

Transmission and Dispersal of *Sphaceloma arachidis* by Crop Debris and Seed from Infected Peanut

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ABSTRACT

Peanut scab (*Sphaceloma arachidis* Bit. & Jenk.) has become epidemic in the southern peanut region of Argentina during the 1997/98 and 1999/00 growing seasons. To determine the potential for transmission of *S. arachidis* by crop debris and seeds and to describe the spread of scab from infected debris, experiments were conducted during two growing seasons. Scab was not observed in field or greenhouse tests planted with seed of cv. Florman obtained from diseased peanut plants. The severity of disease was significantly greater ($P \leq 0.05$) when the debris from diseased peanut plants was applied over the seed rows after planting. Furthermore, disease spread from an inoculum source (debris from infected plants) was generally followed the Río Cuarto's prevailing northeasterly wind, and the fourth-order equation provided good fit to this disease gradient. Results combined over the greenhouse and field experiments indicated that infected residues from the previous peanut crop are a source of inoculum for onset and development scab epidemics in the field.

Key Words: Disease, epidemiology, peanut scab.

Argentina is one of the largest worldwide exporters of peanuts (*Arachis hypogaea* L.) and most of the production (98%) comes from the province of Córdoba (Godoy and Giandana, 1992; Harvez, 1996). Early and late leaf spot caused by *Cercospora arachidicola* Hori. and *Cercosporidium personatum* (Berk. & Curt.) Deighton, respectively, are the major foliar diseases of peanut in Argentina. Although other foliar fungal diseases are frequently observed, only peanut scab caused by *Sphaceloma arachidis* Bit. & Jenk., has become increasingly important (March and Marinelli, 1999).

Peanut scab was first described in Brazil (Bitancourt and Jenkins, 1940); later, it was observed on germplasm collections in the Argentine producing region (Frezzi, 1966; Ojeda, 1966). Since then, peanut scab has been observed with variable intensity in isolated commercial fields (Giorda and Muñoz, 1979; Giorda *et al.*, 1985),

becoming epidemic and causing severe yield losses on peanut crops in the southern peanut region (Río Cuarto and Juárez Celman counties, province of Córdoba) during the 1997/98 and 1999/00 agricultural years; while it was mild in the 1998/99 agricultural year.

The spread of scab in this peanut-producing region raises questions concerning inoculum sources and dissemination. *Sphaceloma arachidis* forms acervuli with two types of conidia, microconidia (1 μm) and conidia (3-4 x 9-20 μm) in the affected plant parts (Bitancourt and Jenkins, 1940; Giorda and Muñoz, 1979). Since the disease affects leaves, petioles, stems, pegs, and shells, crop residues and seeds may be a source of inoculum for the onset of scab epidemics in the field. Infected peanut debris was an efficient inoculum in greenhouse tests (Giorda and Muñoz, 1979). Furthermore, there is general agreement that scab is more serious in monoculture of peanut.

The importance of infested peanut debris as inoculum sources may depend on several variables, including intensity in the preceding peanut crop, rate of residue decay, competitive saprophytic ability, sporulation potential of the pathogen, and weather variables (Fernández *et al.*, 1993; Buchwaldt *et al.*, 1996; Bockus and Shroyer, 1998). In fact, wind and rain have been noted as important agents of dispersal of plant pathogen spores (Fitt and Mc Cartney, 1986; Fitt *et al.*, 1989; Aylor, 1990).

On the other hand, the analysis of the disease gradient can be used to explain the mode of dispersal of the pathogen, to make inferences about the role and location of inoculum sources, and to assist in development control measures (Tresh, 1976; Minogue, 1986). To understand how dispersal of pathogens gives rise to disease gradients, several methods can be used (Jeger, 1983; Campbell and Madden, 1990). The objectives of this study were to determine the potential for transmission of *S. arachidis* in crop residues and seeds from diseased peanut plants, and to describe the spread of scab from infected debris.

Materials and Methods

Field and greenhouse experiments were planted with cv. Florman (runner type) since it makes up 90% of the peanuts growing in the peanut-producing region from Córdoba. The field plots established at the Univ. of Río Cuarto Campus (Córdoba, Argentina) were surrounded by cornfields separated from experimental plots by road ways about 5 m wide.

