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**Decrease by plant development and leaf age of
susceptibility of groundnut to rust (*Puccinia arachidis*)
in a susceptible cultivar**

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Material and methods

Plants and inoculum. Seedlings of a short-cycle, local cultivar were grown in outdoor conditions, in 12-cm plastic pots. The cultivar was highly susceptible to rust, and, in this respect, representative of the varieties traditionally cultivated in Ivory Coast. The inoculum consisted of a local rust isolate collected in the south of Ivory Coast in 1982 and maintained in the laboratory by regular reinoculation on detached leaflets.

Inoculation of leaflets. Detached leaflets, kept on moist filter paper in petri dishes (Cook, 1980a), were used to study the effect of development stage and leaf age on susceptibility to rust. Plants were 15, 26, 34 or 45 days old when leaves were cut for inoculation. This range corresponded to the following stages of plant development: third tetrafoliate of the vegetative stage (I), beginning bloom (II), beginning peg (III), and beginning pod (IV), according to Boote's (1982) scale. Of these plants, 2, 2, 3 and 4 leaf stories, respectively, were used for inoculation (the 1st and 3rd, from the apex of the main stem, for development stages I and II, the 1st, 3rd and 5th for stage III, and the 1st, 3rd, 5th and 7th for stage IV). Leaf age was represented by leaf layer number, counted from the top of the main stem.

potted plants were placed in a tray with some water and kept under plastic bags for 24 h to ensure high humidity (Simkin and Wheeler, 1974), and a 12-h-darkness period was immediately applied to avoid any negative interference of light with the infection process (Zadoks, 1967). Lesion counts and leaf area measurements were made on three leaflets per leaf to calculate the lesion density on attached leaflets (L_{nd}). The fourth leaflet on each test-leaf was used to estimate indirectly the deposited spore density, S_{nd} . Prior to inoculation, leaflets were detached from the second leaf layer (counted from top) of the main axis of spare plants. The reference infection efficiency measured on this vegetal material was: $IE_r = 0.3$ (Savary, 1985a). One of these detached leaflets was inverted and stuck (by moistening its adaxial surface with tap water) on one leaflet of each of the attached test-leaves. After inoculation, the detached leaflets were removed and kept on wet filter paper in separate petri dishes. These provided the estimates of the lesion density on detached leaflets (L_d). Using the reference infection efficiency, the deposited spore density on the three corresponding non-detached leaflets could be calculated: $S_{nd} = L_d/IE_r$, and thus, the infection efficiency: $IE = IE_{nd} = L_{nd} / S_{nd}$.

In a second experiment on potted plants, the latency period (LP) was measured on plants of 26 (flowering stage), 34 (beginning peg) and 42 (beginning pod) days old. On these plants, 160, 280 and 360 mg, respectively, of a mixture containing 260 spores per mg kaolin was dusted. These amounts were calculated according to the mean number of fully expanded leaves, so that about 20 mg of the mixture would be used per leaf. The inoculated plants were incubated as previously. The LP values were estimated as for detached leaflets on 2, 3, and 4 leaf stories of the respective development stages, with three replicates per development stage \times leaf age combination.

Results

IE decreased from younger to older leaves on detached leaflets as well as on non-detached leaflets (Table 1). In both experiments, the data do not indicate a change in IE with development stage. Higher values for IE were obtained with detached leaflets. Inversely, LP increased from younger to older leaves in both experiments. Data also indicate a tendency for LP to increase with increasing development stages. Values obtained with non-detached leaflets were higher than those from detached leaflets. The data for SP did not indicate an effect of leaf age or development stage. The coefficients of variation for LP in both series of measurements were relatively small, suggesting that LP is a reliable response. Studies on partial resistance to leaf rust similarly indicated that the accuracy was higher in measuring LP than IE (Parlevliet, 1975; Parlevliet and Kuiper, 1977).

To analyze the results with respect to possible leaf age, development stage, and leaf age \times development stage effects, a two-way analysis of variance was separately applied to each of the five data sets. As a simplification, the effect of leaf age was considered with only two alternatives, the oldest and the youngest levels per development stage. For IE , a significant effect of leaf age was obtained with detached ($p < 0.001$) and non-detached leaflets ($p < 0.05$). For LP , the effects of leaf age ($p < 0.001$ in both cases), development stage ($p < 0.05$ in both cases) and leaf age \times development stage interaction ($p < 0.001$ with detached and $p < 0.05$ with non-detached leaflets) were significant. For SP , no significant effects was found.

The results for IE and LP were further analyzed according to a split-plot design, *Neth. J. Pl. Path.* 93 (1987)

Table 1. Effects of plant stage and leaf age on the infection efficiency (*IE*), the latency period (*LP*) and sporulation intensity (*SP*) of *Puccinia arachidis* on detached and non-detached leaflets.

