# Stem Rot of Peanut: Relationship Between *In Vitro* Fungicide Sensitivity and Field Efficacy of Fungicides

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**ABSTRACT**

Isolates of *Sclerotium rolfsii* exhibiting varying degrees of *in vitro* fungicide sensitivity were exposed to fungicides in field microplots in 1995 and 1996. Individual peanut (*Arachis hypogaea* L.) plants in 0.9-m microplots were inoculated with isolates of *S. rolfsii* collected from peanut fields throughout Georgia. The 60 isolates used in the study represented the full range of sensitivity to the fungicides tebuconazole, flutolanil, and PCNB. After inoculation, microplots were treated with recommended rates of tebuconazole (0.227 kg ai/ha), flutolanil (0.337 kg ai/ha), PCNB (5.6 kg ai/ha), or were left untreated. Disease ratings were made at harvest, and pod yield from each plant was recorded. In both years, disease severity was significantly lower in treated microplots for all three fungicides. Isolates with lower *in vitro* fungicide sensitivity responded as well to labeled rates of all three fungicides as those with high *in vitro* sensitivity. *In vitro* sensitivity and percent control in treated microplots were not correlated for all three fungicides. Fungicide sensitivity and the level of infection in nontreated microplots also were not correlated for flutolanil and tebuconazole indicating that virulence was not affected by fungicide sensitivity. However, there was a negative correlation between *in vitro* sensitivity to PCNB and the level of infection in nontreated microplots in 1995, indicating that isolates with lower *in vitro* sensitivity were more virulent. However, this trend was not observed when the same isolates were evaluated in 1996. In 1995, plants in PCNB-treated microplots had a significantly higher yield than those in the nontreated microplots. In 1996, all fungicide treatments significantly enhanced yield. Because *in vitro* sensitivity and field efficacy were not correlated for all three fungicides, labeled rates should control stem rot in the field.

Key Words: Groundnut, sensitivity correlation, *Sclerotium rolfsii*, virulence.

Stem rot, also known as white mold or southern blight, caused by the soilborne fungus *Sclerotium rolfsii* Saccardo (1911), is a devastating disease of peanut in the U.S. Of

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all the pathogens affecting peanut in Georgia, stem rot ranks second behind the leaf spot diseases in percent reduction of crop value. The fungus girdles the main stem and lateral branches, often killing the plant. In years when weather conditions are not favorable for above-ground infections to develop, the fungus can infect below ground, severely damaging pegs and pods. For the 3 yr period beginning in 1995, the annual reduction in crop value due to stem rot represented a $31.5 million loss to Georgia growers (Univ. of Georgia Coop. Ext. Serv. estimates, 1995-1997). Prior to 1994, losses often were much higher, primarily because options available to growers for stem rot control were limited to cultural practices and the use of pentachloronitrobenzene (PCNB), which has limited efficacy against this disease (Hagan *et al.*, 1988).

Since 1994, tebuconazole and flutolanil have been registered for control of stem rot and other diseases of peanut. Both products are very effective and tebuconazole in particular is used by many growers (Brenneman and Minton, 1991; Brenneman and Murphy, 1991; Brenneman *et al.*, 1991). Both fungicides have site-specific modes of action, and recent experiences have shown that resistant individuals increase in frequency in fungal pathogen populations after prolonged exposure to such compounds (Hildebrand *et al.*, 1985; Shepers, 1985a; Clark, 1992; Elad, 1992; DeWaard, 1994; Golembiewski *et al.*, 1995). This is of particular concern since tebuconazole is used extensively in peanut production. Tebuconazole is a member of the triazole class of sterol demethylation-inhibiting fungicides (DMIs). Pathogen populations receive additional exposure to DMIs because of the subsequent registration and use of propiconazole on peanut.

