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L-Dopa in Legume Seeds: A Chemical Barrier to Insect Attack

Abstract. *Seeds of the genus* Mucuna *display both a remarkable immunity to attack by seed-eating insects and an unusually high concentration of free* L-*dopa. When seed powders or pure* L-*dopa were incorporated into artificial diets and fed to southern armyworm larvae, mortality increased, and abnormal pupae and adults resulted. At higher concentrations* L-*dopa acted as a feeding repellent.*

Mature seeds of the Central American genus *Mucuna* are conspicuously free from attack by small mammals and insects (*1*). Free L-dopa (3,4-dihydroxyphenylalanine), well known as a drug used for the treatment of Parkinson's disease in man, has been found in the embryos (including cotyledons) of six species of *Mucuna* at concentrations varying from 5.9 to 9.0 percent (*2*). In addition, these species contain varying concentrations of a cyclic imino acid (3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) which is assumed to be a derivative of L-dopa (*3*). We would expect that the metabolic expenditure required to synthesize and store such concentrations of an unusual free amino acid would be offset by some significant advantage conferred on the seeds by its presence. Bell and Janzen (*2*) have suggested that L-dopa serves a protective function in *Mucuna* seeds, discouraging attack by insects and small mammals. We confirm that L-dopa in low concentrations can produce toxic effects in at least one species of insect. At the higher concentrations found naturally in whole seeds of the genus *Mucuna*, this compound inhibits feeding completely.

The insect chosen for the bioassay of *Mucuna* seeds was the southern armyworm, *Prodenia eridania* Cramer (Noctuidae) (*4*). The caterpillar of this species is widely polyphagous with an exceptional ability to detoxify many foreign compounds (*5*) and, presumably, many toxic secondary chemicals encountered in its food plants (*6*). Demonstration of any repellent or toxic effects to an insect with such generalized feeding habits should be of broader interest than similar results with a more specialized insect. Since the armyworm is phytophagous, whole *Mucuna* seeds were ground and offered to larvae as a component of an artificial diet. The diet was a modified version of the one developed by Feeny (*7*). The fungicide methyl-*p*-hydroxybenzoate, which had been incorporated routinely into the diet, was eliminated, since analogs of that compound are inhibitors of tyrosinase activity (*8*). Twenty early fourth instar larvae were reared on each diet under constant conditions (temperature, 21°C during the day, 19°C at night; 30 to 40 percent relative humidity; and a 16-hour light and 8-dark photoperiod).

Table 1. Growth of *Prodenia eridania* larvae on artificial diets containing *Mucuna* seed powders (5 percent) or pure seed chemicals. Initial number of larvae on each diet was 20. Values are expressed as means ± standard error of the mean.

Diet	Mean initial weight (mg)	Mean pupal weight (mg)	Deformed pupae (No.)*	Normal pupae (No.)	Mean adult weight (mg)	Deformed adults (No.)*	Normal adults (No.)
Mucuna pruriens†	21.2 ± 1.0	370.8 ± 11.7	8	9	167.4 ± 6.7	0	15
M. mutisiana†	18.7 ± 0.7	329.7 ± 11.6	4	16	169.5 ± 7.6	1	14
M. holtoni†	10.8 ± .6	321.4 ± 11.3	1	19	163.3 ± 8.3	1	18
M. urens†	10.7 ± .6	309.1 ± 10.9	3	16	163.6 ± 9.6	3	0
Control (5% bean leaf powder)	18.8 ± 1.1	306.6 ± 9.4	0	20	154.7 ± 8.1	0	20
Bean leaf (5%) plus L-dopa (0.25%)	21.3 ± 0.9	328.8 ± 13.6	2	17	157.6 ± 10.0	0	17
M. pruriens (5%) plus L-dopa (0.25%)	19.1 ± .9	417.9 ± 14.0	9	2	176.0 ± 10.1	0	8
L-Dopa (5%)	10.9 ± .6	0‡	0	0	0	0	0
L-Dopa (5%)	62.9 ± 3.5	0‡	0	0	0	0	0
M. pruriens (5%)§	12.5 ± 1.9	335.7 ± 18.0	15	2		1	0
M. pruriens (5%)§	15.7 ± 0.9	347.2 ± 16.1	13	0	185.6 ± 17.9	2	0
Bean leaf (5%) plus L-dopa (0.25%)§	15.6 ± .8	290.7 ± 8.0	14	6	135.7 ± 8.9	3	7
Bean leaf (5%) plus cyclic imino acid (2.0%)‖	14.4 ± .2	281.1 ± 8.1	0	20	131.0 ± 9.2	0	20

* Pupae were deformed through failure of the pupal case to sclerotize over the ventral head, thoracic, and first three abdominal segments; adult deformity was manifested as malformed or stunted wings. † *Mucuna pruriens* seeds were collected on Isla Providencia, Colombia, 25 August 1969, and near Puntarenas, Puntarenas Province, Costa Rica, 11 March 1971; *M. mutisiana* seeds were collected on the Isla Providencia, Colombia, 24 August 1969. Seeds of *M. urens* and *M. holtoni* were gifts of Dr. W. H. Tallent, U.S. Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Illinois. ‡ All larvae starved to death. §Methyl-*p*-hydroxybenzoate (0.1 percent) was added to the diet. ‖ 3-Carboxy-6,1-dihydroxy-1,2,3,4-tetrahydroisoquinoline, isolated from *M. mutisiana* seeds.