Development stage ¹	Leaf age ²	Response type ³				
		detached leaflets		attached leaflets		
		<i>IE</i> (<i>n</i> = 12) ⁴	<i>LP</i> (<i>n</i> = 8)	<i>SP</i> (<i>n</i> = 5)	<i>IE</i> (<i>n</i> = 4)	<i>LP</i> (<i>n</i> = 3)
I	Y	27	10.7	2200	18	— ⁵
	O	17	11.3	3000	10	—
II	Y	24	10.7	2400	20	10.9
	O	10.9	10.9	2500	17	10.9
III	Y	27	10.8	2200	17	11.1
	O	20	11.4	2200	13	13.3
IV	Y	32	10.5	2400	—	11.2
	O	17	11.9	2000	—	12.7

¹ Development stages are: third or fourth tetrafoliate (I), beginning bloom (II), beginning peg (III), and beginning pod (IV).

² Leaf age is considered at two levels: the youngest (Y) and the oldest (O) leaf of the main stem.

³ Entries are mean infection efficiencies (*IE*, %), latency periods (*LP*, days), and sporulation intensities (*SP*, spores lesion⁻¹).

⁴ Number of replicates.

⁵ —: not determined.

considering detached and non-detached leaflets as separate blocks, in which each development stage is a unit including two levels of leaf age (youngest and oldest) as sub-units. The development stages considered for *IE* and *LP* were I, II and III, and II, III and IV, respectively (Table 1). In addition to the effects of development stage, leaf age, and their interaction, this design allowed to calculate the effect of detachment. For *IE*, a significant ($p < 0.10$) detachment effect was found, whereas other effects were found in accordance with previous analyses (development stage: $0.5 < p < 0.75$; leaf age: $p < 0.005$; interaction: $0.10 < p < 0.25$). For *LP*, the effect of detachment may be considered as a trend only: $0.10 < p < 0.25$. The effect of leaf age was found significant ($p < 0.05$), while those of development stage and interaction were found as trends only ($0.10 < p < 0.25$).

Both procedures, two-way analyses of variance on separate experiments and general analysis according to a split-plot design, therefore indicated a significant leaf age effect on *IE*, and, for *LP*, significant leaf age and development stage effects (in the latter case, only a trend when a split-plot design was used).

Discussion

Inoculations of both detached and non-detached leaflets allowed to distinguish leaf

layer which differed in two components: *IE* and *LP*. An increase of *LP* with increasing development of the host was also observed with both plant materials. Therefore, in the case of the highly susceptible cultivar considered, the use of either plant material leads to the same general conclusions.

