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# Reactions of rust-infected groundnut genotypes following treatment with fosetyl-Al, α-aminooxyacetate, and inoculation with *Puccinia sorghi* Schw.

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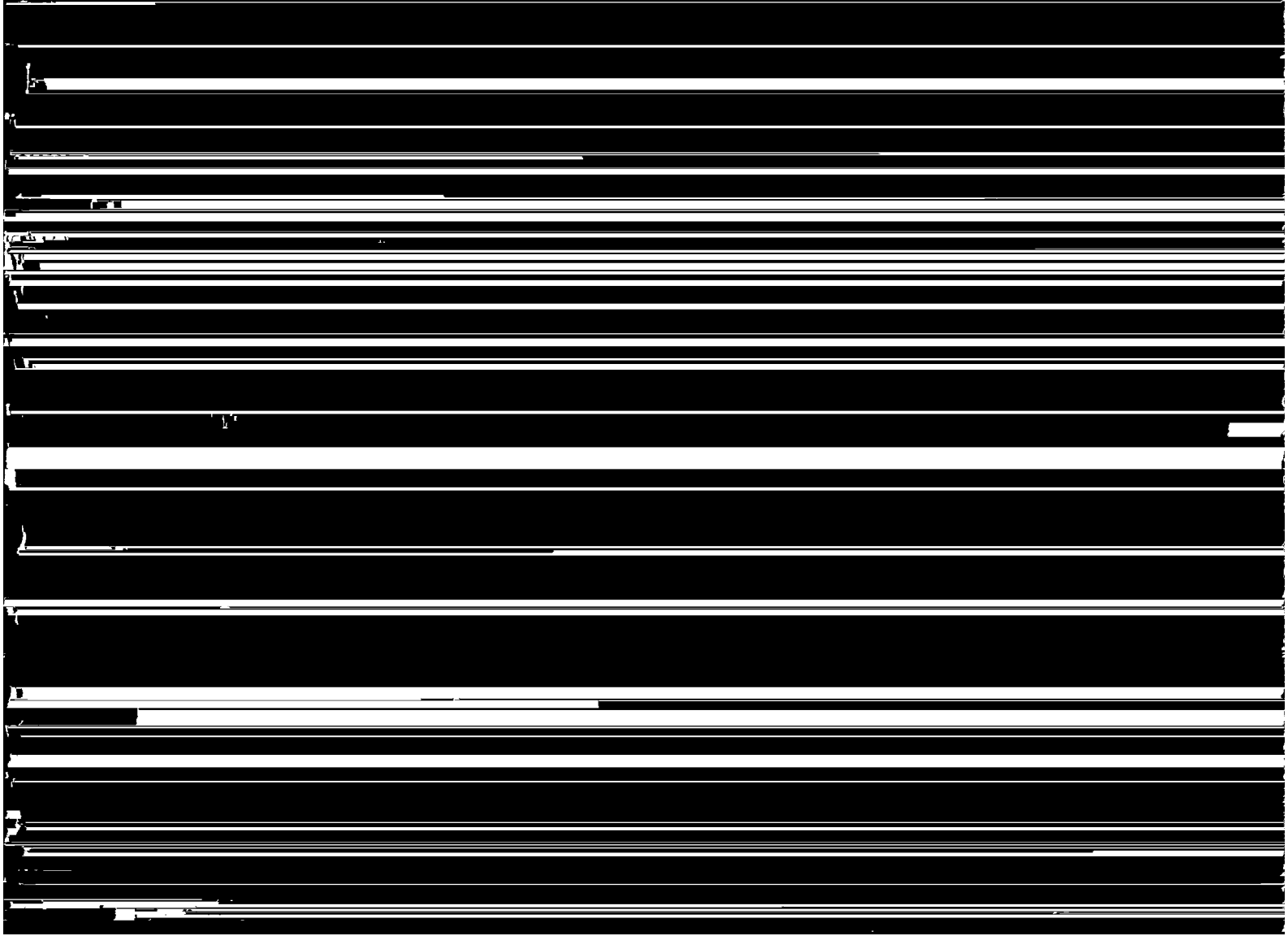
**Abstract.** — Reactions, including variations in the components of resistance (incubation period, latent period, infection frequency, lesion diameter and percent lesions with necrosis) of two rust-resistant and one rust-susceptible groundnut genotypes were studied in glasshouse following artificial inoculations with *Puccinia arachidis* and treatment with fosetyl-Al or α-aminooxyacetate (AOA) or inoculation with *Puccinia sorghi*. Effects of treatments with fosetyl-Al, AOA and inoculation with *P. sorghi* were not consistent on total phenolic content and total concentration of antifungal compounds. Fosetyl-Al inhibited *in vitro* spore germination and germ-tube growth of *P. arachidis* and prolonged latent periods of the treated plants. Prior inoculation with *P. sorghi* had also prolonged latent period besides reducing infection frequency and lesion diameter. AOA treatment had no effect on any of the components of resistance studied. The significance of these effects in host defences against pathogenic infections is discussed.

