

## Microsclerotial Germination of *Cylindrocladium crotalariae* in the Rhizospheres of Susceptible and Resistant Peanut Plants

D. T. Krigsvold, G. J. Griffin, and M. G. Hale

Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg 24061. Present address of senior author: Tela Railroad Co., Research Division, United Fruit Co., LaLima, Honduras.

Based on a Ph.D. dissertation by the senior author.

Research supported in part by a cooperative agreement with the United States Department of Agriculture.

Accepted for publication 22 October 1981.

### ABSTRACT

Krigsvold, D. T., Griffin, G. J., and Hale, M. G. 1982. Microsclerotial germination of *Cylindrocladium crotalariae* in the rhizospheres of susceptible and resistant peanut plants. *Phytopathology* 72:859-864.

Microsclerotia of *Cylindrocladium crotalariae* were tested in artificially infested, unsterile soil for germination in the inner 1-mm of the rhizospheres of plants of peanut cultivars resistant (Argentine) and susceptible (VA-72-R) to the pathogen. Germination was significantly higher in the rhizosphere of the susceptible versus the resistant cultivar. Microsclerotium germination in response to crude root exudates from axenically grown peanut plants also was greater in soil for the susceptible than for the

resistant cultivar. Higher levels of carbon were required for Argentine root exudate than for VA-72-R root exudate to support similar levels of microsclerotial germination in soil. For each cultivar, percentage of germination was related directly to carbon levels in the root exudate. Each of the 14 peanut root exudate components (sugars, amino acids, and organic acids) tested were stimulatory to microsclerotium germination in soil.

*Additional key words:* root infection, soil fungistasis.

*Cylindrocladium* black rot (CBR) of peanut (*Arachis hypogaea* L.), caused by *Cylindrocladium crotalariae* (Loos) Bell and Sobers, is a destructive root disease in the Virginia-North Carolina peanut-growing region when environmental conditions are favorable. The microsclerotium appears to be the principal surviving and infecting propagule of the pathogen in soil (11,14,22), but nothing is known about the behavior of microsclerotia in the peanut rhizosphere or the response of microsclerotia in soil to peanut root exudates. In greenhouse and field experiments, Tomimatsu and Griffin (22) observed numerous *C. crotalariae* infections (1 to >1,000 per plant) on roots of peanut plants growing in naturally infested soils after 3-15 wk. Infections were observed over all portions of

asymptomatic fine, lateral, and taproots. It is likely that constituents in exudates from the peanut root tip (5) are largely responsible for stimulating germination of microsclerotia previously inhibited by soil fungistasis and initiating these root infections. The present study was undertaken to determine the extent and nature of *C. crotalariae* microsclerotium germination in the rhizosphere of peanut plants resistant and susceptible to the pathogen, and to examine the influence of specific root exudate components on microsclerotial germination in soil. Portions of the information reported here were presented in a preliminary report (12).

### MATERIALS AND METHODS

**Germination of *C. crotalariae* microsclerotia in the rhizosphere of resistant and susceptible peanut cultivars.** To study microsclerotium germination in the rhizosphere, a system (10) was designed so that a growing peanut root could be observed

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0031-949X/82/07085906/\$03.00/0

©1982 The American Phytopathological Society

continuously. Glass tubing (0.635 cm in diameter) was bent into a 'V' shape and cemented to a 8 × 16-cm rectangular glass plate (root slide). A 6-g sample of unsterile sandy loam peanut-field soil, artificially infested with microsclerotia, was placed on the plate glass in the bottom of the 'V' formed by the bent glass tubing. The water content of the soil was 10.0% (water content at -0.10 and -0.33 bars = 7.6 and 13.0%, respectively). A thin layer of washed vermiculite was placed above the soil on the glass plate to mark and separate the infested from the uninfested soil. A square of plastic mesh (5 × 5 cm with contiguous 1-mm openings) was placed over the infested soil and secured to the glass plate with two rubber bands. This glass root guide was placed in a 14.5-cm high (0.95 L) plastic container at the most acute angle possible, with the infested soil area aligned so that it could be observed from behind through an observation port. Unsterile, sandy loam peanut-field soil, free of *C. crotalariae*, was then placed in the plastic container so as to cover the root guide to near the top of the container (13 cm high) on the front side of the glass plate and to the bottom of the 'V' on the back side.

To infest soil, microsclerotia of *C. crotalariae* (isolate CC-11), grown for 4 mo in peanut stem liquid medium (10), were harvested and processed by blending and washing (10). Microsclerotia were sized by passing them through nested 105  $\mu\text{m}$  (pore size) (140-mesh) and 50  $\mu\text{m}$  (pore size) (300-mesh) sieves. Microsclerotia retained on the 50- $\mu\text{m}$  sieve were used to infest the soil. A sandy loam, peanut-field soil (pH 5.6; 1.5% organic matter) was artificially infested with  $1 \times 10^4$  microsclerotia per gram of dry soil by hand-mixing the soil and microsclerotia in a plastic bag for 10 min. Infested soil was stored in a plastic bag (with pinholes for gas exchange) in a plastic moist chamber at room temperature (25–27 C); soil water content was monitored at regular intervals. Microsclerotia were in the soil 21 days prior to the first experiment.