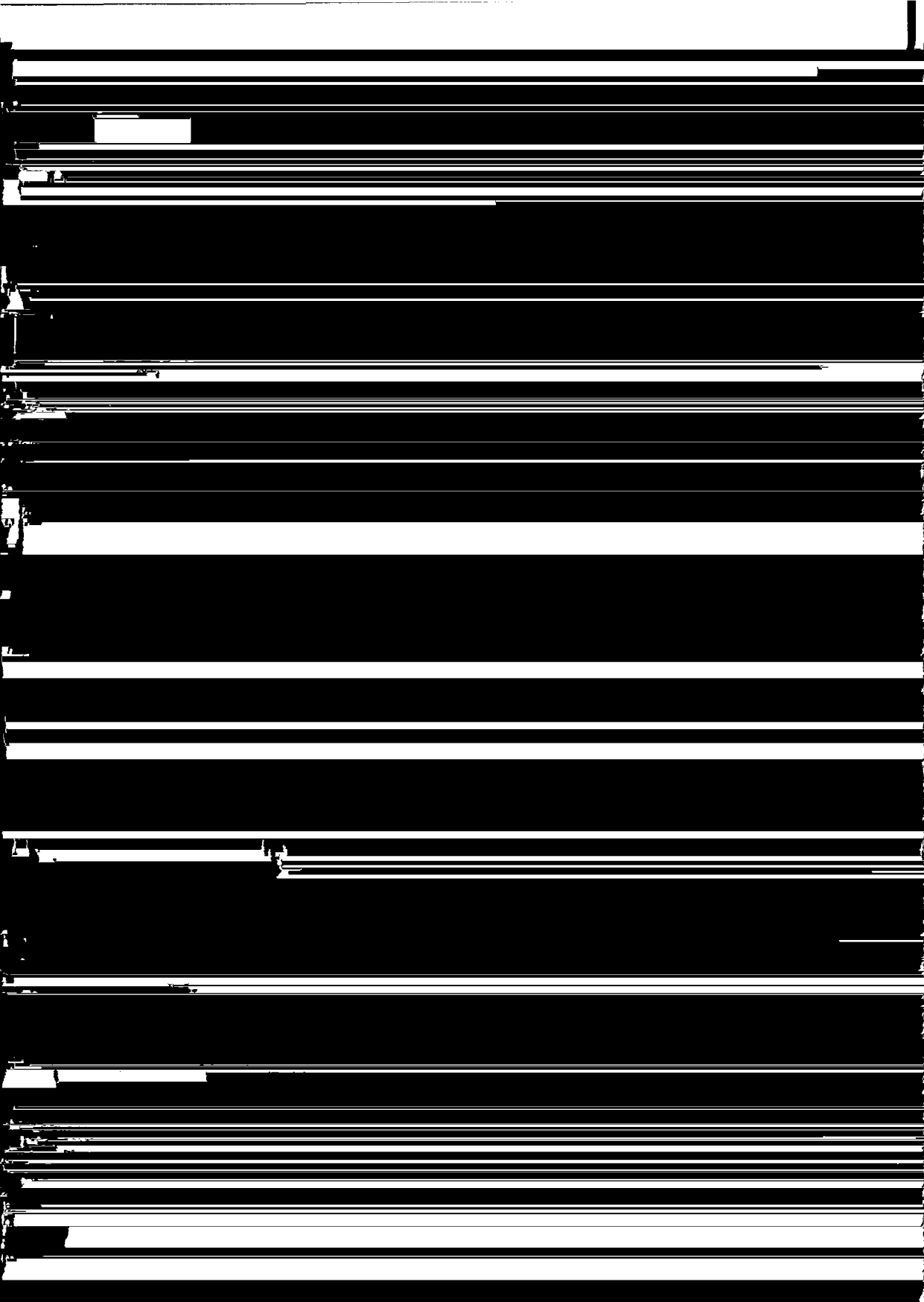
This study suggests that, in the variation of susceptibility with leaf age and development stage of a groundnut cultivar highly susceptible to rust, more factors are involved than just leaf wettability (Cook, 1980 a and b). The effect of plant growth and development on infection by biotrophic foliar fungi was studied intensively. Although an increase in *IE* with leaf age appears in the case of *Puccinia hordei* of barley (Parlevliet and Kuiper, 1977), *IE* frequently decreases with increasing development and leaf age as in *Erysiphe graminis* f.sp. *hordei* of barley (Aust et al., 1980) and in *Uromyces phaseoli* of bean (Schein, 1965), albeit that the existence of a maximum following a steep increase of *IE* at a very young leaf age was demonstrated in the latter case. Parlevliet (1975) reported an increase of *LP* in *P. hordei* during barley development. Studies on *Puccinia recondita* on wheat led to the same conclusions (Tomerlin et al., 1983). On the contrary, Ohm and Shaner's (1976) results indicate a minimum before flowering. In *P. recondita*, a clear reduction in *SP* related to plant maturation was reported by Tomerlin et al. (1983).

In spite of the diversity among pathosystems, several authors (Populer, 1978; Zadoks and Schein, 1979; Vanderplank, 1982) have suggested generalizations to account for the variation in the pathological interactions between fungi and ageing plants. The most general hypothesis for a biotrophic pathogen is, perhaps, that the younger and healthier the host tissues, the easier their recognition and use as a convenient habitat for faster and more intense growth and multiplication. This has led Zadoks and Schein (1979) to suggest that partial resistance against biotrophic fungi would generally increase with age and development.

In the present results, two of the variables studied (*IE* and *LP*), which can be considered as components of resistance to groundnut rust, comply with Zadoks and Schein's (1979) hypothesis, while the third (*SP*) is not demonstrably affected by development stage and leaf age.

Higher values for *IE* were obtained with detached leaflets. In a first approach, this difference may be partly attributed to less favourable conditions (especially lower relative humidity) during the early infection process in the case of potted plants. The experiment with detached leaflets also yielded lower values for *LP* than with potted plants. In a previous study (Savary, 1985 b), such a difference was partly assigned to an extension of the lesion development period on potted plants, when spores are predominantly deposited on the upper leaf surface, as compared to lesion development on detached leaflets, when the lower leaf surface is inoculated.

The results presented here, viz. a reduction of the infection efficiency with leaf age and an extension of the latency period with development stage and leaf age, point to the desirability of further epidemiological studies on groundnut rust to distinguish different layers in the host canopy, and to evaluate the consequences of plant development on epidemics. These variations of susceptibility, as well as, when needed, the effect of leaf detachment, should also be taken into consideration in future studies on groundnut rust resistance.



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