Monitoring fungicide sensitivity in field populations of *S. rolfsii* is one method to assess the development of resistance. Such studies can provide an accurate estimation of the sensitivity of a population to a particular fungicide. However, decreased sensitivity to a fungicide *in vitro* does not mean that "practical resistance" will develop. "Practical resistance" is the term used when the development of resistance leads to a failure of disease control in the field (Delp and Dekker, 1985).

For "practical resistance" to develop, the resistant isolates must be as virulent and competitive as fungicide-sensitive isolates in nature. Several studies have been done to determine how decreases in fungicide sensitivity can affect the fitness of a fungal pathogen. Studies involving *Cercospora beticola* and *Penicillium italicum* have shown that DMI-resistant mutants were less fit than DMI-sensitive strains (DeWaard and VanNistelrooy, 1982; Henry and Trivellas, 1989), while other microplot studies involving *Sclerotinia minor*, *Sphaerotheca fuliginea*, and *Phytophthora infestans* showed that fungicide resistant isolates were equally or more virulent than fungicide sensitive strains in field microplots (Shepers, 1985b; Brenneman *et al.*, 1987; Kadish *et al.*, 1990). The occurrence of resistant isolates that are as virulent as sensitive isolates could have serious implications for use of fungicides with site-specific modes of action, particularly if these isolates are equally fit otherwise.

A fungicide monitoring program has been underway in Georgia since 1994 to determine potential for development of resistance in *S. rolfsii* to tebuconazole and flutolanil. Sampling protocols and baseline sensitivity values to these fungicides have been established *in vitro* (Franke *et al.*, 1998). The rate at which resistance may develop also depends upon the relationship between low *in vitro* sensitivity and the ability of isolates exhibiting that sensitivity to compete with other isolates in the field. This study was undertaken to determine (a) the relative virulence of isolates with different fungicide sensitivity and (b) if adequate disease control will still be attainable in the field when labeled rates of the fungicides are applied.

## Materials and Methods

One hundred twenty-one microplots were used each year of the 2-yr study. Each microplot consisted of a cylindrical aluminum ring (0.9-m diam. × 0.3-m high) buried 15 cm in the soil. In both years, the microplots were fumigated 3 wk prior to planting with metam sodium (Vapam 32%, 1429 L/ha). Ten seeds of Georgia Runner peanut (*Arachis hypogaea* L.) were planted in each microplot on 23 May 1995 and 31 May 1996. After emergence, plants were thinned or transplanted as needed to ensure a population of five plants per microplot. Standard cultural practices and irrigation were applied as recommended by the Georgia Coop. Ext. Serv. both years to promote heavy canopy development which would produce a favorable microclimate for stem rot development.

Isolates of *S. rolfsii* from 10 locations in Georgia (four obtained in 1994 and six in 1995) were used for inoculations. Sensitivity to flutolanil, tebuconazole, or PCNB was determined *in vitro* from a sample of 50 to 76 isolates per location in 1994 and 1995 (Franke *et al.*, 1998) (Tables 1 and 2). Within locations, isolates used in the current study represented the highest and lowest degrees of sensitivity to each fungicide, for a total of six isolates per location (Franke *et al.*, 1998).

Four plants in each microplot were randomly selected for inoculation with a different isolate of *S. rolfsii*, leaving one

**Table 1. Fungicide sensitivity of *Sclerotium rolfsii* isolates collected in 1994 used in the microplot study. Isolates represent the widest range of *in vitro* fungicide sensitivity for flutolanil, PCNB, or tebuconazole observed in a sample size of 71 to 76.**

| Location | Isolate | $ED_{50}$ values | | |
|---|---|---|---|---|
| | | Flutolanil | PCNB | Tebuconazole |
| | | --------- | µg/mL | --------- |
| Tift Co. (Gibbs) | 1-94 | 0.007 | 0.550 | 0.005 |
| | 2-94 | 0.090 | 5.000 | 0.112 |
| Tift Co. (Benson) | 3-94 | 0.020 | 0.490 | 0.007 |
| | 4-94 | 0.130 | 4.460 | 0.090 |
| Sumter Co. | 5-94 | 0.003 | 0.420 | 0.001 |
| | 6-94 | 0.140 | 2.400 | 0.140 |
| Lee Co. | 7-94 | 0.002 | 0.790 | 0.001 |
| | 8-94 | 0.040 | 17.800 | 0.430 |