Peanut cultivars used in this experiment were either resistant (Argentine) or susceptible (VA-72-R) to *C. crotalariae*. None of the seeds of either cultivar were treated with fungicides and all were from the same seed lots. Sound, healthy peanut seeds were placed in plastic moist chambers between layers of moist paper towels and incubated at room temperature (25–27 C). Germinated peanuts with both cotyledons attached were sized by radicle length after 3 days and those nearest to the average lengths of 2.0 cm for Argentine and 1.8 cm for VA-72-R were transplanted into the soil systems. A hole was made in the soil and the germinated peanut was placed carefully in the hole with the cotyledons approximately 1.5 cm above the soil line and, so that the elongating radicle would meet the glass plate, midway between the glass tubing root guides. Each entire system was covered with a ventilated plastic bag and incubated at room temperature (25–27 C) and room light. Shoots of plants had approximately five leaves emerged at the time of soil sampling for microsclerotium germination assays. Root growth on the plate glass guide was monitored through the root observation port, and systems were sampled when the majority of the growing root tips had reached the bottom of the glass-tubing root guide 'V.'

Three experiments were conducted, but soil sampling was slightly different for each experiment. In all cases, however, the inner 1-mm layer of the rhizosphere soil was removed with a microspatula from defined regions of the peanut root tip. The soil from each root tip was smeared on a glass slide and stained with acid fuchsin in lactophenol. Soil smears were examined microscopically at  $\times 125$  and scored for microsclerotium germination. Five to 10 replications per cultivar were made in each experiment with an average of 392, 262, and 365 microsclerotia counted per replication in experiments 1, 2, and 3, respectively.

**Germination of *C. crotalariae* microsclerotia in soil in response to crude root exudates from resistant and susceptible peanut cultivars.** To study microsclerotium germination in the presence of a semicontinuous flow of crude root exudate, an artificial root system was constructed by cementing four Pasteur pipettes together with silicone sealer. The joined pipettes were autoclaved at 121 C to remove acetic acid fumes and to season the sealer. Tips of the pipettes were cut off to leave an orifice approximately 2 mm in diameter. A sandy loam peanut-field soil (pH 5.6), with moisture continuously maintained at near field capacity (-0.33 bars)

previous to use, was placed in a plastic container (14.5 cm high with a volume of 0.95 L) to a height of 9.5 cm. A flat-bottomed bottle was used to smooth a circular area 4 cm in diameter on the soil surface. One-half gram of soil, artificially infested with microsclerotia of *C. crotalariae* (isolate CC-11) at  $1 \times 10^4$  microsclerotia per g dry soil, was placed on the smoothed surface. A 3 × 3-mm-square piece of plastic mesh, with 1-mm openings, was placed firmly atop the infested soil. The artificial root was placed with the Pasteur pipette orifices centered and resting on the plastic mesh. Uninfested soil was placed around the pipettes to a height of approximately 1 cm above the orifices. To conserve moisture, each system was covered with a ventilated plastic bag, loosely closed at the bottom. All glassware used in this experiment was cleaned with acid-dichromate, followed by extensive rinsing with final rinses in doubly distilled water.

To obtain root exudates for bioassays, plants of peanut cultivars Argentine and VA-72-R were grown hydroponically under axenic conditions in germfree plastic chambers. The techniques used for axenic plant growth were modified slightly from those developed and used in the Plant Gnotobiology Laboratory at Virginia Polytechnic Institute and State University (6,7). All glassware used in this portion of the experiment was acid-cleaned and rinsed as described above. Peanut seeds were surface sterilized in NaClO without removal of the cotyledons. Axenic plants in the germfree chambers were grown in 0.25% Hoagland's nutrient solution in 1-L Erlenmeyer flasks at 25–26 C and in continuous illumination supplied by cool-white fluorescent lamps (approximately  $1.5 \times 10^4$  lux at the top of the chambers). Periodic checks of swab samples from plants and growth solution were made on selective media to ensure an axenic condition.

The procedure for obtaining axenic peanut plants involved several transfers of the seedlings before the final axenic growth stage when exudates were collected. The seed was first surface sterilized and then germinated on acidified potato-dextrose agar. Uncontaminated seedlings were transferred to small vessels (transplanting tubes) containing hydroponic growth medium and placed inside the sterilized plastic growth chambers. Seedlings were then transferred to 1-L Erlenmeyer flasks containing hydroponic growth medium for the final growth stage.

