present in significant amount (*8*). Tryptophan was determined separately by the method of Spies and Chambers (*9*). Less than 1 residue per 1000 residues can be attributed to tryptophan.

The possibility that methionine hydrophobic bonds were strong enough to provide effective cross-links that give elastic properties to this insoluble protein has not been resolved. Prolonged exposure (2 weeks) to both iodoacetic acid and iodoacetamide (400 mg protein in 10 ml of 10-percent solution of either reagent at *p*H 2 and *p*H 4.5) at room temperature did not dissolve the ITHL although swelling was observed (*10*). Since the reaction between iodoacetic acid and methionine produces a charged residue, any hydrophobic bonds due to methionine would be disrupted. Homoserine and *S*-carboxymethylhomocysteine were detected in protein hydrolyzates treated with iodoacetic acid, and the methionine peak was diminished; however, further work must be done to determine the effect of these reagents on the methionine residues.

Histological sections showed a distinct morphological difference between the cortical and medullary portion of the ITHL. To determine if there was a difference in protein composition, the two regions were separated by dissection, and amino acid analyses were conducted on each. There is no difference in amino acid concentration in either region, which indicates that the protein is similar throughout the ITHL (Table 1). Treatment with ethylenediaminetetraacetate did not affect the amino acid composition of the ITHL of *Pecten* as it did the inner hinge ligaments of certain other bivalves that we studied (preliminary investigation). No large unknown peaks were found by column chromatography, although a few small unknown peaks were encountered spasmodically; none of these corresponded to desmosine. The possibility remains that material of unknown composition was bound tightly to the ion-exchange resin. The cross-linkages in resilin have been attributed to di- and tri-tyrosine, and Andersen found that these amino acids were held back on the ion-exchange column (*11*). However, these amino acids give rise to a "bright blue fluorescence" in ultraviolet light in intact resilin; in our initial attempts, intact ITHL does not appear to fluoresce (*12*).

These results indicate that the ITHL of *Pecten* is composed primarily of a rubber-like protein distinct from other known elastic proteins, and we propose the name abductin for this insoluble rubber-like protein. The name seems appropriate since the protein functions as the abductor of the valves of the scallop.

Any similarity in the properties of abductin from scallops and the proteins of the inner hinge of a wide range of bivalve mollusks has not yet been determined.

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31 August 1966

Diphenamid Metabolism in Plants

Abstract. *Diphenamid, a herbicide, is metabolized to* N-*methyl 2,2-diphenylacetamide and 2,2-diphenylacetamide by the common soil fungi* Trichoderma viride *and* Aspergillus candidus *within 48 hours. The two metabolites are more toxic than diphenamid to both tomato and barnyard-grass seedlings under sterile conditions. This finding indicates that the phytotoxic moiety is not diphenamid but one of its metabolites—probably the* N-*methyl derivative.*

Diphenamid (*N,N*-dimethyl 2,2-diphenylacetamide) is an effective herbicide for controlling several annual grass and broadleaf species; it is absorbed through the roots and has negligible herbicidal activity when applied to the foliage (*1*). The absorption, translocation, and metabolism of ^{14}C-diphenamid has been studied in tomato plants (*2*). Seven days after treatment with diphenamid, both the MDA (*N*-methyl 2,2-diphenylacetamide) and DA (2,2-diphenylacetamide) metabolites were found in benzene extracts; after 21 days, no diphenamid was detectable in the plants. The *N*-demethylation of diphenamid in tomato plants was proposed as the mechanism of resistance by this species. *N*-Demethylation of methylamides and methylamines in both plants and animals is reported (*3*), but correlation between demethylation and phytotoxicity has not been established with plant species.

The fungi *Trichoderma viride* and *Aspergillus candidus* were cultured in 150-ml flasks containing 100 ml of half-strength Hoagland nutrient solution and 0.02*M* glucose. After 1 week, 0.036 μc of ^{3}H-diphenamid was placed in each flask. After 4- and 48-hour exposures, the diphenamid was extracted with chloroform and subjected to two-directional chromatography (type K301R Eastman thin-layer chromogram sheet), first in a benzene-ethanol (85:15 by volume) solution, and then in a benzene-diethylamine solution (95:5 by volume). The extract was cochromatographed with ^{3}H-diphenamid in the first solvent system and with diphenamid, MDA, and DA in the second.

The extracts exposed to either fungus for 4 hours contain a compound that migrates as MDA (R_F, 0.53) in the benzene-diethylamine system. There is also a spot corresponding to the diphenamid reference at R_F 0.64. Each extract after 48-hour exposure has a larger spot, corresponding to the MDA metabolite, at R_F 0.53, and also a detectable amount, at R_F 0.25, corresponding to the DA metabolite. Fur-

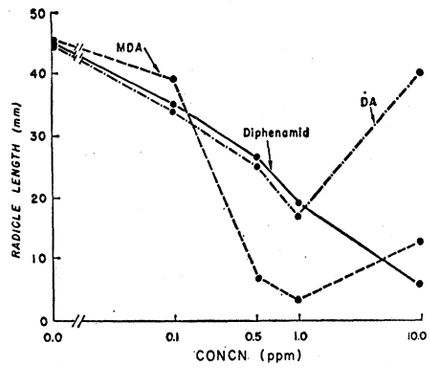

Fig. 1. Effects of diphenamid, MDA, and DA on growth of radicles of barnyard-grass seedlings. The F value for the difference in activity of chemicals at varying concentrations is significant at the 1-percent level.

ther tests and other solvent systems confirm these results. However, development in the second direction with benzene-ethylacetate (140:10 by volume) produces better separation of diphenamid, MDA, and DA. The compounds may be located by ultraviolet light, 10-percent ethanolic phosphomolybdic acid sprays, and determination of the radioactivity after removal of strips from the chromogram and their placement in scintillation vials.

