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The effect of age of the groundnut crop on the development of primary gradients of Puccinia arachidis foci

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Abstract

Three plots with groundnut crops of different ages were inoculated with *Puccinia arachidis* in their centres and the development of primary gradients around these centres was studied. Spontaneous infection of the plots was inevitable and increased with age. After correction for spontaneous infections, significant differences were found between primary gradients, mean values and slopes being higher with increasing plot age. An index for vertical distribution of disease, the relative height of infection *H*, was developed. *H* increased with increasing spontaneous infection. It was also increased by the primary gradient in the older plot. The differences observed are attributed to variations in dispersibility and accessibility, related to age-dependent differences in canopy structure. The relative shallowness of the gradients observed in the experiment indicated an intense and rapid dispersal of groundnut rust.

Additional keywords: Arachis hypogaea, disease dispersal, alloinfection, auto-infection.

Introduction

Groundnut rust, caused by *Puccinia arachidis* Speg., apparently a newcomer in Africa, is another factor reducing yields of groundnut, *Arachis hypogaea* L. (Bromfield, 1974; McDonald and Emechebe, 1978). It was first observed in Ivory Coast in 1976 (M. Lourd, personal communication), where it is omnipresent now. Little is known about groundnut rust dispersal, either over long (Zambettakis, 1980) or over short distances. Short distance within-crop dispersal can be studied by means of artificial foci and disease gradients initiated by these foci (Gregory, 1968).

This paper describes disease gradients in the horizontal plane and disease distribution in the vertical direction around artificial foci of groundnut rust, and their differences in response to age and/or structure of the groundnut canopy.

Material and methods

Experimental plots. A local, short-cycle groundnut cultivar (so-called Spanish type), highly susceptible to rust, was sown in three plots at the ORSTOM experimental station, Adiopodoumé, Ivory Coast. The plots measured 10 × 10 m. The three plots differed in age and canopy structure due to staggered sowing : 2 July (plot A), 17 July (plot B) and 1 August, 1984 (plot C). The plots were separated by distances of at least

200 m. The seed rate was 167 000 seeds ha^{-1}. The spacial design of the sampling scheme followed Berger and Luke (1979): stakes were placed in each plot in eight compass directions (N, NE, E, SE, S, SW, W and NW) to mark the plants to be assessed. These plants were 0.8, 1.6, 2.4, 3.2 and 4.0 m from the plot centre. Each plot had a total of 40 marked plants.

Fields inoculations. The inoculations were performed in the evening of 5 September, 1984, when the plants in the plots had reached the filling-pod (plot A), beginning-peg (plot B) and beginning-pod stage (plot C), respectively, according to Boote's (1982) growth stage scale. The inoculations were performed by dusting dry urediniospores mixed with kaolin onto the plants, as previously described (Savary, 1985a). Foci of nearly equal size, each with equal numbers of infected leaves at equal levels were desirable. Therefore, 260, 200 and 180 mg of a mixture containing approximately 500 spores mg^{-1} were dusted onto 7, 9, and 10 plants in the center of plot A, B and C, respectively. Approximately 9×10^5 spores per plot were applied. The inoculated plants were covered by a heat-sealed plastic tent ($1.0 \times 1.0 \times 0.3$ m), which was removed early in the next morning. Time was counted in days from inoculation.

Spore trapping. Spores were trapped at canopy top level in the center of each plot by means of rotating (Rotorod) spore collectors. Sampling took place from 10.00 to 10.30 a.m. on various days after inoculation. Results were expressed as spore densities (spores m^{-3}), following the manufacturer's instructions.

Disease assessment. Disease severity per sample was determined by combining the disease severities of three chosen leaves along the main stem with disease incidence. Disease severity was assessed on the third (S_3), fifth (S_5) and last (S_L) leaves of the main stem of each marked plant. The mean of these observations, corrected for the proportion (I/T) of infected leaves per main stem, is used as the severity R per plant:

$$R = (I/T) \times (S_3 + S_5 + S_L)/3 \qquad (1)$$

where R: rust severity (%); I: number of rusted leaves per stem (N_{leaves}); T: number of living leaves per stem (N_{leaves}); S_3, S_5 and S_L: diseases severities (%).