**Key words.** — Rust, groundnut, fosetyl-Al, *Puccinia arachidis*, *Puccinia sorghi*, cross-protection, defense reactions, components of resistance.

## INTRODUCTION

Rust caused by *Puccinia arachidis* Speg. is one of the ma-

*et al.*, 1980; Fettouche *et al.*, 1981; Guest, 1984), or by directly affecting the fungus itself (Coffey and Bower, 1984; Fenn and Coffey, 1984, 1985; Derks and Buchenauer, 1986, 1987). Artificial induction of disease resistance...



ched leaflets of the rust-susceptible cultivar maintained in moist petri plates. Uredospores of *P. sorghi* were collected from infected maize leaves in the farmer's field and were harvested using a sterile razor blade. Inoculum mixture was prepared using kaolin powder (Sigma, USA) at the rate of 1000 mg/15 mg of uredospores (Savary, Comm. Pers.). The Kaolin-spore mixture was dusted onto the labelled leaves using a hand dust depositor. Care was taken to limit the inoculum to the target leaves. Following the inoculation, plants were incubated in a humid chamber at  $25.5 \pm 1^\circ\text{C}$  for 24 h in dark, and later were transferred to light with 12 h photoperiod, where they remained for the rest of the experimental period. Humid chambers were removed 48 h after inoculation. The light intensity was maintained at 200 lux (Philips fluorescent tubes) during the experimental period.

#### □ Experiment involving treatment with AOA and fosetyl-Al

Treatment with fosetyl-Al began from 6 days before inoculation. Fosetyl-Al was applied at the base of the plant every six days until 10 days after inoculation with *P. arachidis*. Two concentrations of fosetyl-Al (5 or 10 mg in 25 ml water per pot per application) were used in the study.

AOA was applied in a similar way at the rate of 1.25 mg in 25 ml water per pot, per application, every two days during the same period as described for fosetyl-Al. Effect of AOA and of fosetyl-Al on the components of resistance to *P. arachidis* was studied in two separate experiments. In the first experiment, plants received either five AOA applications or two fosetyl-Al applications while, in the second one, they received eight and three applications respectively. Experimental design, number of replications, mode of application and the experimental material remained the same for both the experiments.

#### □ Cross protection experiment

The following treatments were included:

- 1- Groundnut plants - healthy (uninoculated)
- 2- Maize plants - healthy (uninoculated)
- 3- Groundnut plants - inoculated with *P. arachidis* only
- 4- Groundnut plants - inoculated with *P. sorghi* only
- 5- Maize plants - inoculated with *P. sorghi* only
- 6- Groundnut plants - inoculated simultaneously with *P. arachidis* and *P. sorghi*
- 7- Groundnut plants - inoculated first with *P. sorghi* followed by inoculation with *P. arachidis*, 48 h later.

While inoculating groundnut plants with both *P. sorghi* and *P. arachidis* at the same time, standard spore mixtures of *P. arachidis* and *P. sorghi* prepared separately were mixed in 50:50 W/W ratio and were dusted onto the target leaves.

