THE ROLE OF OXALIC ACID AND POLYGALACTURONASE IN THE PATHOGENICITY OF *PYTHIUM APHANIDERMATUM* ON DIFFERENT COWPEA VARIETIES

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The amounts of oxalic acid and polygalacturonase (PG) produced in the tissues of cowpea (*Vigna unguiculata* Walp.) plants infected by *Pythium aphanidermatum* were higher in vars. IT81D-1020 and VITA 5 than in vars. IT82E-32 and TVX3236. Oxalic acid accumulated early at the infective stage of the disease but its production decreased as the plants became older or as the disease developed. At the peak of oxalic acid and PG production (8-10 days after infection) the pH of the tissue fell from 7.2 to 3.1 and thereafter rose to 4.3 (within 18 days). It seems, therefore, that the combination of oxalic acid and PG and the accompanying reduced pH of infected tissue play an important role in the pathogenesis of susceptible varieties of cowpea by *P. aphanidermatum*. In the two varieties found to be resistant in this study, oxalic acid and PG production were lower than in the susceptible varieties and the pH did not fall as low. Early accumulation of oxalic acid in cowpea tissue during pathogenesis may be a useful tool for monitoring disease severity and hence susceptibility or resistance to *P. aphanidermatum*, when there is a compatible interaction between host and pathogen.

KEY WORDS: *Pythium aphanidermatum*; cowpea varieties, susceptible and resistant; oxalic acid; polygalacturonase.

INTRODUCTION

Among the 40 species of fungi that are pathogenic to cowpea (*Vigna unguiculata* Walp.) is *Pythium aphanidermatum*, which causes the damping-off disease of cowpea seedlings and stem rot of established plants (2,18). The disease has been reported in

Received Aug. 25, 1986; received in final form Sept. 8, 1987.
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many parts of Nigeria (12,13,15,16). At its peak, up to 70% seedling mortality has been reported within 21 days of sowing seeds in the field (15).

Some characteristics of the damping-off disease of cowpea seedlings, wet stem rot and diseases caused on other host plants have been described in the literature (2,6,12,13,15,16). There has been at least one study of the cell wall-degrading enzymes produced by *P. aphanidermatum* (17), but oxalic acid production during pathogenesis by this fungus has not been reported, to the best of our knowledge. Thus, no correlation between the production of pectolytic enzymes and oxalic acid by this organism has been established.

This work was therefore designed to study the production of pectolytic enzymes by *P. aphanidermatum in vivo* and *in vitro* and the relation of the production of the enzymes and oxalic acid to pathogenesis. The study would provide information on the difference in the production of these substances in susceptible and resistant varieties of cowpea.

MATERIALS AND METHODS

Pythium aphanidermatum was isolated from the base of infected cowpea plants obtained from the Crop Collection Garden of the Department of Agricultural Biology, University of Ibadan, and was grown on potato dextrose agar (PDA) at 28°C. Thereafter, for the production of infective zoospores for inoculation purposes, the fungus was grown on 5% corn meal broth (CMB) as still culture at 28°C. A 7-day-old culture of the fungus on CMB was observed under a light microscope for the presence of sporangia and zoospores. The cultures were then filtered through a two-layer muslin cloth and the concentration of the zoospore inoculum was adjusted to 2.0×10^5/ml of suspension using a Neubauer haemocytometer. Approximately 10 ml of inoculum was poured at the base of cowpea plants that had been wounded (stem puncture) with an inoculating needle to facilitate infection.

Cowpea seeds of varieties TVX 3236, IT81D-1020, VITA 5 and IT82E-32 obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, were used for the study. Each variety was sown at the rate of four seeds/5-liter pot and watered regularly until the seedlings were inoculated 7 days after emergence. The surface of the soil in each pot was covered with polyethylene sheets to prevent excess moisture loss. Plants were observed regularly to record susceptibility or resistance to infection, which was rated on a scale of 0-4 (Table 1).