This rating system is similar to that used by Emge and Schrum (1976) in their studies on *Puccinia striiformis* of wheat.

Height of infection. A quantitative characterization of the vertical disease distribution seemed desirable. The relative height of infection ($\bar{H}$) along the main stem (which is taken to represent the plant) can be calculated as follows:

$$\bar{H} = \sum_{i=1}^{I} i \times S_i \, / \, (I \times \sum_{i=1}^{I} S_i) \qquad [1] \qquad (2)$$

where i is the ranking number of the leaf layer, with i = 1 for the first emerged leaf and $I = i_{max}$ for the top layer, and S_i is the rust severity of leaf in percent.

In the present experiment, only three leaf layers were assessed.
The equation for H becomes:

$$H = [(I-d+1)\times S_L + (I-4)\times S_5 + (I-2)\times S_3] \, / \, [I \times (S_L+S_5+S_3)] \qquad (3)$$

where d is the number of dead leaves. In both cases H is a proportion with $0 < H < 1$.

Corrections for background noise. During the experiment, inevitable spontaneous infections occurred. To correct rust severity (R) for spontaneous infections (R_b), the background noise (spontaneous infections) was subtracted from the signal (dispersal from the inoculated centre):

$$R_c = R - R_b \qquad [\%] \qquad (4)$$

where R_c is the corrected rust severity, R the severity recorded from the sample plant and R_b the severity representing the background noise. Background noise was estimated from the sample plants, by using the last observations made before t = 2p, there p is the latency period as observed in the inoculated centre. The increase in background noise during the delay between the two observation days was disregarded.

To correct the relative height of infection (H) for background noise (H_c), the following formula was employed:

$$H_c = (R \times H - R_b \times H_b) / (R - R_b) \qquad [1] \qquad (5)$$

where symbols have the same meaning as before.

Analysis of results. The variation of rust severity in the three plots with direction and distance was analyzed according to a split-plot design, considering each of the eight compass directions as a unit, and each of the five distances as a sub-unit (Cochran and Cox, 1957). The split-plot ANOVA was applied to the data of day 22. Day 22 corresponds roughly with the beginning of the third latency period after inoculation, the first latency period being needed to initiate the focus, and the second to initiate the primary gradient (Gregory, 1968).

Results

Description of the foci. On inoculation day (t = 0) plot ages were different (Table 1), and canopy structure, as expressed by the number of leaf layers, varied accordingly. As older plots were longer subjected to spontaneous infection than younger ones, the infection already present on inoculation day differed also as illustrated by Fig. 1 (especially Fig. 1A), Fig. 2 (t = 2), and by the spore catches during the first sporulation wave after inoculation.

Part of the spontaneous infection was alloinfection (Robinson, 1976), but especially in plot A there was plenty of time for auto-infection. A significant effect of plot age on the relative height of infection (H) was found on day 17 (Table 1). As the rate of leaf emission did not differ significantly between plots (Table 1), the varying amounts of alloinfection and auto-infection seem to explain the differences in vertical distribution of disease in the plot centres. The low value of H in plot C on day 17 is surprising in view of the care spent in obtaining a regular deposit of inoculum over plants and leaf layers.

When corrected for background noise (Table 1), the relative height of infection on day 17 (H_c) did not deviate significantly ($p >$ 0.99). This indicates an even vertical distribution of the inoculum applied in the plot centres. Apparently, the differences in H observed at t = 17 were due to variations in spontaneous alloinfections and auto-infections between plots.

Table 1. Characteristics of foci of groundnut rust established in three plots of different ages. Data refer to the inoculated centres of the plots.