Tomato (*Lycopersicon esculentum* Mill.) and barnyard-grass (*Echinochloa crusgalli*) seedlings were used for study of the effects of diphenamid, MDA, and DA on the growth of resistant and susceptible species. Each of the three compounds was applied to seeds, disinfected with sodium hypochlorite, before they were placed on filter paper in sterile petri dishes; each was applied in 5 ml of sterile distilled water containing 0.1, 0.5, 1.0, or 10.0 parts per million. The seedlings were grown for 4 days in a dark incubator at 26°C before the radicle and hypocotyl of each were measured.

Diphenamid at up to 10 ppm does not inhibit growth of tomato seedlings. The MDA metabolite at 0.5 or 1.0 ppm inhibits the growth of tomato radicles without causing acute toxicity. The growth of barnyard-grass seedlings decreases linearly with increasing concentrations of diphenamid (Fig. 1); radicles are severely injured by MDA at 0.5 or 1.0 ppm, but this phytotoxicity is partially overcome at 10.0 ppm. The DA metabolite causes similar response in both plant species but is less toxic than MDA.

In a similar study, sandy loam soil was placed in 10-cm clay pots, two-thirds of which were then autoclaved; the remainder were not sterilized. Straight from the autoclave, the sterilized pots were placed in large plastic bags to prevent contamination. The following day all pots were planted with surface-disinfected seeds of tomato and barnyard grass and treated with 300 ml of sterilized nutrient solution containing diphenamid at concentrations of 0.0, 1.0, 10, or 100 ppm. Half the sterilized pots were inoculated with *A. candidus* and *T. viride*. The plastic bags were left on all sterilized pots until seedlings emerged but were never placed over other pots. After 3 weeks the grass plants were rated between 1 and 9 for injury, 1 indicating no injury; 9, complete kill.

In unsterilized soil, barnyard-grass seedlings are severely injured or killed by all concentrations of diphenamid (Fig. 2). In sterilized soil inoculated with fungi, the grass is severely injured at 10 or 100 ppm of diphenamid; in sterilized soil diphenamid causes injury only at 100 ppm. Tomato seedlings are not injured at two lower rates but are stunted by the 100-ppm concentration under all three conditions.

Both MDA and DA metabolites of diphenamid have been found in tomato plants grown in soil under nonsterile conditions (*2*). However, the possibility of demethylation by microorganisms and subsequent plant absorption and translocation seems more feasible than metabolism within the tomato plant itself. To test this hypothesis, tomato and barnyard-grass seeds were surface-disinfected by 30-minute immersion in 1-percent solution of sodium hypochlorite before being placed in autoclaved test tubes each containing 2 ml of distilled, deionized water and a folded sheet of 90-mm Whatman No. 1 filter paper. The tubes were stoppered with sterile cotton and incubated in the dark at 26°C. After germination, the tubes were subjected to a 16-hour light, 8-hour dark cycle until the radicles were about 7 cm long. The seedlings were then transferred to sterile nutrient solution in 50-ml flasks containing diphenamid at 10 ppm. Half the plants of each species were kept in the dark throughout this stage, while the other half were subjected to 16-hour light, 8-hour dark cycling. Plants of both species were removed from these solutions 1, 2, 4, 8, or 16 days after treatment and extracted with benzene; the extracts were subjected to two-directional chromatography, first in benezene-ethanol (85:15 by volume) and then in benzene-ethylacetate (140:10 by volume). In the second solvent system, diphenamid, MDA, and DA have R_F values of 0.48, 0.30, and 0.17, respectively.

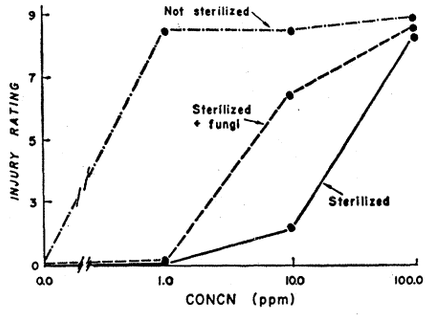

Fig. 2. Growth of barnyard grass in sterile and nonsterile soil treated with diphenamid. The F value for the difference in response to treatments with increasing concentrations is significant at the 1-percent level.

After 16 days, diphenamid is still the only compound found in these extracts, with no trace of either metabolite, regardless of exposure to light. These findings indicate that under sterile conditions neither tomato nor barnyard grass metabolizes diphenamid within 16 days of its application. An extract of an acetone-dried powder of *T. viride* converted diphenamid to MDA, indicating the presence of enzymes capable of demethylating diphenamid.

There are two successive metabolites of diphenamid after its exposure to *T. viride* and *A. candidus* for 48 hours; both are more toxic than diphenamid to tomato and barnyard-grass seedlings. Soil studies indicate metabolism of diphenamid under nonsterile conditions, resulting in production of a more toxic compound. We believe that diphenamid is not phytotoxic at the concentrations used herbicidally, but must be metabolized in the soil to the more toxic MDA and DA compounds.

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