Nutrient solutions with root exudates were collected approximately every 7 days for 46 days. Crude root exudate solutions were flash-evaporated and passed through a Nucleopore filter (pore size 0.44  $\mu\text{m}$ ) apparatus to remove sloughed cells and to maintain sterility. The solutions were freeze-dried, weighed, and stored in screw-cap bottles in a desiccator until used. At the end of 46 days, all plants were removed from the chambers, freeze-dried, and dry weights were taken. The number of major lateral roots was counted for each root system.

Crude root exudates, redissolved in doubly distilled water, were put into each artificial root system at four drops per system (one drop per pipette) at hourly intervals to a total of 6.6 ml over a 24-hr period. Each 6.6 ml contained 303.0 mg crude root exudate for the susceptible cultivar (VA-72-R) and 252.5 mg crude exudate for the resistant cultivar (Argentine), based on a 1.2:1 (susceptible: resistant) ratio. Since microsclerotium germination in situ occurs near the root surface, the ratio of crude root exudates utilized was based on a determination of the average root dry weight produced by each peanut cultivar growing in axenic hydroponic culture and on the average amount of crude root exudate produced by each cultivar. The susceptible cultivar produced 20% more crude exudate per gram dry weight of root tissue than the resistant cultivar; 50% more crude exudate was produced by the susceptible cultivar on the basis of numbers of root tips, however. Since the exudate used was derived from both root tips and mature root regions, the exudate per gram dry weight of root tissue was considered to be the better basis for the experimental design. Preliminary tests indicated what levels of crude exudate were sufficient to stimulate microsclerotium germination in the artificial root system. The pH for each exudate sample was measured at  $6.0 \pm 0.2$ .

Treatments consisted of the exudates collected from 10 plants for each of three sampling periods (0–7 days, 8–22 days, and 23–46

days after transplanting or start of growth of the peanut plant in hydroponic culture) for each cultivar, with three replicate artificial root systems used per sampling period. The age of the axenic peanut plant at 0 days in the sampling period was actually 20 days from germination. Doubly distilled water was used in place of root exudates for the control systems. Another control received no treatment. After 24 hr, the systems were carefully dismantled and a sample (approximately 0.25 g) of infested soil was removed from each system, smeared on a glass slide, and stained with acid fuchsin in lactophenol. An average of 283 microsclerotia were counted from each sample. Soil moisture content was determined gravimetrically for soil samples taken directly below the infested soil and averaged  $8.0 \pm 0.5\%$  for all samples; soil moisture content for this soil near field capacity ( $-0.33$  bars) is 7.6%.

**Determination of total carbon, nitrogen, and hydrogen in crude root exudates.** Samples to be analyzed were taken from filtered, freeze-dried, root exudates of gnotobiotically grown peanut plants. Assays were made for three sampling periods per cultivar as outlined. The analyses were done on a Perkin-Elmer 240 carbon-hydrogen-nitrogen analyzer.

**Germination of *C. crotalariae* microsclerotia in soil in response to selected root exudate components: Sugars, amino acids, and fatty acids.** A sandy loam, peanut-field soil (pH 5.6) was artificially infested with microsclerotia of *C. crotalariae* (isolate CC-11) as described. The root exudate components tested were sugars (15,19), amino acids (Hale, M. G., unpublished), and fatty acids (21) found to be present in root and fruit exudates of gnotobiotically grown peanut plants of cultivars Argentine (sugars, amino acids, and fatty acids) and Florigiant (amino acids), resistant and susceptible, respectively, to infection by *C. crotalariae*. Components were tested at equivalent carbon and the concentration used was 1.9 mg of carbon per gram of dry soil. All components were put into solution in doubly distilled water and the pH was adjusted to approximately 6.0 with KOH or HCl. The soil used contained  $3.54 \mu\text{g NH}_4^+\text{-N}$  and  $6.25 \mu\text{g NO}_3^-\text{-N}$  per gram of soil.

One-gram samples of infested soil were allowed to air-dry partially on glassine weighing paper before 0.2 ml of component solution was added to the soil. The component solution was mixed thoroughly into the soil with a microspatula while the soil was drying to approximate field capacity ( $-0.33$  bars). The treated soil

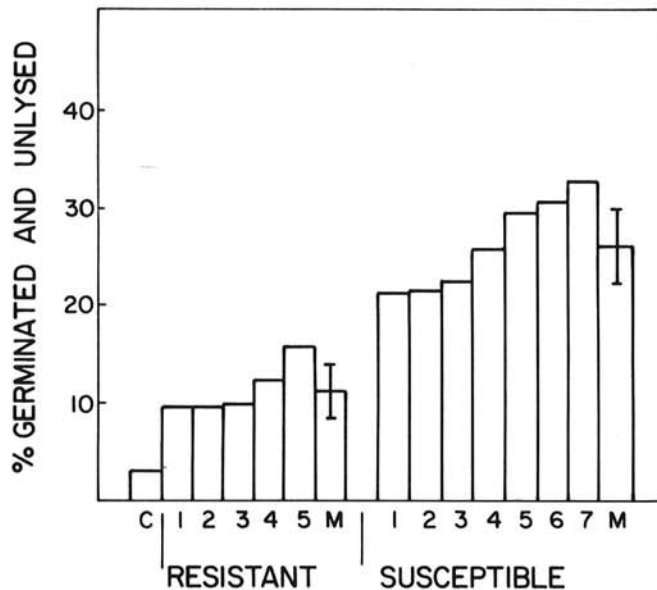
was halved and placed into separate watch glasses (25-mm diameter) which were in turn placed into glass petri dishes ( $10 \times 2$  cm) containing doubly distilled water to maintain high humidity. After 24 hr incubation at 25–27 C, the systems were sampled and soil smears were prepared as described and examined for microsclerotium germination.