Potential for Transmission. To evaluate the potential for transmission of *S. arachidis* by crop residues and seeds from diseased peanut plants, a field experiment was established at the Univ. of Río Cuarto Campus on 5 Nov. 1998. The trial site had not been cropped to peanut in the previous 10 yr. Plots consisted of four 10-m rows of cv. Florman peanut spaced 0.70 m apart and treatments were replicated four times in a randomized complete block design. The

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cv. Florman was planted by hand at the rate of 15 seeds per 1 m of a row, and seed was treated with recommended rates of carboxin + thiram (20-20 flowable). Standard cultural practices and weed and leaf spot control were followed as recommended by the Agric. Exp. Sta. of INTA Manfredi (Pedelini and Casini, 1996).

Ten kilograms of mature peanut plants (aboveground portion) with severe symptoms of peanut scab were harvested the last week of March 1998 (about 150 d after planting), air-dried in the laboratory (23 C), and stored in paper bags at laboratory temperature (18-24 C) until used. The dried plant material was grounded in a hand mill (2-mm mesh) before using as inoculum in the field experiment. Furthermore, seed from healthy and diseased plants were harvested by hand and stored separately in paper bags until planted.

The treatments included (a) seeds from healthy peanut plants, (b) seeds from diseased peanut plants, (c) seeds from healthy peanut plants mixed with the inoculum, and (d) inoculum applied at 30 g/m in a 0.2-m band over the row after planting. In treatment c), seeds were sprayed with distilled water and then mixed with the inoculum at a rate of 10 g/100 seed, by shaking for 15 min in Erlenmeyer flasks. Seeds were covered with about 0.5 g of inoculum.

Two weeks before harvest, disease severity was calculated for each plot using the following severity grades: 0 = healthy plant; 1 = small spots on young leaflets and petioles; 2 = small spots on leaflets, petioles and stems; 3 = leaflet margins curl upward, and cankers on stems and pegs; and 4 = stems and petioles are sinuous and plants appear to be burned. The average frequency for each severity grade was determined, and a disease severity index was calculated for each treatment using the following formula: $S = (X_0Y_0 + X_1Y_1 + X_2Y_2 + X_3Y_3 + X_4Y_4)/100$ where S = severity index, X = severity grade, and Y = % of diseased plants in each severity grade. Data were subjected to analysis of variance and means were compared by Duncan's Multiple Range Test.

According to the results of this experiment, residues and seeds obtained from diseased peanut plants were analyzed separately to evaluate the role that they play in the transmission and dispersal of *S. arachidis*.

Spread of Scab. A field plot measuring 60 x 60 m was planted with cv. Florman at the Univ. of Río Cuarto Campus on 15 Nov. 1999. The plot was seeded in rows 0.70 m apart at the rate of 15 seeds per 1 m of a row, and the rows were planted north to south. The trial site had not been cropped to peanut in the previous 10 yr. Agricultural techniques were as previously described.

After emergence, and before the appearance of infections, we carefully examined the plot area to eradicate peanut plants of cv. Manfredi BM68 because it is highly susceptible to *S. arachidis* (Giorda and Muñoz, 1979) and because Florman seed planted is frequently contaminated with Manfredi BM68 (Cavallo and Lörincz, 1997). Ten kilograms of mature peanut plants (aboveground portion) with severe symptoms of peanut scab were harvested the last week of March 1999. Stems were cut into 15-cm pieces, air-dried in the laboratory (23 C), and stored in paper bags at laboratory temperature (18-24 C) until used. Six kilograms of these crop residues were placed in a 1-m diameter circle in the center of the field plot at crop emergence. Crop residues were layered 0-1 cm deep in soil to prevent its dispersal by wind or runoff from rainfall.

The dispersal of *S. arachidis* was indirectly assessed by

measuring the disease gradient. Observation points were established along lines radiating from the inoculum source. These lines were established in the eight compass directions, and observation points along these lines were marked at 1, 5, 10, 15, 20, and 25 m from the edge of the inoculum source. Each observation point marked was performed of 10 peanut plants near the line-row intersection, and the scab incidence was determined.