#### □ Estimation of total phenolic content

Total phenolic contents of different genotypes with different treatments were estimated using Folin-Ciocalteu method with chlorogenic acid as reference compound and the concentration is expressed in  $\mu\text{g}$  of phenols equivalent to chlorogenic acid per mg of fresh host tissue.

#### □ Estimation of total concentration of antifungal compounds

Total concentration of antifungal compounds was estimated in terms of unit areas covered by the peaks of these compounds in a HPLC analysis as described by Subba Rao (1987).

#### □ *In vitro* inhibition of spore germination and germ-tube growth by fosetyl-Al

*In vitro* inhibition tests of *P. arachidis* spore germination were conducted using the method described earlier (Subba Rao, *et al.*, 1988) at fosetyl-Al concentrations ranging from 10  $\mu\text{g}/\text{ml}$  to 250  $\mu\text{g}/\text{ml}$  in two experiments. In the first experiment, in addition to spore germination, inhibition of germ-tube growth was also measured at concentrations ranging from 10  $\mu\text{g}/\text{ml}$  to 50  $\mu\text{g}/\text{ml}$  by comparing the lengths of uredospore germ-tubes in fosetyl-Al treatments with the controls and calculating percentage values from these data.

From eight days after inoculation with *P. arachidis*, the inoculated leaves were observed daily and numbers of total and ruptured uredosori were recorded. Lesion diameter was measured using an ocular micrometer of 30 lesions per leaf selected at random when the increase in total number of uredinia ceased. Infection frequency (number of lesions per  $\text{cm}^2$  of leaf area) was calculated by dividing the total lesion number with the leaf area (A) obtained by the formula  $A = \pi(L \cdot l)/4$  where, L and l represent the length and breadth of the leaflet (Savary, 1985). Percentage of lesions with necrosis was obtained by estimating number of lesions with necrosis using an optical hand lens and calculating their percentages out of total lesion numbers.

Since the experimental unit was one plant, each inoculated/treated plant was considered as one replication. All treatments in the experiments with fosetyl-Al and AOA were replicated thrice, while the ones in cross-protection experiment had four replications. The experimental data was subjected to analysis of variance and the percentage values were transformed using angular transformation before analysis. Duncan's multiple range test and Newman-Keuls tests were used to separate treatment means.

## RESULTS

#### □ Treatment effect on total phenolic content and on the total concentration of antifungal compounds

None of the three treatments (fosetyl-Al, AOA and inoculation with *P. sorghi*) had consistent effect on either total phenolic content or total concentration of the antifungal compounds, rendering the data difficult to interpret.

#### □ *In vitro* inhibition of uredospore germination and germ-tube growth of *P. arachidis* by fosetyl-Al

Uredospore germination was adversely affected by fosetyl-Al when tested *in vitro* at concentrations ranging from 10  $\mu\text{g}/\text{ml}$  to 250  $\mu\text{g}/\text{ml}$ . Percent inhibition of spore germination increased with increase in fosetyl-Al concentrations up to 150  $\mu\text{g}/\text{ml}$  and beyond that concentration the inhibition was total (Table I). Similarly, inhibition of germ-tube growth also increased as fosetyl-Al concentrations increased and there was nearly 50 % inhibition of germ-tube growth at a concentration of 20  $\mu\text{g}/\text{ml}$  of fosetyl-Al (Table I).

#### □ Influence of treatment with fosetyl-Al and with AOA on components of rust resistance

The influence of treatment with fosetyl-Al and AOA on the components of resistance was studied in two similar but separate experiments which differed in the number of fosetyl-Al or AOA applications only. Increase in number of application from two (in Experiment 1) to three (in Experiment 2) with fosetyl-Al and from five (in Experiment 1) to eight (in Experiment 2) with AOA did not have any additional influence on the components of resistance considered in this study. Hence data from Experiment 2, which also included study of the effects of increased fosetyl-Al concentration on

TABLE I. — *In vitro* inhibition of uredospore germination and germtube growth of *Puccinia arachidis* by fosetyl-Al at increasing concentrations

