	M	48.5 abc	15.3 bc	51.0 a-e
NC 8C	Va	M+	30.5 de	26.9 a	58.0 a
GK-7	Ru	M	59.5 a	12.9 c	45.7 c-g
Florunner	Ru	M	58.2 ab	15.4 bc	47.7 b-f
Tifrun	Ru	M-	39.8 cd	26.8 a	57.0 ab
Sunbelt Runner	Ru	M-	48.2 abc	22.8 abc	58.3 a
Sunrunner	Ru	M	63.5 a	18.3 abc	53.7 a-d
NC 7	Va	M-	58.8 a	27.4 a	51.0 a-e
Florigiant	Va	M	62.8 a	22.5 abc	55.3 abc
NC 6	Va	M	62.5 a	28.1 a	53.0 a-d

[a] Sp = spanish, Va = virginia, Ru = runner.

[b] Maturity classes are relative to Florunner. M = medium maturing, M- = -7 days, M+ = +7 days, ME = -14 days, ML = +14 days, E+ = -14 to -21 days.

[c] Rhizoctonia rating is a visual rating of % branches infected at six, 30-cm locations in each two-row plot after digging, where 0 = 0% infection and 100 = 100% infection by R. solani.

[d] Values are averages of four, six, and five replications in 1986, 1987, and 1988, respectively. Means within each column followed by the same letter do not differ significantly according to Waller-Duncan multiple range test, (P = 0.05).

moderately severe and very similar to that in 1986 (Table 1). VA 81B with a decrease rating of 25.7% was significantly more resistant than all cultivars, except Langley. Sunbelt Runner, NC 8C, Tifrun, Florigiant, and Sunrunner were some of the more susceptible cultivars in 1988. Due to the cultivar x year interactions, 3-yr means could not be analyzed statistically, and therefore, are not presented.

Discussion

SBI fungicides represent a relatively new class of systemic fungicides with excellent activity against many important plant pathogenic fungi, including *R. solani* (2, 5, 7, 8, 15). Csinos *et al.* (8) demonstrated that diniconazole is about 30 times more active than PCNB at inhibiting radial growth of two *R. solani* isolates from peanuts. In that study, radial growth was inhibited by 0.1 µg/mL diniconazole and 3.0 µg/µL PCNB in water agar. The mean EC_{50} for isolates used in this study was 0.025 µg/mL diniconazole in fresh PDA; however, depending on the isolate, EC_{50}s may range from 0.008 to 0.114 ppm.

The SBI fungicides used in this study were more active on *R. solani* than the two fungicides, PCNB and chlorothalonil, most commonly used in peanut production in Georgia. Diniconazole, cyproconazole, and tebuconazole were 145, 72.5, and 24.5 times more effective at inhibiting radial growth in laboratory tests than PCNB, respectively.

All limb rot isolates used in this study were collected from a small area (300m^2). Realizing this, it was surprising that such great variability in fungicide sensitivity existed in the *R. solani* population. Since these fungi have never been exposed to a SBI fungicide, it appears that a large natural variation to SBIs exists in the *R. solani*, AG-4 populations; this may partially explain variable results in controlling Rhizoctonia limb rot with SBIs under field conditions (8).

A technique was developed to reproduce the disease on greenhouse-grown peanuts and screen cultivars for resistance to Rhizoctonia limb rot. Wounding significantly increased lesion length on all except three cultivars. This supports a recent report by Brenneman and Sumner (6) that wounding (tractor traffic) significantly increases Rhizoctonia limb rot incidence under field conditions.

The incidence of Rhizoctonia limb rot in field plots varied greatly from 1986 through 1988. Toalson, although evaluated only 1-yr, was among the most resistant cultivars screened, with a disease incidence of 3.8% in 1986. Toalson is a spanish market type with an upright growth habit. This possibly allows for a drier, less favorable environment for *R. solani* under the canopy. Branch and Csinos (4) reported that Toalson was also among the most resistant cultivars to southern stem rot. In 1988, VA 81B was among the most resistant cultivars screened. VA 81B also has a very erect, bunch-type growth habit, possibly allowing for fewer limbs to come in contact with *R. Solani* propagules on the soil surface. However, VA 81B was also the most resistant cultivar after direct limb inoculation in the greenhouse. It appears that other mechanisms such as morphological barriers or active plant responses may also be associated with resistance in this cultivar. Susceptibility of NC 6 and NC 7 in the field trial also supports results from the greenhouse inoculations. No association between market type and susceptibility was evident from either field or greenhouse tests.

Cultivars such as Toalson, VA 81B, NC 9 and Langley have earlier maturity dates as compared to Florunner, and consequently, these cultivars were dug and inverted 1-3 weeks before Florunner. Early maturing cultivars tended to have lower incidence ratings across all years. Since Rhizoctonia limb rot is a late season, cool temperature disease, the use of early maturing cultivars may be a beneficial approach in escaping the disease by digging them before environmental conditions are suitable for disease development.

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