Growth Inhibition, Peroxidase and 3-Indoleacetic Acid Oxidase Activity, and Ethylene Production in Cowpea Mosaic Virus-Infected Cowpea Seedlings

B. E. Lockhart and J. S. Semancik

Former Research Fellow and former Assistant Professor, respectively, Department of Plant Pathology, University of California, Riverside 92502. Present address of authors: Postdoctoral Fellow and Associate Professor, respectively, Department of Plant Pathology, University of Nebraska, Lincoln 68503.

During investigations on virus multiplication in etiolated cowpeas (8), it was observed that cowpea mosaic virus (CPMV)-infected cowpea seedlings did not elongate as much as did healthy ones. This apparent growth inhibition was the only visible effect of CPMV infection in etiolated cowpea seedlings, and an attempt was made to relate symptom production to metabolic events associated with virus infection.

Cowpea (*Vigna unguiculata* L. Walp 'Early Ramshorn') seeds were germinated in moist vermiculite, and the seedlings kept in the dark at 30 C. When the hypocotyls were about 8 cm long, they were inoculated with a buffer-Celite suspension containing or lacking 50 μg/ml-purified CPMV in 0.05 M potassium phosphate, pH 7.0. At this stage, the epicotyls had not yet begun to elongate. No differences in the growth of healthy and infected seedlings were apparent until 4-5 days after inoculation (days p.i.), and by 6 days p.i. both epicotyl and hypocotyl had attained their maximum lengths. Measurements of epicotyl and hypocotyl wt and lengths made at 6 days p.i. are shown in Table 1. When either parameter (dry wt, length) is considered, growth inhibition in etiolated CPMV-infected cowpea seedlings is related to retardation of epicotyl development.

The growth inhibition accompanying viral infection could possibly result either from restriction of energy supply or from interference with growth factor metabolism. Since the tissues consist of nondividing cells, the inhibition must be one of cell elongation only. In view of the opinion (3) that in the mature plant cell energy supply probably does not pose a barrier to continued cell extension, it was considered more likely that some effect on growth factor metabolism was involved.

Among current ideas on growth regulation in plants is one that implicates an interaction between auxin, peroxidase—which is known to act as an auxin (IAA) oxidase, and ethylene (5). Attempts to extract auxin from etiolated cowpea tissue were unsuccessful, probably due to the low endogenous level. Peroxidase activity was determined colorimetrically (6), and 3-indoleacetic acid oxidase activity measured manometrically (12). All assays of enzyme activity were performed, using the 10,000 *g* supernatant fraction of phosphate buffer extracts of healthy or infected seedlings. Ethylene content was determined in samples of air from stoppered 25-ml flasks in which 2.5 g of healthy or infected tissue had been incubated for 24 hr at 30 C. Ethylene production and peroxidase and IAA oxidase activities are higher in infected tissue (Fig. 1). The differences appear at 2-3 days p.i., sufficiently early to be involved in the growth inhibition observed at 6 days p.i.

TABLE 1. Average lengths (cm) and dry wt (mg) of hypocotyls and epicotyls of healthy and cowpea mosaic virus-infected, etiolated cowpea seedlings at 6 days after inoculation. Averages of two experiments of 50 samples each

	Average length (cm)		Average dry wt (mg)	
	Hypocotyl	Epicotyl	Hypocotyl	Epicotyl
Healthy	14.1	18.5	44	33
Infected	14.3	10.5	46	15
Infected as % healthy	102	57	101	35

Growth retardants can increase the level of peroxidase and IAA oxidase activity in plant tissue (7). Peroxidase activity increases in several plant virus infections (6, 9, 16), and increased peroxidase activity has in many instances been associated with stunting and dwarfing (5, 9). Both increases (10) and decreases (11, 14) in auxin content have been reported for virus-infected tissue, and one report (15) describes an apparent interference with auxin transport following viral infection, an effect also reported to be produced by ethylene (2). Increased ethylene evolution in virus-infected tissue has also been reported (13); more recently, the effects of ethylene on auxin transport (2) and peroxidase activity (4, 16) and the possible interaction between auxin and ethylene in the regulation of plant growth (1, 5) have been postulated.

The data presented above suggest that growth inhibition in CPMV-infected cowpea seedlings results from either a destruction of auxin by peroxidase acting as an auxin oxidase, or an interference with auxin trans-

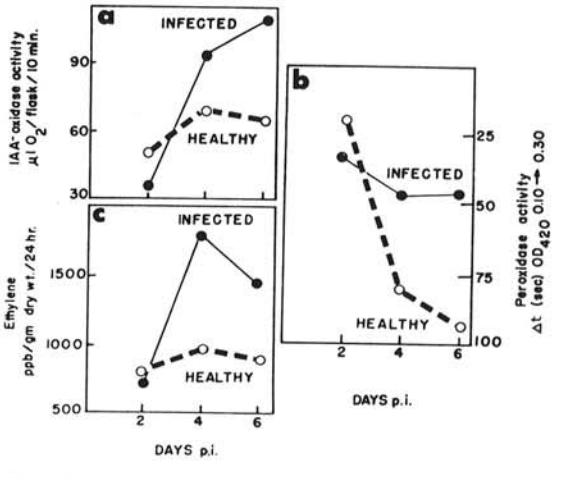

Fig. 1. **a)** IAA-oxidase and **b)** peroxidase activities of crude extracts of healthy and CPMV-infected etiolated cowpea seedling tissue at 2, 4, and 6 days after inoculation (days p.i). **c)** Ethylene production by healthy and CPMV-infected etiolated cowpea seedling tissue at 2, 4, and 6 days after inoculation.

port or metabolism mediated by ethylene. The effect is seen only on epicotyl elongation, because at the time of inoculation the hypocotyl has already attained more than half its maximum length. This hypothesis invokes a direct role for peroxidase in symptom production, thereby differing with a previous report (16) in which increased peroxidase activity accompanying virus infection was considered a probable secondary effect rather than the cause of symptom expression.

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