Expt No. <sup>(1)</sup>	Fosetyl-Al concentration (ug/ml)	Spore germination (%)	Inhibition of spore germination (%) <sup>(2)</sup>	Germtube length (mm)	Inhibition of germtube growth (%) <sup>(2)</sup>
1	Control	76.3 a <sup>(3)</sup>	—	0.18 a	—
	10	60.4 ab	20.8	0.12 b	33
	20	65.3 ab	14.4	0.09 c	50
	30	56.6 b	25.8	0.08 d	55
	50	9.3 c	87.8	0.05 e	72
	SEM <sup>(4)</sup> ±	5.45		0.004	
	SD <sup>(5)</sup>	9.44		0.007	
	CV (%) <sup>(6)</sup>	17.6		6.1	
2	Control	54.3 a	—	—	—
	50	8.4 b	84.5	—	—
	100	6.1 b	88.8	—	—
	150	0.01 c	99.98	—	—
	200	0.01 c	99.98	—	—
	250	0.01 c	99.98	—	—
	SEM ±	1.355			
	SD	2.347			
	CV (%)	20.5			

(1) Experiment number

(2) With reference to control

(3) Values followed by the same letter within a column are not significantly different according to Duncan's multiple range test at  $\alpha = 0.05$

(4) Standard Error of Means

(5) Standard Deviation

(6) Coefficient of variation (%)

TABLE II. — Influence of fosetyl-Al or  $\alpha$ -aminooxyacetate on components of rust resistance in two groundnut genotypes

Treatment	Components of resistance <sup>(1)</sup>									
	IP <sup>(2)</sup>		LP <sup>(3)</sup>		IF <sup>(4)</sup>		LD <sup>(5)</sup>		LN <sup>(6)</sup>	
	L <sup>(7)</sup>	NC <sup>(8)</sup>	L	NC	L	NC	L	NC	L	NC
F <sup>(9)</sup> alone	11.0 <sup>(10)</sup>	21.3	14.3	28.3	9.3	4.8	0.64	0.46	—	72.4

In+fosetyl-Al <sup>(12)</sup> 11.0 a 21.3 a 14.3 a 28.3 a 9.3 a 4.8 a 0.64 b 0.46 b — 72.4 a

Control <sup>(13)</sup>

TABLE III. — Analysis of variance for "latent period", a component of rust resistance measured to estimate the effect of treatment with fosetyl-Al or  $\alpha$ -aminooxyacetate in two groundnut genotypes in a glasshouse experiment

Source of variation	DF	Sum of suares	Mean square	"F" value	Probability of "F"	Level of significance
Genotype	1	1312.76	1312.76	881.29	0.0000	**
Treatment with fosetyl-Al or with $\alpha$ -aminooxyacetate.	3	14.28	4.76	3.20	0.0514	*
Genotype $\times$ treatment	3	5.11	1.70	1.14	0.3619	NS
Error	16	23.83	1.49			
Total	23	1355.99	58.96			

DF : Degrees of freedom    \* : 5 %    \*\* : 0.1 %    NS : Not significant

TABLE IV. — Effect of cross-protection with *Puccinia sorghi* on components of resistance in two groundnut genotypes artificially inoculated with *Puccinia arachidis*

Inoculation with	Treat No. <sup>(6)</sup>	Components of resistance <sup>(1)</sup>							
		IP <sup>(2)</sup>		LP <sup>(3)</sup>		IF <sup>(4)</sup>		LD <sup>(5)</sup>	
		L <sup>(7)</sup>	PI <sup>(8)</sup>	L	PI	L	PI	L	PI
<i>P. arachidis</i> alone	3	9.0 a <sup>(9)</sup>	14.6 a	13.0 a	18.1 a	11.5 a	1.9 a	0.77 a	0.69 a
<i>P. arachidis</i> + <i>P. sorghi</i> simultaneously	6	8.8 a	15.6 a	12.8 a	22.0 b	11.9 a	2.3 a	0.65 a	0.69 a
<i>P. sorghi</i> followed by <i>P. arachidis</i> 48h later	7	10.8 a	15.4 a	14.8 a	22.5 b	5.6 b	0.5 b	0.62 b	0.47 b
SEM <sup>(10)</sup> $\pm$			0.544		0.499		1.078		0.028
CV (%) <sup>(11)</sup>			12.4		7.4		54.3		11.8