At weekly intervals for 10 weeks, five plants evincing damping-off and stem rot symptoms were collected; the upper parts of the stems were cut off while the lower 10 cm, including the roots, was washed in running water and later placed in envelopes in which it was dried at 60°C to a constant weight and then used for investigations on the *in vivo* production of oxalic acid. A second set of infected plants was collected and homogenized in 0.1 M citrate-phosphate buffer solution at pH 5.0 and then examined

for polygalacturonase (PG) activity (*in vivo* production). Control plants (uninoculated) were also collected, at the same regular intervals as infected plants, for bioassays. The pH of healthy and infected plants was obtained by blending 50 g cowpea tissue in 50 ml glass-distilled water and measuring the pH with an EIL pH meter.

For the oxalic acid content of the infected plant part (*in vivo* production), samples were ground into fine powder in a mortar after drying to constant weight at 60°C. Oxalic acid extractions and subsequent estimations were carried out using the methods of Andrews and Viser (1) as modified by Faboya *et al.* (8). The amount of oxalate present was calculated as percent oxalic acid (14).

The PG activity *in vivo* was studied by the methods of Ikotun (10). The *in vitro* enzyme preparation was elaborated according to Ikotun (10) and the assay for enzyme activity was carried out following the viscometric methods of Winstead and McCombs (17) and of Bateman (3).

RESULTS

Results given in Table 1 showed that cowpea variety IT81D-1020 was the most susceptible to *P. aphanidermatum*. The area between the soil level and the first leaves was soft rotted. Within 7 days after inoculation, disease rating was 3. By the second week all inoculated plants of this variety attained the maximum disease score – 4 – but were sampled for oxalic acid content until the tenth week. VITA 5 plants inoculated with *P. aphanidermatum* attained the maximum disease rating by the third week after

TABLE 1

DISEASE RATING* OF FOUR COWPEA VARIETIES INFECTED BY *PYTHIUM APHANIDERMATUM*

Cowpea varieties	*Weeks after inoculation*				
	1	2	3	4	5
IT81D-1020	3	4	4	4	4
VITA 5	1	2	4	4	4
IT82E-32	1	1	1	2	2
TVX 3236	0	1	1	1	1
S.D. ±0.23					

*0 = No infection.
1 = Slow spreading water-soaked lesion at the point of wounding.
2 = Large water-soaked lesion and incipient wilting of plant.
3 = Part of plant from soil level to first leaves soft-rotted, and wilting of plants apparent.
4 = Wilting and death of whole plant.

inoculation. Varieties IT82E-32 and TVX 3236 merely showed a slightly expanded stem lesion which did not develop further. After 5 weeks callus tissue developed around the lesion and there was evidence of wound healing.