Scab gradients were assessed weekly beginning 6 wk after sowing and ending near harvest the last week of March 2000. Incidence at each observation point along each direction away from the inoculum source was expressed as the number of diseased peanut plants. The relationship of incidence to distance from the inoculum source was then examined by regression analysis for models of disease spread (Jeger, 1983; Campbell and Madden, 1990). Polynomial equations also were fitted to both, transformed (ln, log), and nontransformed incidence and distance data.

Role of Peanut Seeds. To determine whether transmission of *S. arachidis* takes place by planting seed of cv. Florman from infected peanut plants, two field tests were planted near Córdoba city (IFFIVE-INTA) and Quines city (province of San Luis), respectively, the second week of Nov. 1999. Each site had never been planted with peanut. Furthermore, a greenhouse test was planted near Córdoba city (IFFIVE-INTA) the first week of Dec. 1999. Seed used in these studies were obtained from a crop of cv. Florman exhibiting symptoms of scab near Río Cuarto State Univ. the last week of March 1999.

Diseased plants with the greatest severity grades of scab were dug up by hand, placed in burlap sacks, and taken to the greenhouse. Pods measuring 1.5 cm or longer were hand-picked, dried on a greenhouse bench at 25 ± 3 C, and later hand-shelled for seeds and stored in paper bags at laboratory temperature (18-24 C) until used. Six hundred seeds untreated with fungicides were planted by hand at a rate of 10 seeds/m in six, 10-m rows with 0.70 m between rows in both field trials. Peanut plots were maintained using agricultural techniques including local recommendations for weed and leaf spot control. The experiments were monitored weekly for scab symptoms from emergence to harvest.

One hundred seeds untreated with fungicides were used in the greenhouse test. Each seed was planted in a 10-cm plastic pot containing a steam-pasteurized mixture of soil (sandy loam) and sand (2:1, v/v). After sowing, pots were placed close to each other on greenhouse benches and watered daily to maintain soil moisture at field capacity and provide the humid conditions necessary for scab development. Greenhouse temperature and relative humidity ranged from 20 to 30 C and 80 to 100%, respectively. These conditions are necessary for disease development when inoculum is present (Giorda and Muñoz, 1979). The test was monitored weekly for scab symptoms from emergence to 8 wk later.

Results

Potential for Transmission. The disease severity ranged from 0 to 2 when seeds from both healthy or diseased peanut plants were planted; furthermore, the higher frequencies of healthy plants were recorded. Conversely, diseased plants showed a higher frequency of both 3 and 4 severity grades, and only 2% of healthy

plants, when inoculum was applied over the seed row after planting (Fig. 1).

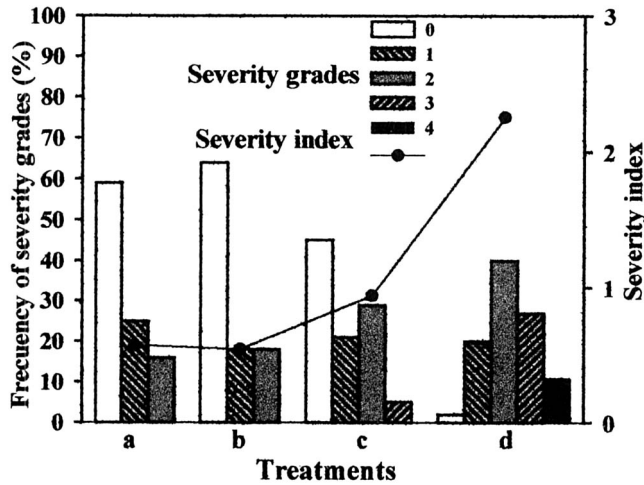


Fig. 1. Frequency of severity grades and severity index of peanut scab (*Sphaceloma arachidis*). Severity grades: 0 = healthy plant; 1 = small spots on young leaflets and petioles; 2 = small spots on leaflets, petioles and stems; 3 = leaflet margins curl upward, and cankers on stems and pegs; and 4 = stems and petioles are sinuous and plants appear to be burned. Severity index = $(X_0Y_0 + X_1Y_1 + X_2Y_2 + X_3Y_3 + X_4Y_4)/100$ where X = severity grade and Y = % of diseased plants in each severity grade. Treatments: a = seed from healthy plants, b = seed from diseased plants, c = seed from healthy plants + grounded infected debris, and d = grounded infected debris over seed row after planting.