(1) Mean of four replications

(2) Incubation period (days)

(3) Latent period (days)

(4) Infection frequency (Number of lesions/cm<sup>2</sup> leaf area)

(5) Lesion Diameter (mm)

(6) Treatment number

(7) Local (susceptible variety)

(8) PI 259747 (resistant genotype)

(9) Values within a column followed by the same letter are not significantly different according to Newman-Keuls test at  $\alpha = 0.05$ 

(10) Standard Error of Means

(11) Coefficient of variation

Results of the investigations on the effect of inoculation with *P. sorghi* on four components of rust resistance are presented in table IV. Simultaneous inoculation with both fungi (Treatment No. 6) did not have significant effect on any of the components of resistance estimated except latent period. The effect on latent period was however significant ( $P \leq 0.05$ ) only in case of the resistant genotype, PI 259747. On the other hand, both susceptible and resistant genotypes had significantly longer latent periods, lower infection frequencies and smaller lesion diameters when inoculated first with *P. sorghi* followed by inoculation with *P. arachidis*, 48 h later (Treatment No. 7). Interestingly, this treatment had no significant effect on incubation period (Table IV).

## DISCUSSION

Since total phenolic content and accumulation of antifungal compounds were considered to be some of the important host resistance factors (Kosuge, 1969; Ingham, 1982), investigations were carried out to understand their possible role in host resistance in relation to pathogen development either by stimulating their production (using treatment with fosetyl-Al and inoculation with a non-pathogen) or by suppressing them (using  $\alpha$ -aminooxyacetate).

Many workers (Vo-Thi-Hai *et al.*, 1979; Bompeix *et al.*, 1980; Guest, 1984; Derks and Creasy, 1989) have observed significant increase in phenolic content and accumulation of antifungal compounds following treatment with fosetyl-Al. The present investigations also recorded similar trend but, the effect was not consistent. During our investigations, it

was observed that the effect of fosetyl-Al on total phenolic content was similar when either sprayed on the foliage or fed through roots indicating that host reactions did not vary irrespective of the mode of application.

In addition to its influence on two components of rust resistance in two groundnut genotypes, indications of marked effect of fosetyl-Al were observed on one unidentified compound (with a retention time of 2.1 min., localized in the antifungal compounds zone in a HPLC analysis) during the present investigations. However, further studies are necessary to confirm these observations. Fosetyl-Al also had an inhibitory effect on uredospore germination and germ-tube growth suggesting that in case of *A. hypogaea*-*P. arachidis* interactions, fosetyl-Al could act both directly and indirectly on the pathogen development and spread. Our results concerning the direct mode of action of fosetyl-Al are in agreement with those of Coffey and Bower, 1984; Fenn and Coffey, 1984, 1985; Derks and Buchenauer, 1986, 1987 but differ with those of others (Vo-Thi-Hai *et al.*, 1979; Bompeix *et al.*, 1980; Fettouche *et al.*, 1981; Guest, 1984). The dual mode of action of fosetyl-Al in groundnut-rust interactions suggests that an effective check is operating on the rust development. This suggestion was further strengthened by the observation of significant reduction in disease levels (Subba Rao, 1987) calculated by fitting the data obtained in the present investigations into a preliminary simulation model developed by Savary *et al.*, (1988). Nevertheless, influence of fosetyl-Al was limited to either a few specific compounds and/or specific components of rust resistance.

Reasons for the observed inconsistency in the effect of AOA on the total phenolic content and on the total concentrations of antifungal compounds could be either that the

concentrations used during the present investigations were insufficient or that its take-up via the root-system was inefficient. It is to be investigated, if a different mode of appli-

lexin were involved in the process of cross-protection. Kochman and Brown (1975) attributed the protection of plants to physical blockage of the infection sites by the non-pathogen.

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