Plot	n[1]	Number of leaf layers on day 0[2]	Rate of leaf emission[3]	Rust severity[4]	Relative height of infection			Plot age in days on day 0
					H_8[5]	H_{17}[6]	H_{C17}[7]	
A	7	12.4±1.0[9]	0.31±0.18	26.2±7.7	0.43±0.08	0.50±0.06	0.46±0.14	64
B	9	8.7±0.8	0.22±0.09	8.5±2.1	0.43±0.15	0.48±0.06	0.46±0.08	49
C	10	6.7±0.4	0.23±0.12	10.5±2.2	0.19±0.09	0.39±0.04	0.43±0.06	35
F[8]	—	71**	0.78	28**	4.0*	3.8*	0.08	—

[1] Number of plants in the inoculated centre assessed for rust severity.
[2] Per main stem, mean over n plants.
[3] In leaf day^{-1}, per main stem, mean over n plants and between day 0 to 17.
[4] In percent, on day 17.
[5] Relative height of infection (unitless, see text) on day 8, mean over n plants.
[6] Relative height of infection on day 17.
[7] Relative height of infection, corrected for spontaneous infection, on day 17.
[8] Fisher's F-values (one-way analysis of variance), when followed by * or ** significant at $p<0.05$ or $p<0.01$, respectively.
[9] Each entry is followed by its confidence interval at $p<0.05$.

Aerial spore density above the foci. Strong differences between plots in aerial spore densities at canopy top level were observed during most of the duration of the experiment (Fig. 2). An important difference between plots is observed on day 2 due to the initial discrepancy in spontaneous infection between the three plots. A significant ($p<0.05$) difference was found between the mean densities over the period. t = 12 to t = 18, which represents the first sporulation wave (Table 2). This wave creates – after a second latency period – the primary rust gradient.

Disease development in the plot. Background noise was highest in plot A, where the differences in rust severity between the inoculated central area and the surrounding plants remained visible for few days only. In the younger plots (B and C), the artificial foci were more conspicuous. The variation of disease level with distance from the centre could still be observed at harvest time in plot C, where the inoculated plants and their neighbours showed a high proportion of wilted (lower) leaves.

Horizontal variation of rust severity The variation of rust severity (R, z-axis) with distance to the centre (x-axis) is shown in Fig. 1. The horizontal variation of rust severity in the three plots was studied by means of an analysis of variance, applying a split-plot design, for t = 17 and 22. Significant (p < 0.01) plot effects (P) for R at t = 17 and t = 22, and for R_c at t = 22 were found. The effect of direction (D) on both R and R_c was not significant (p> 0.1). In other words, the three foci were isodiametric but they differed in average severity. The difference, due to spontaneous infection prior to inoculation corresponds with the difference in age of the plot at inoculation time (t = 0).