The severity index was significantly greater ($P \leq 0.05$) when the debris from diseased peanut plants was applied as inoculum at 30 g/m in a 0.2-m band over the seed row after planting (severity index: 2.25). However, the severity index was lower when either seeds from healthy or diseased peanut plants were planted (severity index: 0.57 and 0.54, respectively). When seeds from healthy peanut plants were mixed with debris from diseased peanut plants, the severity index was intermediate (0.94), and significantly different from other treatments (Fig. 1).

Spread of Scab. Diseased peanut plants were observed at the observation points located 1 m from the inoculum source in the eight compass directions in the assessment on 25 Jan. 2000 (Fig. 2). All the peanut plants in the observation points located at 1, 5, 10, 15, 20, and 25 m from the inoculum source toward the southeast were diseased in the last assessment on 31 March 2000, and there were also diseased plants in the other compass directions (Fig. 2).

Disease incidence assessment on 2, 11 and 23 Feb., 6 and 17 March decreased sharply from the observation point at 1 m from the inoculum source up to 5 m toward southeast (Fig. 3). From this observation point, disease incidence increased greatly up to 15 m from the inoculum source, and decreased sharply again to the observation point located at 25 m from the inoculum source on 25 Jan., and 2, 11, and 23 Feb. (Fig. 3).

The models of disease spread frequently fail to fit the incidence data on the first observation date. However,

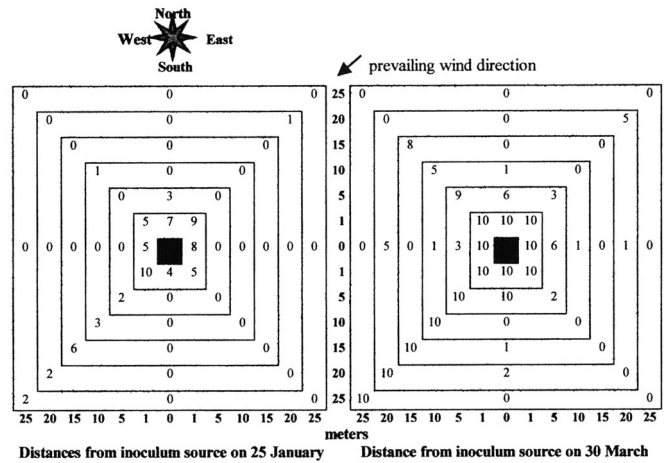


Fig. 2. Number of peanut plants with scab (*Sphaceloma arachidis*) at 1, 5, 10, 15, 20, and 25 m from inoculum source (■) along the eight compass directions on 25 Jan. and 30 March 2000, first and last date of disease assessed.

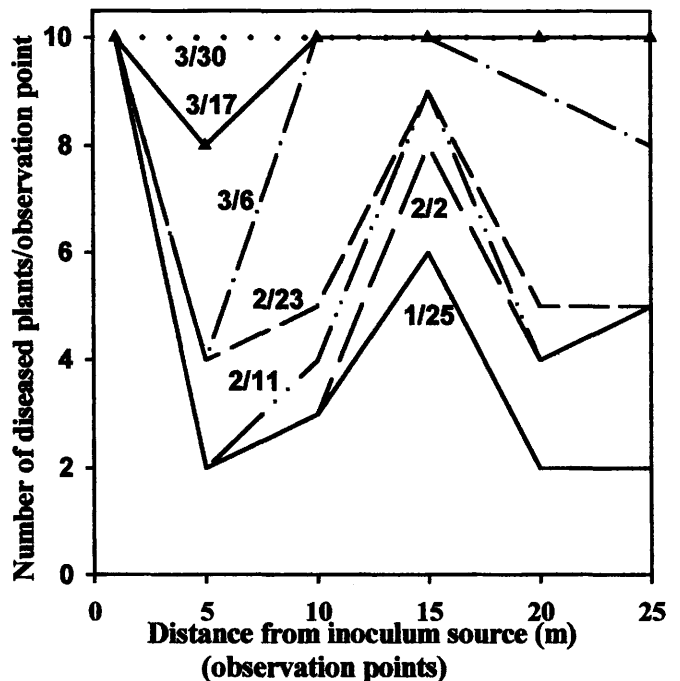


Fig. 3. Number of peanut plants with scab (*Sphaceloma arachidis*) at 1, 5, 10, 15, 20, and 25 m from inoculum source toward southeast on different dates.