**Table 2. Fungicide sensitivity of *Sclerotium rolfsii* isolates collected in 1995 used in the microplot study. Isolates represent the widest range of *in vitro* fungicide sensitivity at one discriminatory dose of flutolanil (0.03 mg/mL), PCNB (2.00 mg/mL), or tebuconazole (0.02 mg/mL) observed in a sample size of 38 to 50.**

| Location | Isolate | Sensitivity[a] | | |
|---|---|---|---|---|
| | | Flutolanil | PCNB | Tebuconazole |
| | | ---------- | % | ---------- |
| Colquitt Co. | 9-95 | 54.00 | 93.00 | 79.00 |
| | 10-95 | -11.10 | 38.00 | -19.00 |
| Coffee Co. | 11-95 | 56.00 | 88.00 | 38.00 |
| | 12-95 | 15.00 | 14.00 | 3.00 |
| Tift Co.(Braxson) | 13-95 | 62.00 | 92.00 | 73.00 |
| | 14-95 | 0.00 | 5.00 | -25.00 |
| Miller Co. | 15-95 | 45.00 | 69.00 | 59.00 |
| | 16-95 | 14.00 | -8.00 | 7.00 |
| Seminole Co. | 17-95 | 53.00 | 87.00 | 45.00 |
| | 18-95 | -31.00 | 0.00 | -54.00 |
| Berrien Co. | 19-95 | 46.00 | 91.00 | 43.00 |
| | 20-95 | 10.00 | 4.00 | 6.00 |

[a]Percent inhibition was calculated as [100-(colony diameter on amended medium divided by colony diameter on controls) × 100]. Negative percent inhibition indicates more colony growth on amended medium than controls.

plant as a noninoculated control. There were four-replicate paired treated and nontreated microplots randomly selected for each fungicide evaluated. The experiment was analyzed as a split-plot design with fungicide treatment (treated or nontreated) as the whole plot and isolate as the subplot. Five plots per treatment were used to accomodate all isolates tested and isolates were rerandomized for each treatment. Isolate × plot interactions were determined to see if differences among plots had an effect on disease levels. The fungicides and their corresponding most and least sensitive isolates were evaluated separately. Inoculum consisted of agar plugs (1.0 cm diam.) taken from the edge of actively growing colonies on potato dextrose agar (PDA). An agar plug was placed placed between a primary branch and the main stem above the soil line in the crown of 113-d-old plants in 1995 and 71-d-old plants in 1996. In both years, microplots were irrigated prior to inoculation with overhead sprinklers that applied approximately 1.3 cm of water. Immediately after inoculation, a leaf spot cover spray of chlorothalonil (Bravo 720, 1.3 kg/ha) was applied plus an application of acephate (Orthene 75S, 1.1 kg/ha) insecticide to prevent fire ants from eating the agar plugs. The microplots were then irrigated again to prevent PCNB toxicity and facilitate movement of the fungicide through the plant canopy to the soil surface. An additional 1.3 cm of water was applied 24 hr later in 1996 to maintain high relative humidity within the plant canopy.

In 1995, tebuconazole (Folicur 3.6F, 0.227 kg/ha), flutolanil (Moncut 50W,0. 337 kg/ha), or PCNB (Terraclor 4F, 5.6 kg/ha) was applied 24 hr after inoculation to the treated microplots. In 1996, fungicide applications were made 72 hr after inoculation. Peanuts in all microplots were sprayed at approximate 14-d intervals with chlorothalonil, which is only active against foliar peanut pathogens. Fungicides were broadcast using a $CO_2$-powered back-pack sprayer equipped with a broadcast boom with three D2-23 nozzles per row at a rate of 186.9 L of water per ha at $3.1 \times 10^5$ Pa. In 1996, due to an earlier inoculation date and ideal conditions for stem rot development, two additional applications of each fungicide were needed as per label recommendations to protect the plants until maturity.