All solutions containing fatty acids had to be heated to the melting point of the particular fatty acid before adding to the soil. Concurrent tests were run with doubly distilled water at the same temperatures and at room temperature to test the possible effects of momentary heat on microsclerotial germination. No effects were noted at the temperatures used (73 C maximum).

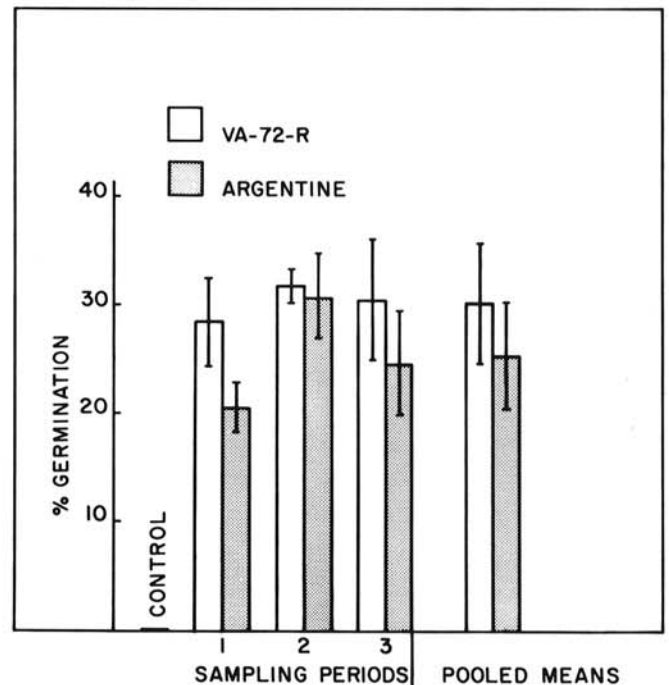
## RESULTS

**Germination of *C. crotalariae* microsclerotia in the rhizospheres of resistant and susceptible peanut cultivars.** In all three experiments, the mean percentage microsclerotium germination was higher in the inner 1-mm of rhizosphere soil of susceptible versus resistant peanut plants. In experiment 1, microsclerotia germinated with unlysed germ tubes at 26.0 and 11.4% in the inner 1 mm of rhizosphere soil (collected from approximately 0 to 3.0 cm from the root tip) of susceptible and resistant peanut plants, respectively (Fig. 1). This difference was highly significant ( $P < 0.001$ ), according to Student's *t*-test.

The data in experiments 2 and 3 (based on at least five and 10 plants of each cultivar for experiments 2 and 3, respectively) were pooled, and an analysis of variance was done. The means of percentage germination (unlysed germ tubes) were significantly different at  $P < 0.05$ . Microsclerotia germinated at 39.8 and 33.9% in the inner 1-mm of rhizosphere soil (collected from 1.0 to 4.0 cm from the root tip) of susceptible and resistant peanut plants, respectively. Although soil samples were taken from two root zones (1.0–2.5 and 2.5–4.0 cm from the root tip) in experiment 3, an analysis of the data showed only a very slight difference in percentage microsclerotial germination between the two zones (37.4% in the 1.0–2.5 cm zone versus 36.5% in the 2.5–4.0 cm zone).



**Fig. 1.** Germination in situ of *Cylindrocladium crotalariae* microsclerotia in artificially infested soil in the inner 1-mm of rhizosphere soil of resistant (Argentine) and susceptible (VA-72-R) peanut cultivars. Control (C) was artificially infested soil without peanut plants. Replications (1–5 or 1–7) represent individual plants. M = mean of replications within each cultivar group. An average of 392 microsclerotia were counted for each replication. Error bar represents standard deviation.



**Fig. 2.** Effect of axenically collected crude root exudates from resistant (Argentine) and susceptible (VA-72-R) peanuts on germination of *Cylindrocladium crotalariae* microsclerotia in a soil-artificial-root system. Each bar represents microsclerotia germinated and unlysed and is the mean of three replications with an average of 283 microsclerotia observed per replication. Sampling periods represent: 1 = 0–7, 2 = 8–22, and 3 = 23–45 days after seedlings were transplanted. Controls were double distilled water in soil. Error bar represents standard deviation.