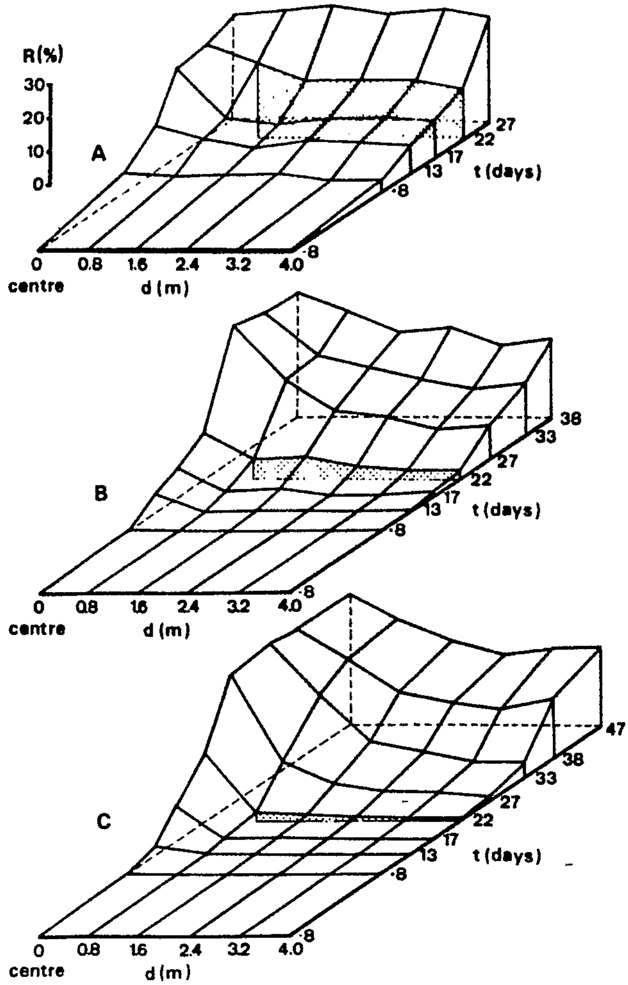

Fig. 1. Development of rust severity in space and time in three groundnut plots with artificial foci of rust established simultaneously in crops of different ages. The primary gradients on $t = 22$ are shaded. R: rust severity in percent; d: distance from focal center in m; t: time in days from inoculation day; A: sowing at $t = -64$; B: sowing at $t = -49$; C: sowing at $t = -35$.

The effect of distance (d) on R at $t = 17$ was not significant ($p > 0.1$), but was significant on both R and R_c at $t = 22$ ($p < 0.001$). No significant interaction effect between compass direction and distance was found ($D \times d$). In other words, primary gradients were well established on day 22, possibly in plot A and certainly in plot B and C. They were still visible at $t = 27$, plot A excepted (Fig. 1).

Correcting the primary gradient (day 22) for background noise (as measured as rust severity at $t = 17$) resulted in a reduction of the plot effect (P), which nevertheless remained significant (at $t = 22$, the F-value for P was 422 with R, but 33 with R_c). The corrected severity data therefore indicated an increase of the primary gradient with

Table 2. Urediniospore density in the air at canopy top level of artificial foci of groundnut rust, established in three plots of different ages. The period of spore trapping corresponds to the first sporulation wave after inoculation.

Plot	Mean spore density[1]
A	203 ± 179
B	44.7 ± 31.0
C	9.2 ± 6.6
F[2]	5.8

[1] Entries are means of 7 days (day 12 through day 18 after inoculation); they are followed by their confidence interval at $p<0.05$.
[2] Fisher's F-values for one-way analysis of variance: $F_{0.95}(2, 18) = 3.6$; $F_{0.99}(2, 18) = 6.0$.

age. Correcting the primary gradient also resulted in an increase of the F-values of the distance effect (8.2 instead of 5.8). Fig. 1 suggests that the primary disease gradients are the steeper in the older plot, but this suggestion (since no $P \times d$ interaction is available) cannot be corroborated by statistical analysis.

Vertical disease distribution in the three plots. A split-plot analysis of variance of the relative height of infection (H) indicated that plot effects were significant ($p<0.01$) at $t = 17$ and 22 without, and at $t = 22$ with correction for spontaneous infection. Direction (D) and distance effect (d) nor their interaction ($D \times d$) were significant ($p>0.1$).

The plot effect P increased between $t = 17$ and 22 (F-value at $t = 22$ was: 28.0, instead of 8.5 at $t = 17$), i.e. after the first wave of spores emitted by the inoculated centres. This is confirmed by the significance ($p<0.001$) of the plot effect after correction

