The Effect of Oxycarboxin on Amino Acid Metabolism by Phaseolus vulgaris var. Pinto

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Missouri Agricultural Experiment Station Journal Series 6447.

Accepted for publication 13 December 1972.

ABSTRACT

Amino acid metabolism was investigated in bean plants (*Phaseolus vulgaris* var. 'Pinto') treated with oxycarboxin. Plants were grown hydroponically in solutions containing 4.0 and 32 μg/ml of oxycarboxin. After treatment, discs were removed from the primary leaves and the free amino acids were extracted and quantitatively determined. The effect of oxycarboxin on incorporation of ^{14}C-labeled CO_2 into the cation, anion and neutral metabolites was also investigated. The data obtained from these investigations though not conclusive suggest that biochemical alteration of the host is not a primary mode of action by oxycarboxin for controlling bean rust caused by *Uromyces phaseoli*.

Phytopathology 63:686-688

Additional key words: oxycarboxin, fungicide.

Current evidence indicates that the mode of fungicidal action of the oxathiins involves a direct effect on the plant parasite (2, 3, 5, 8). However, there are a few reports which refer to physiological effects on plants after treatment with the oxathiins. Reyes et al. (6) found that foliar application of carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) and oxycarboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide 4,4-dioxide) increased protein content in wheat seed. They also observed that amino acid metabolism is slightly stimulated in wheat, corn, sorghum, and soybean plants by both compounds. In addition, nitrate absorption and nitrate reductase induction are stimulated when the nitrate supply and/or the temperature are less than optimum. Newby & Tweedy (4) found that oxycarboxin treatment resulted in a decrease in the quantity of amino acids washed from 'Pinto' bean leaves. Thorn & Richardson (10, 11) treated tomato plants with 1,000 ppm carboxin for 24 hr and observed little change in total volume of exudate from the stumps and they stated that there was as much as a 26% increase in total amino acid exudation. Carlson (1) studied the effects of carboxin on chlorophyll content, photosynthesis and respiration of barley leaves and suggested that the compound affects cell permeability.

In general, these studies suggest that the oxathiins have an effect on the metabolism of the host. This study was conducted to further delineate the effects of oxycarboxin on the metabolic activities of the plant. The specific objectives were: (i) to determine qualitative and quantitative changes in the essential free amino acids after treating bean plants with oxycarboxin; and (ii) to observe changes in $^{14}CO_2$ incorporation into ionic and neutral plant fractions after oxycarboxin treatment.

MATERIALS AND METHODS.—Twelve-day-old bean plants (*Phaseolus vulgaris* var. 'Pinto') in vermiculite were treated with 8 μg/ml oxycarboxin in Hoagland's solution for 96 hr. Then two leaf discs (16 mm) were removed from the central portion of each of 48 primary leaves. The discs were weighed, immediately submerged in boiling 80% ethanol, and refluxed in 80% ethanol for 24 hr. The extract was subsequently filtered and the filtrate was concentrated to about 20 ml using a rotary evaporator at room temp. The volume was adjusted to about 50 ml with warm 0.1 N HCl which precipitated and/or flocculated chlorophyll and other macromolecules. This mixture was centrifuged at 10,000 *g* for 15 min and the supernatant fraction was decanted. The residue was washed twice by suspension in 20 ml of 0.1 N HCl and recentrifuged. The combined supernatant fractions were concentrated to approximately 20 ml.

The extract was added to a 0.9-cm i.d. column containing 10 ml of Dowex 50 X 8 resin (H^+, 50-100 mesh). The neutral and anionic fractions passed through the column with 40 ml of water while the free amino acids were retained. The amino acids were subsequently eluted with 30 ml of 4.0 N ammonium hydroxide. The eluants were collected and evaporated to dryness and the residue was solubilized in 2 ml of 0.1 N HCl. The ninhydrin-positive materials were analyzed by a modified method of Spackman et al. (9). The experiment was repeated four times in duplicate.

To study the effect of oxycarboxin on the incorporation of ^{14}C-carbon dioxide by the leaves, plants were removed from the rooting medium and placed into 100 ml of nutrient solution. One container was used as a control and two were treated with 4 (1.5 μM) and 32 (12 μM) μg/ml oxycarboxin, respectively, for 24 hr and then exposed to $^{14}CO_2$. The entire experiment was repeated three times. Following the fungicide treatment, all plants were placed inside a clear plastic bag in a laboratory hood when stomatal opening was at a maximum. Light intensity was approximately 900 ft-c and temp was

30 C. Generation of $^{14}CO_2$ in the plastic bag was accomplished by adding 0.2 ml of sulfuric acid to 20 mg of ^{14}C-barium carbonate (ca. 100 μC). After exposure to $^{14}CO_2$ for 1 hr, the leaves were removed, weighed, placed in boiling 80% ethanol, and refluxed in this solution for 24 hr.

The solution was filtered and the residue homogenized in 50 ml of 80% ethanol for 2 min at high speed in a Virtis homogenizer. The homogenate was centrifuged for 10 min at 800 *g* and the supernatant fraction added to the original extract. The residue was washed with 20 ml of 80% ethanol by centrifugation.