Plants were dug manually at 142 d after planting (DAP) in 1995 and 134 DAP 1996 and disease ratings were made. Individual plants were rated on a 1-8 scale with each number representing a range of percentage stem area that was infected (1 = 0%, 2 = 1-3%, 3 = 4-5%, 4 = 6-15%, 5 = 16-25%, 6 = 26-50%, 7 = 51-75%, and 8 = 76-100%). Approximately 3-4 wk after digging plants were cured in the field, pods were removed manually from each plant and the dry weight (g) was recorded. Disease severity ratings were transformed using the midpoint of each percentage class prior to analysis. Disease ratings and yield were analyzed by ANOVA using the General Linear Models Procedure of PC-SAS (SAS, 1996). The effects of fungicide treatment, year × treatment, and isolate × treatment interactions for disease severity and yield were determined for each fungicide.

Correlation coefficients between isolate sensitivity (expressed as $ED_{50}$ values or percent inhibition) and percent disease control of plants from treated microplots were calculated. Similarly, correlations between isolate sensitivity and disease severity in nontreated microplots were calculated. Because the measure of sensitivity was different for isolates collected in 1994 compared to 1995, they were grouped by year collected before correlation analysis.

## Results

In both years, disease was significantly ($P < 0.05$) more severe in nontreated than in treated microplots for all fungicides tested. Mean percent infection for plants in treated microplots was 4.3 for flutolanil, 15.3 for PCNB, and 8.5 for tebuconazole, as compared with 16.0, 22.6, and 22.6 % infection, respectively, in nontreated microplots (Fig. 1). Isolate × treatment interactions for disease severity were not significant for any of the fungicides and isolate × plot interactions were only significant for flutolanil. There also was not a significant ($P > 0.05$) correlation between sensitivity *in vitro* and percent control (percent control compared to noninoculated plants) in treated microplots for any of the fungicides in either year. *In vitro* fungicide sensitivity and percent infection in nontreated microplots also was not correlated for flutolanil and tebuconazole, indicating that *in vitro* sensitivity did not affect virulence. However, there was a significant negative correlation ($r = -0.74$, $P = 0.006$) between *in vitro* sensitivity to PCNB and percent infection for isolates collected in 1995, indicating that less sensitive isolates were more virulent.

Due to a significant year × treatment interaction for flutolanil, yield data from both years were not combined for this presentation. In 1995, only PCNB significantly

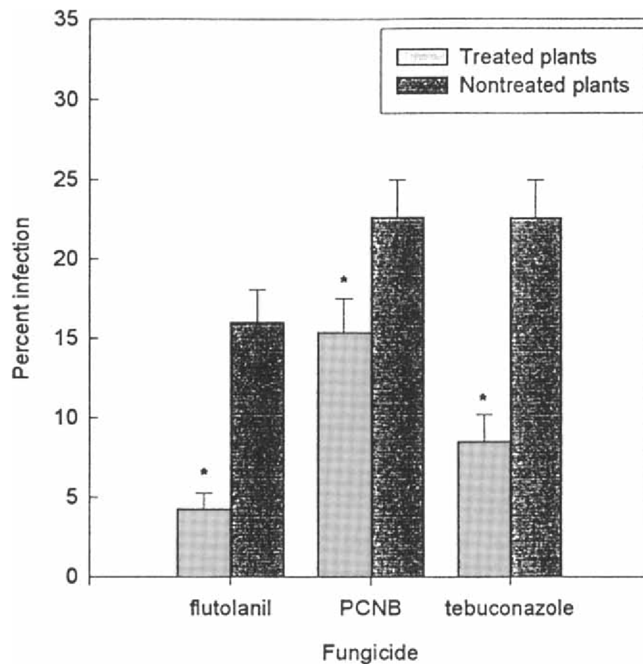


**Fig. 1. Combined mean percent infection of Georgia Runner peanut plants inoculated with different isolates of *Sclerotium rolfsii* from fungicide-treated and nontreated microplots in 1995 and 1996. *denotes a significant difference (P ≤ 0.05) between treated and nontreated plants for each fungicide.**