Also, percentage of microsclerotia with lysed germ tubes was not greater in the 2.5–4.0 cm zone than in the 1.0–2.5 cm zone; overall, each zone averaged 12.0% microsclerotia with lysed germ tubes. In these experiments, the percentage of microsclerotia with lysed germ tubes in the rhizosphere soils was similar to that in the control soil (11.5%) with each experiment. This lysis is considered to result following previous microsclerotium germination (after artificially infesting the soil with microsclerotia).

The maximum time of exposure for any microsclerotium to the growing tip of the peanut plant root was 28 hr in all three experiments. The minimum time was 0, 13.9, and 9.5 hr for experiments 1, 2, and 3, respectively.

**Germination of *C. crotalariae* microsclerotia in soil in response to axenically collected crude root exudates from resistant and susceptible peanut cultivars.** For each of the three sampling periods tested, microsclerotia of *C. crotalariae* germinated at higher percentages in the crude root exudates from peanuts susceptible (VA-72-R) to *C. crotalariae* than from peanuts resistant (Argentine) to the fungus (Fig. 2). Overall, microsclerotia exposed to root exudates from susceptible peanuts germinated at 30.2 versus 25.3% for microsclerotia exposed to root exudates from resistant peanut plants. A *G*-test for independence indicated that overall percentage germination was not independent of peanut cultivar ( $P < 0.01$ ). However, when germination percentages in resistant and susceptible systems were compared within each sampling period, a departure was noted. Germination in sampling period 2 (8–22 days) was found to be independent of peanut cultivar, but germination in periods 1 (0–7 days) and 3 (23–45 days)

was not independent of peanut cultivar at  $P < 0.005$  and  $P < 0.05$ , respectively. Overall, the controls treated with doubly distilled water only had 0.1% microsclerotial germination with germ tubes holding stain (unlysed).

The number of germ tubes per microsclerotium was slightly higher overall in the systems exposed to root exudates of susceptible peanuts than in systems exposed to root exudates from resistant peanuts (1.36 versus 1.28 germ tubes per microsclerotium). However, these differences were not significant in a *t*-test at  $P = 0.05$ .

The systems with root exudates from susceptible peanuts averaged 2.9% microsclerotia with lysed hyphae versus 4.0% in the resistant exudate systems. These figures are comparable with the 5.8% that was found in untreated and undisturbed soil.

**Determination of total carbon, nitrogen, and hydrogen in crude root exudates.** These analyses were done to investigate the causes of the artificial-root experiment results reported above. The percentage of carbon in crude root exudates varied from sampling period to sampling period for both cultivars. When the data were pooled by cultivar and presented on the basis of mg carbon per gram dry root, Argentine plants exuded 1.14 times (48.79 mg carbon per gram of dry root) as much carbon as VA-72-R plants (42.70 mg carbon per gram of dry root). Using the carbon analysis data, the carbon content was calculated for the crude root exudate of each cultivar from each sampling period used in the artificial-root experiment. Although there was an average of 33.9% more carbon in Argentine (resistant) crude exudate used than in VA-72-R (susceptible) crude exudates used, the percentage germination in response to Argentine root exudate was less than for VA-72-R at all sampling periods (Fig. 3). Conversely, much more exudate carbon was required for Argentine than for VA-72-R to promote the same levels of germination. Increases in percentage microsclerotium germination were directly associated with increases in the amount of carbon in exudates of both cultivars. The overall amount of nitrogen in exudate samples appeared to be fairly uniform between cultivars (average 6.7% for VA-72-R versus 6.1% for Argentine). Some of the nitrogen and hydrogen may be associated with inorganic salts.

**Germination of *C. crotalariae* microsclerotia in soil in response to selected root exudate components: sugars, amino acids, and fatty acids.** Addition of any single root exudate component promoted microsclerotial germination (Table 1). Mixtures of components, with and without fatty acids, promoted the highest percentages of germination (48.7 and 55.6%, respectively). Stearic acid and proline were the most stimulatory of the single components tested. With a few exceptions, components that supported higher germination percentages supported a greater number of germ tubes per microsclerotium. The fatty acids, stearic and palmitic, promoted better than average quality germination (thick, branched germ tubes) while oleic acid promoted very poor quality (thin germ tubes) germination. Mixtures of sugars alone or amino acids and sugars fostered the best quality germination in soil.

## DISCUSSION

Differential germination of soilborne propagules in the rhizosphere or in root exudates of resistant versus susceptible plants has been hypothesized (13,16,18), but yet has not been convincingly demonstrated for any plant-fungus interaction. Buxton's (1,2) results, reported to show differential germination of physiologic races of *F. oxysporum* f. sp. *pisi* in the root exudates and rhizosphere leachates of resistant and susceptible pea cultivars in vitro, have been cast in doubt by the unsuccessful attempts of Kommedahl (8) in vitro, Whaley and Taylor (24,25) in vitro and in soil, and Schippers and Voetberg (17) in plant rhizospheres, to reproduce the fungal responses. Turner (23) reported no difference in sporangial germination of *Phytophthora palmivora* in vitro in the root exudate of cacao selections showing differential susceptibility to pod infection. However, zoospore germination and mean germ-tube length were considerably greater in root exudates from the more susceptible selections.