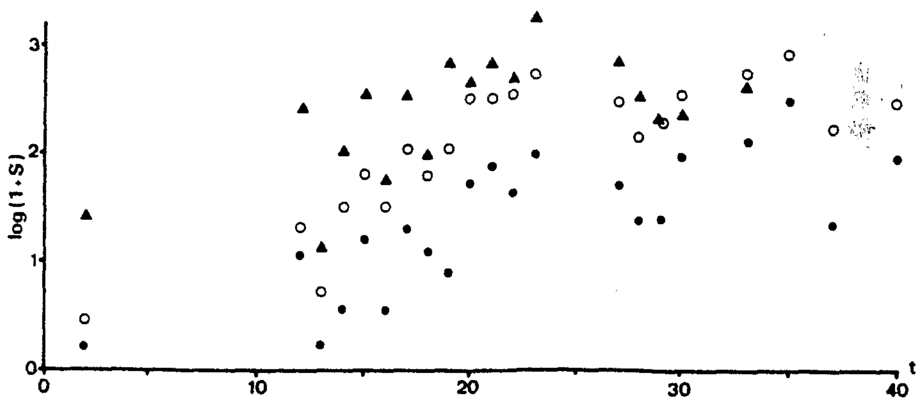

Fig. 2. Variation of spore density in the air at canopy top level in three groundnut plots of different ages. The results of day 2 represent spore density due to spontaneous infection only. ▲: plot A; ○: plot B; •: plot C. Abscissa: time t in days from inoculation day; ordinate: S = spore density (spore m^{-3}).

for spontaneous infection. The relative height of the lesions caused by spores originating from the initial artificial infections therefore was larger in the older plots.

Discussion

Regression equation of primary gradients. The primary gradient of a focus is the gradient of disease severity along the radius of the plot from its inoculated centre to its circumference as a result of inoculum dispersing from the inoculated centre during its first sporulation wave. Consequently, the primary gradient must be assessed during the third latency period after inoculation. Day 22, at the beginning of the third latency period (Savary, 1985b), was taken to be representative for a cross-sectional analysis (Zadoks, 1972). On day 27, the esodemic in the plot became troublesome and precludes any longitudinal analysis.

Gregory (1968) employed a double logarithmic transformation to study regression lines derived from observed gradients. A simple exponential equation was chosen here to analyse the gradients. Regression equations are given in Table 3, column 1. They have the general shape:

$$y = a + b \times \log d \tag{6}$$

where a is the intercept of the y-axis and b is the regression coefficient (slope of log-line). The equations based on $n = 5$ distances have significant correlation coefficients. At least 81 percent (R^2) of the variation is explained by regression.

Differences in intercepts are significant between plots because of differences in spontaneous infections related to different plot ages. The significant difference in slopes, the older plot having the steeper gradient, is due to the same reason and/or to differences between canopies.

Regression equations of corrected primary gradients. The equation employed by Gregory (1968; $\log y = a + b \log d$) does not allow y (disease severity) to take zero value; y tends to 0 when d (distance from the centre) tends to infinite. The distance limit of a measurable effect of a focus was considered by Van der Plank (1960), who called it the horizon of infection. This is a theoretical concept, which should be taken as a metaphor by field observers (Zadoks and Schein, 1979).

When the focus is the only source of inoculum, the use of equations such as those of Table 3 implies that at some distance from the infected centre, where the regression line intercepts with the abscissa, there is no more disease. This distance is:

$$d_{max} = 10^{-a/b} \tag{7}$$

In the case of spontaneous contaminations, the end of the gradient is hidden. Therefore, background noise, in addition to reducing the slope b of the gradient, should lead to an increase of d_{max} and of the ratio $-a/b$. As an effect of correcting for background noise, equation (6) should thus lead to (1) an increase of the regression coefficients b, (2) a decrease of the ratio $-a/b$, and (3) more accurate estimates of a and b accompanied by an increase of the significance of the regressions.

The regressions equations for the corrected ($R_c = R_{22} - R_{17}$) primary gradients are given in Table 3, column 2. In addition to the expected effects of correcting for background noise, the difference between the slopes is increased, the gradient being steeper in the older plot (A).

Table 3. Regression equations for primary gradients of three foci of groundnut rust, established in plots of different ages.

Plot	Equations for rust severity (R) on day 22[1]	Equations for corrected rust severity ($R_c = R_{22} - R_{l'}$) on day 22
A	$R = 20.2(\pm 3.1) - 9.1(\pm 5.0) \times \log d$[2] $R^2 = 0.86 \quad -a/b = 2.2$	$R_c = 9.7(\pm 0.9) - 10.0(\pm 2.2) \times \log d$ $R^2 = 0.98 \quad -a/b = 1.0$
B	$R = 5.9(\pm 1.6) - 5.9(\pm 3.9) \times \log d$ $R^2 = 0.81 \quad -a/b = 1.0$	$R_c = 4.0(\pm 1.1) - 4.5(\pm 2.7) \times \log d$ $R^2 = 0.84 \quad -a/b = 0.9$
C	$R = 2.4(\pm 0.6) - 2.8(\pm 1.4) \times \log d$ $R^2 = 0.89 \quad -a/b = 0.9$	$R_c = 1.8(\pm 0.5) - 2.4(\pm 1.2) \times \log d$ $R^2 = 0.84 \quad -a/b = 0.8$