(P = 0.003) increased the yield of treated versus nontreated plants (Fig. 2). The only significant interactive effect of isolate and treatment was for PCNB in 1995, but there was no apparent relationship between isolate sensitivity and pod yield of inoculated plants. In 1996, yield of treated of plants was significantly greater than nontreated plants for all three fungicides. Pod yield was 32.9 g/plant for PCNB, 38.6 g/plant for tebuconazole, and 45.9 g/plant for flutolanil as compared to 20.8, 23.6, and 24.4 g/plant, respectively, in nontreated microplots. Treatment × isolate interactions were not significant for any of the fungicides evaluated.

## Discussion

The absence of isolate × treatment interactions for disease severity indicates that isolates responded similarly to fungicide treatments. Disease severity was significantly reduced by the fungicides, indicating that both the most and least sensitive isolates were adequately controlled in the field. Application of fungicides after inoculation may have influenced the disease data. Both tebuconazole and flutolanil have protective and curative properties, but studies have not been done to determine the degree of curative action at different time intervals after inoculation. However, disease severity was reduced by all the fungicides indicating that the length of time from inoculation to the first fungicide application was not long enough to nullify the efficacy of the fungicides. Significant isolate × plot interactions for flutolanil may have been caused by smaller plants or improperly functioning irrigation sprinklers which created unfavorable conditions for disease development.