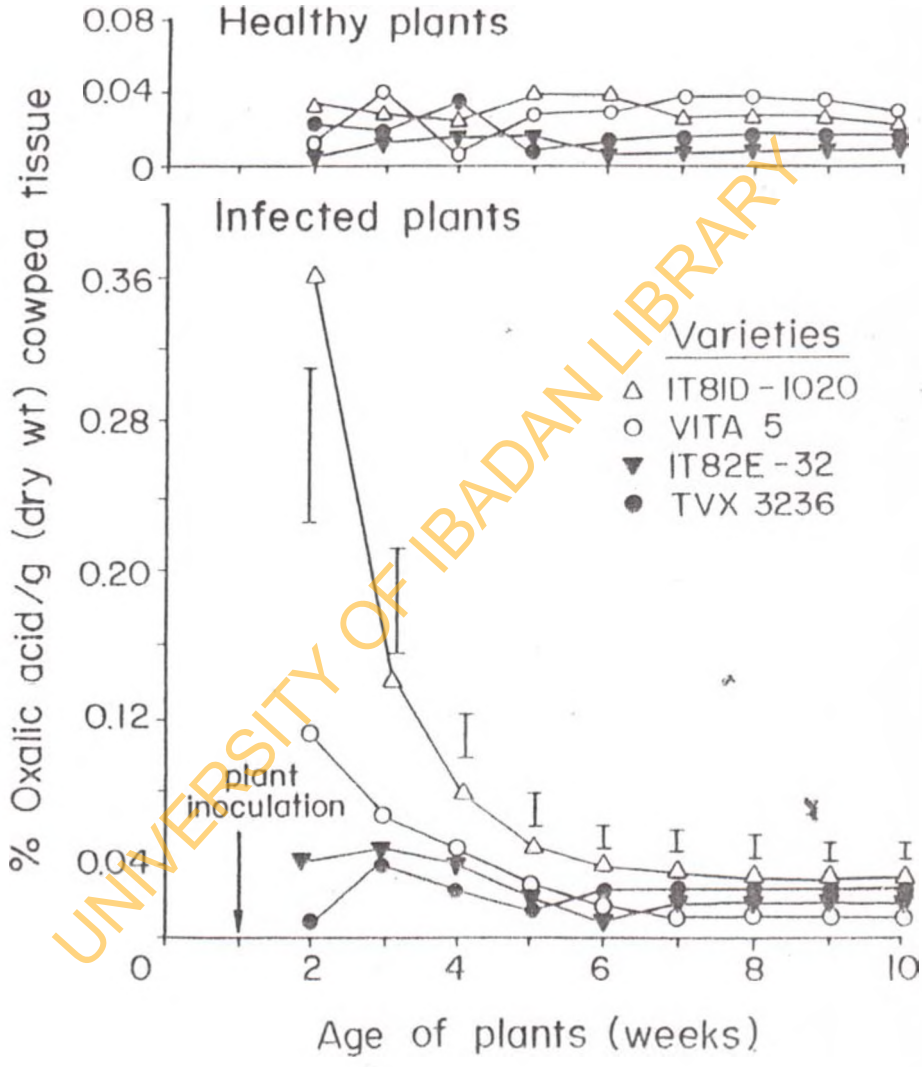

Fig. 1. Oxalic acid content of healthy and infected plants of four varieties of cowpea. Bars denote S.D. for all varieties on a given day.

Results given in Figure 1 show the difference between the oxalic acid content in diseased and healthy plants of the four varieties. There was no significant difference ($P = 0.05$) in the oxalic acid content of the different healthy cowpea varieties throughout the period of study, which spanned 10 weeks. In the inoculated and infected cowpea varieties there was a marked significant difference ($P = 0.05$) in the oxalic acid content among the varieties 7 days after inoculation. Oxalic acid content of var. IT81D-1020 was significantly higher than that of the other varieties for 4 weeks, after which there was no significant difference between infected and healthy cowpea varieties. VITA 5 was next in oxalic acid content and its trend was similar to that of IT81D-1020. The remaining two inoculated plants did not contain an appreciable amount of oxalic acid.