the fourth-order equation provided good fit to the disease gradient on the first observation date. The relationship between disease incidence and distance from inoculum source on 25 Jan. 2000 is given as $Y = 0.38x^4 - 5.73x^3 + 30.43x^2 - 65.32x + 50.33$ ($R^2 = 0.964$), where Y = incidence as nontransformed data and x = distance as nontransformed data. Thereafter, the disease incidence gradient tended to become flatter with increasing time. Plants of cv. Manfredi BM68 were not present in the trial and there was no evidence of contamination from outside

the experimental plot.

Role of Peanut Seeds. Scab was not observed in field trials or the greenhouse test.

Discussion

Young tissues of nearly all plant peanut parts are susceptible to *S. arachidis*. The results of this work showed that debris from peanut plants infected with *S. arachidis* from the previous agricultural year was an inoculum source in initiating disease epidemics. The severity index was higher when infected debris was applied over the seeded rows compared to mixing it with seeds at planting. This may be attributed to differences in inoculum quantity and/or to a microbial decomposition of peanut residues when it was grounded and buried. It has been noted in different crop systems that buried residues may be decomposed by microbial activity during the growing season, while the crop residue on the soil surface delays its decomposition (Bockus and Shroyer, 1998). On the other hand, the majority of microbial antagonists are restricted to the upper centimeters of soil (Baker and Cook, 1982; Lumsden, 1992).

Scab gradients showed a reversal of the initial negative slope with increasing distance from inoculum source. It has been noted that "false or anomalous" gradients may occur in virus and fungal diseases, when different dispersal forms coexist and each contributes to disease spread, or there is a secondary inoculum source (Gregory, 1968; Tresh, 1976). In fact, *S. arachidis* has conidia of two sizes, microconidia and conidia (Bitancourt and Jenkins, 1940; Giorda and Muñoz, 1979). The observed gradients of scab probably represent the superimposition of two separate distributions. The greater conidia size may have been more frequently splash-dispersed causing a steeper gradient than that of smaller microconidia which were mainly carried downwind to greater distances (Steadman, 1979, 1980; Mc Cartney and Fitt, 1987; Aylor, 1990). The significance of wind has been noted in the dispersal of pathogens from the inoculum source in splash droplets becomes greater and the size of the inoculum particles becomes smaller. However, the distinction between rain splash or wind as the dispersal mechanism for spores is not always clear (Fitt *et al.*, 1989; Aylor, 1990). The effect of Río Cuarto's prevailing north-easterly warm and wet wind from December through February was obvious because disease incidence was greater in the direction of southwest. The flattening of the disease gradients as distance increases from a point source of inoculum has been reported previously (Gregory, 1968; Lim, 1978; Berger and Luke, 1979; Aylor, 1990).

In the peanut scab pathosystem a fourth-order equation provided best fit to the gradient disease on the first observation date. However, when an unique dispersal form is present, the spread of fungal disease generally tends to decrease steeply with increasing distance from inoculum source (Cammack, 1958; Lim, 1978; Ries and Royse, 1978; Berger and Luke, 1979; Kable *et al.*, 1980; Jeger *et al.*, 1983; Minogue and Fry, 1983; Alderman *et al.*, 1989; Buchwaldt *et al.*, 1996).

Sowing seeds of cv. Florman obtained from diseased

peanut plants did not result in scab symptoms caused by *S. arachidis*. Similar results were reported with peanut seeds from cv. Blanco Manfredi 68 (Giorda and Muñoz, 1979).

The analysis of the results combined over the field and greenhouse experiments indicated that infected residues from the previous peanut crop are a source of inoculum for the onset and development of scab epidemics. Windborne inoculum from nearby fields also may be a source of infection. On the other hand, diseased peanut plants can become secondary inoculum source during the growing season (Bitancourt and Jenkins, 1940; Giorda and Muñoz, 1979). Monitoring the longevity of *S. arachidis* on peanut debris affected by scab either on the soil surface or buried in soil throughout the year would help elucidate the length of time of crop rotation for an effective control of residue-borne inoculum of the pathogen.

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