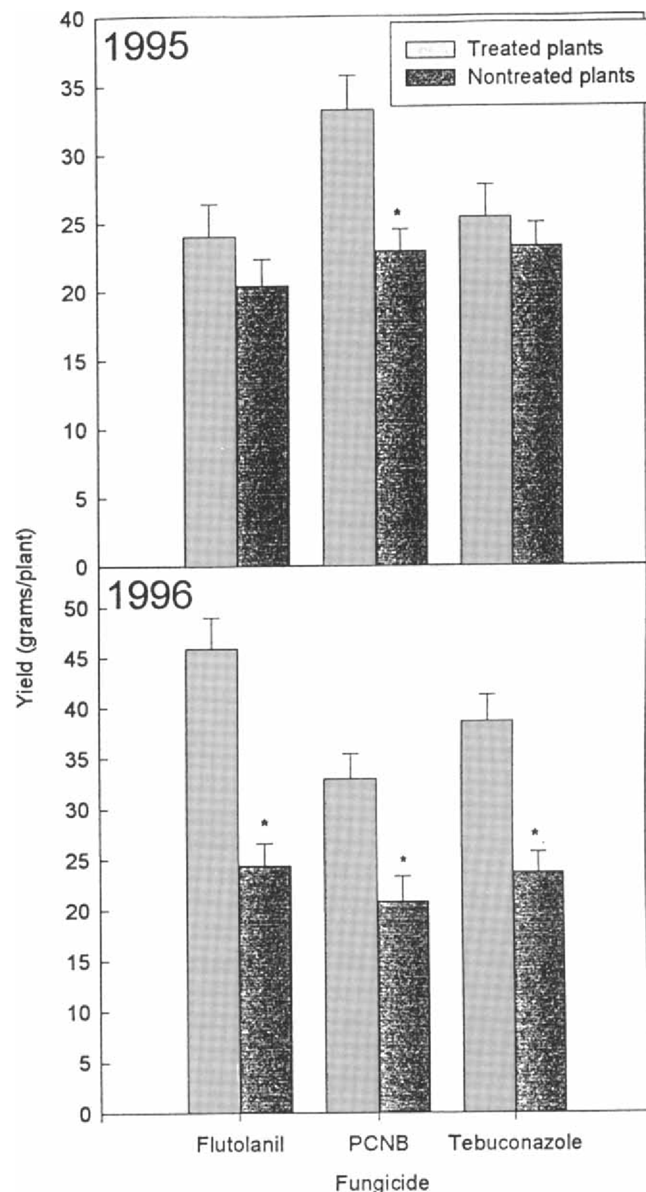


**Fig. 2. Average pod yield of Georgia Runner peanut plants inoculated with different isolates of *Sclerotium rolfsii* from fungicide-treated and nontreated microplots in 1995 and 1996. *denotes a significant difference (P ≤ 0.05) between treated and nontreated plants for each fungicide.**

Based on *in vitro* sensitivities alone, it is impossible to determine if changes in virulence are associated with low fungicide sensitivity. In 1989, Henry *et al.* (1989) found that 60% of *Cercospora beticola* isolates with lower *in vitro* sensitivity to flusilazole, etaconazole, and fenarimol also had reduced fitness and virulence. Of the isolates with reduced fitness, 16% were nonpathogenic. In a more recent study, Shim *et al.* (1998) evaluated virulence of isolates of *S. rolfsii* with varying degrees of sensitivity to PCNB and found that the most insensitive isolate (Sr4/26) also was the least virulent. However, a significant negative correlation between virulence and sensitivity was not present. In our study, correlation analysis was done separately for isolates collected in 1994 or 1995

because different measures of *in vitro* sensitivity were used. We did find a significant negative correlation between *in vitro* sensitivity to PCNB and virulence. However, this trend was not observed for the isolates collected in 1994. It is important to note that variability in disease severity and yield for PCNB-treated peanuts in microplots is similar to the modest and often erratic control of stem rot by PCNB in replicated field plots (Hagan *et al.*, 1988). Variability in isolate sensitivity along with environmental effects and timing of PCNB applications could be responsible for erratic results seen with PCNB.

Disease severity proved more valuable than yield data in assessing the relationship between *in vitro* sensitivity and field efficacy of the fungicides. Yields were higher only for plants that received treatment with PCNB in 1995. This is somewhat surprising since PCNB gave the least disease suppression of the three fungicides. In 1995, plants were inoculated late in the season when conditions were not ideal for disease development. This could be responsible for the lack of differences between treated and nontreated microplots for the other fungicides. Plants were inoculated earlier in the season and conditions were more favorable for disease development in 1996. Because yield data was collected from single plants, inherent variability may account for the lack of statistically significant differences in yield between treated and nontreated microplots for tebuconazole and flutolanil.

Based on the results of this experiment, isolate aggressiveness was not correlated with *in vitro* sensitivity to tebuconazole or flutolanil. Henry *et al.* (1989) suggested that any loss of virulence associated with reduced fungicide sensitivity may be further compounded by loss of fitness in saprophytic or survival stages. If changes in virulence are related to fungicide sensitivity, then additional studies will be needed to determine if these changes remain stable over time.

The lack of correlation between *in vitro* fungicide sensitivity and field efficacy indicates that labeled rates of the three fungicides are capable of controlling stem rot caused by isolates with a lower *in vitro* sensitivity. Additional studies to evaluate the response of these isolates to reduced rates of fungicides should be performed. It could be possible that the labeled rates of fungicides used in this study were high enough that isolates with a lower *in vitro* fungicide sensitivity would still respond to the treatment. Using lower rates of the fungicides may reveal a differential response among isolates correlated with *in vitro* sensitivity. Essentially, this would model the response of even less sensitive isolates to full labeled rates of fungicide. If such responses cannot be quantified, then *in vitro* testing may not be an accurate method of assessing resistance risks with these fungicides.

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