Characteristically deformed pupae developed from larvae reared on a diet of 5 percent *Mucuna* seed powder (Fig. 1, center row); the frequency of deformity varied with the species of *Mucuna* (Table 1). In deformed pupae the pupal case failed to sclerotize over the ventral head, thoracic, and first three abdominal segments. This large unsclerotized area was especially vulnerable to puncture with subsequent loss of hemolymph. Surviving adults of both sexes sometimes emerged with such stunted wings that mating was impaired.

L-Dopa (Nutritional Biochemicals, Cleveland, Ohio) was added to a control diet of bean (*Phaseolus vulgaris*) leaf powder at a concentration (0.25 percent) approximating that found in diets of 5 percent *Mucuna* seed powder (*9*). Two of the resulting pupae were deformed in the same manner (Fig. 1, bottom row), but a 0.5 percent L-dopa diet produced no abnormal effects. A diet of 5 percent *M. pruriens* seed powder fortified with 0.25 percent L-dopa resulted in increased pupal deformity and adult mortality for larvae reared on that diet (Table 1). Diets of 5 percent L-dopa, prepared to approximate the concentrations found in *Mucuna* seeds, were completely unacceptable to both fourth and fifth instar larvae (Table 1). Whereas larvae had readily eaten diets with low concentrations of L-dopa and had gained more weight than larvae reared on control diets, no feeding was observed on diets of 5 percent L-dopa, and mean larval weight on death was approximately half the initial mean larval weight. The *p*H of the diet (5.0) did not differ from that of the control diets.

Under alkaline conditions L-dopa may be oxidized. Our experimental diets, however, were mildly acidic and contained ascorbic acid, an antioxidant (*3*). Agar gels containing 0.25 percent and 5 percent L-dopa plus 0.15 percent ascorbic acid were extracted and analyzed by paper chromatography (*10*). No decomposition of L-dopa could be detected up to 14 days after preparation, the maximum time any diet was stored.

Incorporation of methyl-*p*-hydroxybenzoate, a suspected tyrosinase inhibitor, into test diets resulted in substantial increases in the incidence of pupal deformity in larvae reared on diets of *M. pruriens* seed powder or L-dopa (Table 1). We suspect that L-dopa, or some metabolite generated after ingestion, may interfere with the action of

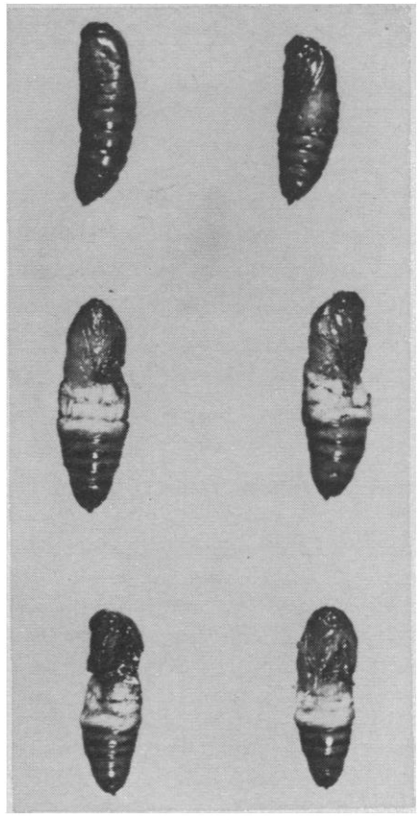

Fig. 1. Pupae of *Prodenia eridania* larvae reared on artificial diets. (Top row) Larvae fed 5 percent bean (*Phaseolus vulgaris*) leaf powder diet (control); (center row) larvae fed 5 percent *Mucuna pruriens* seed powder diet; and (bottom row) larvae fed a diet of 5 percent bean leaf powder plus 0.25 percent L-dopa.

tyrosinase, an enzyme essential to the hardening and darkening of insect cuticle (*11*). The variation in incidence of pupal deformity caused by different species of *Mucuna* remains unexplained. The percentage of L-dopa per whole seed is roughly the same for all *Mucuna* species tested. The cyclic imino acid concentration does vary among species; however, a diet of 2.0 percent cyclic imino acid, the concentration found in *M. mutisiana* seeds, did not produce characteristic deformity or mortality in southern armyworm larvae reared on it (Table 1). It seems likely that manifestation of the effects of L-dopa at low concentrations is subtly dependent upon the ratios of its concentration to those of other chemical constituents, possibly including the cyclic imino acid.

In addition to a few species of Lepidoptera, the major predators of Central American legume seeds include many beetle species of the family Bruchidae (*12*). Incomplete sclerotization of the well-armored adults could well be a lethal reaction to L-dopa in potential hosts such as *Mucuna* seeds. However, direct repulsion of the beetle larvae would not be an adequate defense against damage; the larvae would have drilled through the seed coat and would have encountered the embryo before the L-dopa could influence it. Protection through sequestering of L-dopa in the seed probably operates through selection against those mutant strains of insects that would oviposit on *Mucuna* seeds or pods.

Storage of high concentrations of unusual free amino acids in legume seeds may also provide developing embryos with a ready supply of nitrogen (*13*). Like tannins, mustard oils, and some other secondary plant chemicals (*14*), L-dopa may thus confer more than one adaptive advantage on plants containing it.

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