[1] The shape of the equations for primary gradients is: $y = a + b \times \log d$ (see text). a and b are followed by their confidence intervals at $p<0.05$.
[2] $\log d = {}^{10}\log d$.

Comparison of the development of the epidemics. Disease development, after correction for spontaneous infection, differed between plots in several respects. Mean severity of the primary gradients and their slopes increased with increasing plot age. These results indicate that dispersal from the inoculated plot centres increased with increasing plot age. As H_c, the corrected relative height of infection, was similar in the three plot centres, accessibility of the foliage to spores dispersed over a short distance may have been the better in the older plots (Zadoks and Schein, 1979). As the older plots had more leaf layers, without differing in H_c, spore liberation and dispersibility in the older plots may have been better too. Differences in dispersibility and accessibility, as determined by age-dependent differences in canopy structures, might together explain the differences in dispersal between plots.

The ANOVA of the relative height of infection shows an increase in the differences between plots due to the appearance of the lesions generated by the inoculated centres (t = 22). Previous differences (t = 17) were, however, significant. The relative height of infection was smaller in the younger plots, where auto-infections were less numerous. The hypothesis is forwarded that, in groundnut rust, the esodemic tends to increase the relative height of infection H. The effect of the exodemic, represented in this experiment by the primary gradients, would depend on the canopy structure.

Natural epidemics. The primary gradients observed during this experiment on groundnut rust are shallow in comparison to those usually obtained in similar experiments. If the double logarithmic transformation (Gregory, 1968) is applied to the corrected primary gradients, b-values ranging from −0.6 to −0.8 are obtained for their slopes, which approaches the usual range, −1 to −3 (Zadoks and Schein, 1979).

The difference between focal and general epidemics can be assigned to the initial amounts of inoculum from which they derive (Zadoks, 1961; Zadoks and Schein, 1979). In the traditional groundnut fields of Ivory Coast, the pattern of rust epidemics is rarely of the focal type, and, if so, for brief, transient periods only. This may be due to intense and rapid dispersal of the rust from its primary foci within the fields, and/or to uniform infection from strong distant or numerous nearby sources outside the fields. The relative shallowness of the gradients observed in the experiment supports the first of these two hypotheses.

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Samenvatting

Het effect van de leeftijd van het aardnootgewas op de ontwikkeling van primaire gradiënten in haarden van de aardnootroest (Puccinia arachidis)

Drie aardnootveldjes van verschillende leeftijd werden in het centrum geïnoculeerd met aardnootroest, teneinde de ontwikkeling te bestuderen van de primaire gradiënten rond de inoculatiecentra. De eerste infectiegolf na inoculatie produceert de puistjes in de geïnoculeerde centra. De tweede sporulatiegolf, uitgaande van deze puistjes, produceert de primaire gradiënt rondom de geïnoculeerde centra. Spontane infectie bleek onvermijdelijk te zijn; zij nam toe met de leeftijd van de veldjes. Na correctie van waargenomen aantastingen voor spontane infectie werden significante verschillen gevonden tussen primaire gradiënten. Hun gemiddelde waarden en hellingshoeken namen toe met de leeftijd van de veldjes. Een index H, de relatieve hoogte van de infectie, geeft de verticale verdeling van de ziekte aan. Deze waarde nam voor de primaire gradiënt eveneens toe met de leeftijd van het veldje. De geconstateerde verschillen worden toegeschreven aan variaties in verspreidbaarheid en toegankelijkheid, gerelateerd aan leeftijdsafhankelijke verschillen in de gewasstructuur. De betekenis van de resultaten voor het begrijpen van spontane epidemieën wordt besproken.

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