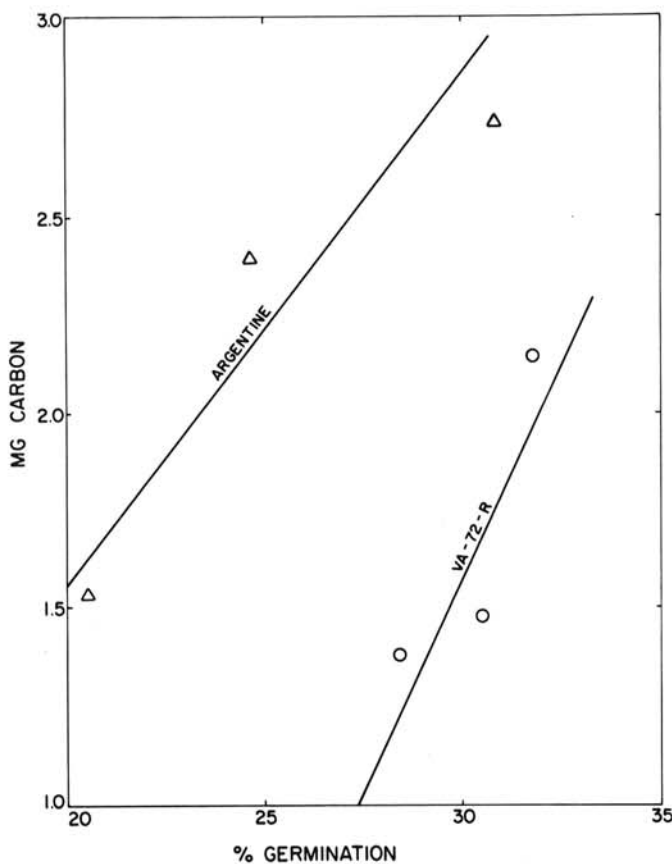


Fig. 3. Percent germination of *Cylindrocladium crotalariae* microsclerotia and total carbon levels in crude peanut root exudates obtained from resistant (cultivar Argentine) and susceptible (cultivar VA-72-R) plants and used in the artificial root system. Each data point represents the amount of carbon for each of three sampling periods of crude root exudate from Argentine and VA-72-R, respectively. Sampling periods in ascending order for both cultivars on the graph are 1, 3, and 2. The amount of carbon is plotted on the Y-axis to show the levels of exudate carbon required to support a specific level of microsclerotium germination for each peanut cultivar.

The results in this study support the hypothesis that peanut root exudates effect differential germination of *C. crotalariae* microsclerotia in soil. The in situ rhizosphere experiments are probably the most representative of what occurs in the peanut rhizosphere in nature. Significantly, germination of *C. crotalariae* microsclerotia was greater in the rhizosphere of susceptible plants in all three experiments conducted. Further, there was no evidence that germ tubes lysed in the rhizosphere of either peanut cultivar following microsclerotial germination. Only the rhizosphere germination experiments of Schippers and Voetberg (17), with *F. oxysporum* f. sp. *pisi* on resistant and susceptible pea cultivars, utilized a similar approach for a root pathogen. The data obtained in the artificial root experiment with crude root exudates also indicate that root exudates of susceptible cultivar VA-72-R are more stimulatory to *C. crotalariae* microsclerotia than exudates of resistant cultivar Argentine. The lower percentage of microsclerotium germination obtained in rhizosphere experiment 1 than in rhizosphere experiments 2 and 3 was probably due to the differences in rhizosphere sampling. In experiment 1, the rhizosphere soil sample probably included many microsclerotia in the latent period of germination, because soil sampling included the extreme tip of growing roots.

The mechanisms by which microsclerotia are stimulated more by exudate of the susceptible than by exudate of the resistant cultivar are not clear. Carbon analyses indicated that axenic Argentine plants produced 14% more exudate carbon per gram of root than did VA-72-R plants, but analysis of the data presented in Fig. 3 indicates that almost twice as much Argentine exudate carbon than of VA-72-R exudate carbon was required to support 30% microsclerotium germination in soil. Similar results were obtained in an experiment conducted preliminarily to that reported in Fig. 3, except that percentages of microsclerotium germination supported by both cultivar exudates were somewhat higher. In both experiments, percentage microsclerotium germination was related directly to the amount of root exudate carbon supplied by plants of