The combined extract was concentrated to approximately 50 ml, diluted with 0.1 N HCl and centrifuged for 20 min at 10,000 *g*. The supernatant solution was decanted and the pellet washed three times with 10 ml of 0.1 N HCl. Little radioactivity remained in the solid material. The combined solutions were concentrated to about 40 ml under vacuum at 40 C. The extract was diluted to a known volume from which four 25-μliter aliquots were removed for radioactive determinations with a liquid scintillation counter. Quenching was determined and the data converted to dpm.

Separation into neutral, anionic, and cationic fractions was then performed by passing each sample through a cation exchange column (Dowex 50 X 8, H^+, 50-100 mesh) and an anion exchange column (Dowex 1 X 8, formate, 100-200 mesh) connected in tandem with Tygon tubing. The eluant from the anion column was restricted to a flow rate of approximately 1.25 ml/min. Under these conditions, the neutral fraction passed through both columns and the cation and anion fractions were bound to their respective columns.

The columns were washed with 50 ml of distilled, deionized water, separated, and the cations from the Dowex 50 column were eluted with 4.0 N ammonium hydroxide and the anions from the Dowex 1 eluted with 6.0 N formic acid. The volume of each fraction was adjusted to 10 ml from which aliquots were removed and radioactivity determined as previously described.

RESULTS AND DISCUSSION.—The analytical data concerning the amino acids are summarized in Table 1. Tryptophan was not determined because of its destruction under analytical conditions used in this study. In addition, the isolation procedure failed to separate aspartate from threonine. Nevertheless, it is apparent that the quantities of one or both of the two amino acids decreased significantly in the plants treated with oxycarboxin. Other than these two amino acids, oxycarboxin appears to have little or no effect on amino acid metabolism.

The results of $^{14}CO_2$ incorporation into the neutral, cation and anion plant fractions are recorded in Table 2. Virtually no effect was observed when plants were treated with 4 and 32 ppm of oxycarboxin and the plants subsequently exposed to $^{14}CO_2$. The ratio of the percentage radioactivity in the cation fraction to neutral plus anion fractions were 0.192, 0.196, and 0.174 for the 0, 4, and 32 ppm treatments, respectively. The slight reduction observed at 32 ppm was not statistically significant.

Sisler (7) points out that selective actions by a systemic compound may take advantage of certain fundamental differences between plant and pathogen. Alternatively protoplasts of host plants simply do not accumulate the compound as rapidly as the pathogen. Reports (4, 10) that oxycarboxin treatment of plants causes changes in amino acid metabolism are not supported by our data. Nevertheless, the evidence against biochemical alteration of host metabolism as

TABLE 1. The effect of oxycarboxin on free amino acid content of 'Pinto' bean leaves

Amino acid	μg/g Fresh weight	
	NT[a]	T[a]
Aspartic Acid[b] + Threonine	202	120
Lysine	6	4
Methionine	2	1
Isoleucine	7	5
Glutamic Acid	109	145
Ornithine	5	3
Citrulline	Tr[c]	Tr
Arginine	2	3
Proline	15	13
Tyrosine	3	Tr
Phenylalanine	8	7
Tryptophan	No analysis	
Histidine	6	4
Alanine	21	23
Leucine	7	5
Valine	9	8
Serine	32	36
Glycine	9	8
Cysteine	Tr	Tr
Total a. a.	445	485

[a] NT represents "no treatment" controls and T represents treatment with 8 ppm oxycarboxin for four days.

[b] Aspartic acid and threonine were not separated sufficiently to yield individual quantities.

[c] Tr indicates trace amount present.

TABLE 2. The effect of oxycarboxin on $^{14}CO_2$ incorporation into plant fractions

Oxycarboxin exposure (ppm)[b]	Percentage radioactivity[a]		
	Neutral fraction	Cation fraction	Anion fraction
0	78.6	16.0	4.9
4	81.7	16.4	1.9
32	81.9	14.8	3.2

[a] The percentage radioactivity is based on total radioactivity eluted from ion exchange columns. The average total radioactivity recovered for the 0, 4, and 32 ppm was 1.45×10^7, 1.33×10^7, and 1.22×10^7/g fresh wt, respectively.

[b] Plants were treated with oxycarboxin for 24 hr prior to exposure to $^{14}CO_2$ for one hr.

the primary mode of action of oxycarboxin is not conclusive. However, with the exception of aspartate and/or threonine, alterations in amino acid metabolism, as we have monitored them, do not seem to be significant. It may well be that changes involving protein synthesis (6) do occur over longer periods of time, but are probably not associated with a mechanism of action per se.

Mathre's (2, 3) reports of slow accumulation of oxycarboxin by sensitive fungi may apply as well to plants, thus adding support to Sisler's (7) concept concerning slower accumulations by host protoplasts. It seems reasonable to postulate that oxycarboxin at the concentrations and treatment periods used in this study, is generally confined to intercellular areas of bean leaves thus allowing direct contact with the invading fungus. Literature reports of changes in amino acid exudation (3, 9) could be explained by the reported effect on permeability (1), thereby allowing such changes to be observed without altering concentrations of total free amino acids.

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