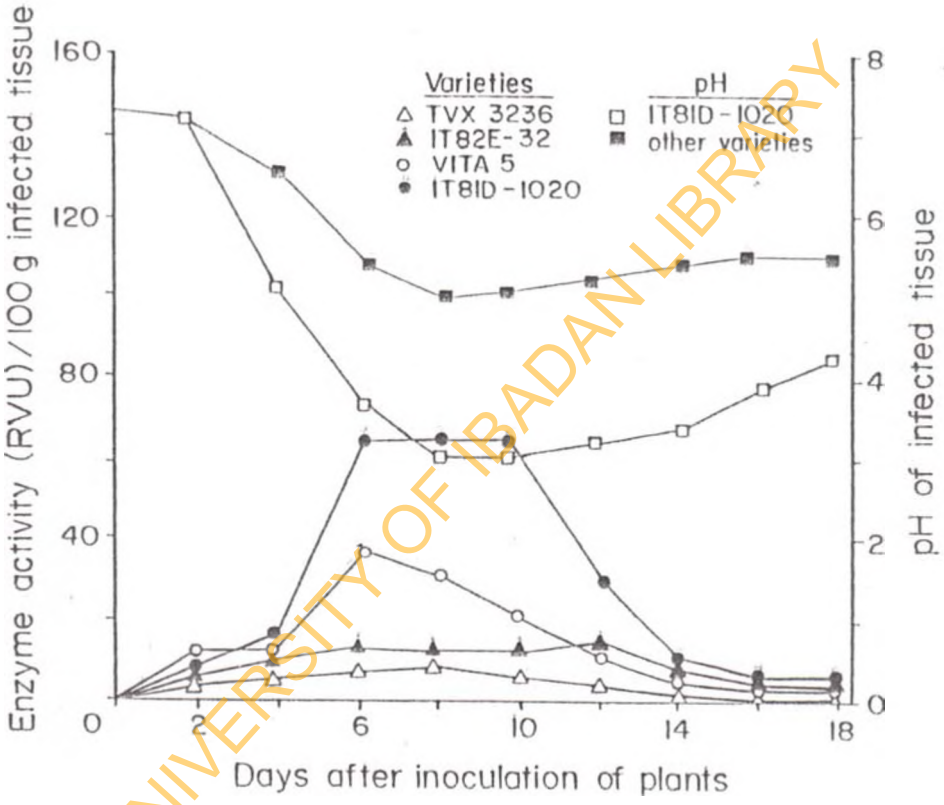

Fig. 2. Polygalacturonase activity in cowpea plants infected by *Pythium aphanidermatum*, and the pH of tissues.

*RVU = relative viscometric unit.

The *in vivo* production of PG (Fig. 2) was again highest in var. IT81D-1020 between 6 and 10 days after plant inoculation; this was followed by var. VITA 5, though at a much lower level. The other two varieties did not show any appreciable amount of PG in their tissue even after 30-fold concentration by $(NH_4)_2SO_4$ precipitation.

Figure 2 also shows the pH profile of infected tissues of IT81D-1020 and the other varieties from inoculation until the 18th day after. The pH of IT81D-1020 fell from 7.2 to 3.1, 8-10 days after inoculation; in the other varieties the lowest pH of infected plant tissue was 5.0, on the 8th day.

DISCUSSION

In the greenhouse, damping-off of seedlings and stem rot of established plants caused by *P. aphanidermatum* were most severe in var. IT81D-1020 and moderately severe in VITA 5, but vars. IT82E-32 and TVX 3236 seemed to have some resistance to the disease.

While there is sufficient evidence to show that a small amount of oxalic acid was produced in healthy cowpea varieties as a by-product of respiration in the tricarboxylic acid cycle, a significantly higher amount was produced in the infected vars. IT81D-1020 and VITA 5. These two varieties were the most susceptible of the four tested. Increased oxalic acid content of the tissues of the two susceptible varieties appears to be a consequence of a successful and compatible interaction between the host plants and the pathogen.

In assaying for PG production by *P. aphanidermatum* in infected plant tissues, the highest enzyme activity was detected in the tissue of IT81D-1020, followed by VITA 5; it was very low in the other varieties. PG activity was highest in var. IT81D-1020 between the 6th and 10th days after inoculation, while oxalic acid production was highest in the same variety 7 days after inoculation. Peak production of oxalic acid and of PG seemed to coincide in this study and the two may have acted simultaneously to increase disease severity on vars. IT81D-1020 and VITA 5.

The pH of infected tissue of var. IT81D-1020 fell to 3.1, 8 days after inoculation. Thus three factors seem to act in conjunction in the pathogenesis of susceptible cowpea plants by *P. aphanidermatum*: (i) production of polygalacturonase and (ii) of oxalic acid, and (iii) lowering of the pH of infected tissues. These three factors were less apparent in the resistant cowpea varieties.

Oxalic acid is known to chelate calcium and magnesium ions present as pectates in the middle lamella of plant cell walls (4,5,10), lowering the pH of the cell wall microenvironment to a more favorable level for the cell wall degrading enzymes (mainly PG) to hydrolyze the pectates, which are the cementing substance between cell walls (7,8). The lowered pH would further enhance the activities of hemicellulases (9) and cellulases (11). Polygalacturonase and cellulases were reported to be produced by *P. aphanidermatum* (17). These explanations fit very well the results presented here.

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