each cultivar. Thus, some other factor(s) appears to be operative than simply the level of carbon-containing exudates to account for the differential germination responses observed. Possibly an inhibitory or specific stimulatory substance, such as found for *S. cepivorum* (4), is involved. None of the known peanut root exudate components examined in this study could account for the differential germination, although all of the components stimulated microsclerotium germination in soil over controls. Further, the results of amino acid analyses of crude exudates (10) did not indicate that any of these compounds were responsible for the differential germination results. Statler (20) reported a direct correlation of resistance of bean to *F. solani* f. sp. *phaseoli* with the amount of phenolic compounds in extracts of seed coats of nongerminated seeds, in germinated seeds and in healthy hypocotyls and tap roots. Clauss (3) observed the same relationship with phenols from seed coats of peas resistant to *Ascochyta pisi*. However, Kraft (9) could find no correlation between amounts of phenols in exudates from germinating pea seeds and seedlings and resistance to *P. ultimum* and *F. solani* f. sp. *pisi*. Phenolic constituents of root exudates were not examined in the present study. If there is a specific stimulatory or inhibitory substance in the root exudate, it would appear to be present from the seedling stage until at least 65 days of plant age; seedlings were used in the rhizosphere tests, whereas crude root exudates were collected from plants 20–65 days old.

The possibility exists of an indirect effect of exudate on the pathogen due to a direct exudate effect on the antagonistic rhizosphere microorganisms. The greater quantities of carbon substrate in resistant cultivar exudate may stimulate greater populations of microorganisms antagonistic to microsclerotium germination.

The results of this work best support the hypothesis that there are stimulatory and/or inhibitory substances in the root exudates of Argentine and VA-72-R peanut cultivars which foster differential germination of *C. crotalariae* microsclerotia in soil. This selective stimulation or inhibition of microsclerotial germination and growth in soil could act as the first stage or one component of host resistance, but other mechanisms are likely to be of equal or greater importance in determining resistance in toto. It is not likely that the differences in percentage microsclerotium germination found here in rhizosphere tests (1.2- to 2.3-fold greater germination for VA-72-R) could account for the differences in resistance found in the field tests (3.0- to 5.8-fold more diseased plants for VA-72-R).

TABLE 1. Germination of *Cylindrocladium crotalariae* microsclerotia in artificially infested soil in response to peanut root exudate components<sup>a</sup>

Root exudate components <sup>b</sup>	Microsclerotia germinated <sup>c</sup> and:		Germ tubes per microsclerotium <sup>c</sup>
	Unlysed (%)	Lysed (%)	
Control <sup>d</sup>	5.1	7.7	1.1
Oleic acid (A)	13.8	5.8	1.7
Methionine (AF)	14.1	4.8	1.5
Ribose (A)	18.5	3.3	2.4
Arabinose (A)	19.4	5.0	1.3
Mannitol (A)	21.3	3.9	2.6
Lysine (AF)	21.6	3.8	1.5
Serine (AF)	23.4	4.2	3.6
Phenylalanine (F)	23.8	4.3	1.7
Glucose (A)	27.4	5.4	2.3
Palmitic acid (A)	30.2	3.6	3.0
Mannose (A)	31.8	7.6	3.3
Galacturonic acid (A)	34.3	2.1	3.9
Proline (A)	38.0	3.6	2.8
Stearic acid (A)	39.3	4.5	1.5
Mix with fatty acids	48.7	4.2	2.6
Mix without fatty acids	55.6	2.4	3.0
	Average = 4.5 ± 1.5		

<sup>a</sup>Root exudate component data are from work done by Shay and Hale (19), Thompson (21), and M. G. Hale (*unpublished*) in the Plant Gnotobiology Laboratory at Virginia Polytechnic Institute and State University, Blacksburg. Capital letters in parentheses following component names represent peanut cultivars Argentine (A) and Florigiant (F) from which these components have been identified and which are resistant and susceptible, respectively, to *C. crotalariae*. Microsclerotia were produced in peanut-stem broth.

<sup>b</sup>All components were added to soil at 1.9 mg C/g soil.

<sup>c</sup>Observations were made 24 hr (25–27 C incubation temperature) after addition of components to soil.

<sup>d</sup>Control consisted of soil treated with doubly distilled water.

<sup>e</sup>Mean of two experiments with two replications each with an average of 248 microsclerotia counted per replication.

#### LITERATURE CITED

- Buxton, E. W. 1957. Some effects of pea root exudates on physiologic races of *Fusarium oxysporum* f. *pisi* (Linf.). Snyder and Hansen. Trans. Br. Mycol. Soc. 40:145-154.
- Buxton, E. W. 1957. Differential rhizosphere effects of three pea cultivars on physiologic races of *Fusarium oxysporum* f. *pisi*. Trans. Br. Mycol. Soc. 40:305-317.
- Clauss, E. 1961. Die phenolischen Inhaltsstoffe der Samenschalen von *Pisum sativum* L. und ihre Bedeutung für die Resistenz gegen die Erreger der Fusskrankheit. Naturwissenschaften 48:106.
- Coley-Smith, J. R. 1976. Some interactions in soil between plants, sclerotium-forming fungi, and other microorganisms. Pages 11–23 in: J. Friend and D. R. Threlfall, eds. Biochemical Aspects of Plant-Parasitic Relationships. Academic Press, New York. 354 pp.
- Griffin, G. J. 1969. *Fusarium oxysporum* and *Aspergillus flavus* spore germination in the rhizosphere of peanut. Phytopathology 59:1214-1218.
- Hale, M. G. 1969. Loss of organic compounds from roots. I. Cultural conditions for axenic growth of peanut *Arachis hypogaea* L. Plant Soil 32:463-472.
- Hale, M. G., Lindsey, D. L., and Hameed, K. M. 1973. Gnotobiotic culture of plants and related research. Bot. Rev. 39:261-273.
- Kommedahl, T. 1966. Relation of exudates of pea roots to germination of spores of races of *Fusarium oxysporum* f. *pisi*. Phytopathology 56:721-722.
- Kraft, J. M. 1974. The influence of seedling exudates on the resistance of peas to *Fusarium* and *Pythium* root rot. Phytopathology 64:190-193.
- Krigsvold, D. T. 1979. The ecology of *Cylindrocladium crotalariae* microsclerotia in soil and in the rhizosphere of peanut. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg. 116 pp.

11. Krigsvold, D. T., and Griffin, G. J. 1975. Quantitative isolation of *Cylindrocladium crotalariae* microsclerotia from naturally infested peanut and soybean field soils. *Plant Dis. Rep.* 59:543-546.
12. Krigsvold, D. T., Griffin, G. J., and Hale, M. G. 1981. Germination of *Cylindrocladium crotalariae* microsclerotia in rhizospheres of susceptible and resistant peanut cultivars. (Abstr.) *Phytopathology* 71:232.
13. Park, D. 1963. The ecology of soil-borne fungal disease. *Annu. Rev. Phytopathol.* 1:241-258.
14. Phipps, P. M., Beute, M. K., and Barker, K. R. 1976. An elutriation method for quantitative isolation of *Cylindrocladium crotalariae* microsclerotia from peanut field soil. *Phytopathology* 66:1255-1259.
15. Rittenhouse, R. L., and Hale, M. G. 1971. Loss of organic compounds from roots. II. Effects of O<sub>2</sub> and CO<sub>2</sub> tensions on release of sugars from peanut roots under axenic conditions. *Plant Soil* 35:463-472.
16. Rovira, A. D. 1965. Plant root exudates and their influence upon soil microorganisms. Pages 170-184 in: *Ecology of Soilborne Pathogens*. K. F. Baker and W. C. Snyder, eds. University of California Press, Berkeley, 571 pp.
17. Schippers, B., and Voetberg, J. S. 1969. Germination of chlamydo-spores of *Fusarium oxysporum* f. *pisi* race I in the rhizosphere and penetration of the pathogen into roots of a susceptible and resistant pea cultivar. *Neth. J. Plant Pathol.* 75:241-258.
18. Schroth, M. N., and Hildebrand, D. C. 1964. Influence of plant exudates on root-infecting fungi. *Annu. Rev. Phytopathol.* 2:101-132.
19. Shay, F. J., and Hale, M. G. 1973. The effect of low levels of calcium on exudation of sugars and sugar derivatives from intact peanut roots under axenic conditions. *Plant Physiol.* 51:1061-1063.
20. Statler, G. D. 1970. Resistance of bean plants to *Fusarium solani* f. *phaseoli*. *Plant Dis. Rep.* 54:698-699.
21. Thompson, L. K. 1978. Effects of cytokinins on distribution and exudation of free sterols and free fatty acids in *Arachis hypogaea* L. cv. Argentine under axenic conditions. M.S. thesis, Virginia Polytechnic Institute and State University, Blacksburg. 77 pp.
22. Tomimatsu, G. S., and Griffin, G. J. 1982. Inoculum potential of *Cylindrocladium crotalariae*: infection rates and microsclerotial density-root infection relationships on peanut. *Phytopathology* 72:511-517.
23. Turner, P. D. 1963. Influence of root exudates of cacao and other plants on spore development of *Phytophthora palmivora*. *Phytopathology* 53:1337-1339.
24. Whaley, W. M., and Taylor, G. S. 1973. Influence of pea-root exudates on germination of conidia and chlamydo-spores of physiologic races of *Fusarium oxysporum* f. *pisi*. *Ann. Appl. Biol.* 73:269-276.
25. Whaley, W. M., and Taylor, G. S. 1976. Germination of chlamydo-spores of physiologic races of *Fusarium oxysporum* f. *pisi* in soil adjacent to susceptible and resistant pea cultivars. *Trans. Br. Mycol. Soc.* 66:7-13.