The Antimetabolite

IMIDAZOLE AS A PESTICIDE

ROY J. PENCE

Imidazole, an antimetabolite of histamine and nicotinic acid, has been found to be a safe and effective pesticide and is now undergoing field tests. Developed at U.C., Los Angeles, the new material has been patented and given the trade name "Imutex." Imidazole, when synergized with boric acid, may be employed at low levels to proof fabric satisfactorily against insect attack. When synergized with 2-Aminopyridine, along with several newer synergists, and combined with base oil, imidazole is capable of controlling a number of insects and related arthropod species. Effectiveness is significantly increased by adding two surfactants to base oil. A hydrophilic surfactant increases the physiological activity of imidazole without improving the physical properties of the oil carrier, while perchloroethylene directly increases solubility. The particularly exciting quality about the material is its low mammalian toxicity.

THE METABOLIC EFFECTS of antimetabolites within the systems of fabric-feeding insects have been studied for the last six years. Some of these compounds, when formulated in suitable lipid-soluble carriers, have recently been found to perform as effective contact pesticides in addition to their already proven metabolic values. One of the most promising of the antimetabolites is imidazole (glyoxaline or 1,3-diazole), which has an extremely low mammalian toxicity.

Antimetabolites closely resemble a vitamin or other growth substance in chemical structure but, by design, are slightly altered in their manufacture. Perhaps a single atom has been omitted, or added where it does not belong. With such a chemical structure the deceptive resemblance to a vitamin may cause it to smell or even taste like a vitamin to a fabric-feeding insect, but it will never serve the same purpose. Once taken into the system of the insect, the antimetabolite becomes an inhibitor antagonistic to the growth regulator it resembles. The antimetabolite does not fit into the digestive processes, and physiological complications develop and interrupt normal metabolism. When low-level antimetabolites are applied to test fabrics, early instar larvae driven by hunger to eat increasing amounts of the pseudo-vitamins soon die of either a vitamin deficiency or related forms of hastened starvation while allowing minimum losses in fiber weight.

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Field tests

Imidazole has been tested in the field by a few accredited pest control operators, who are reporting a sufficient knockdown of insects and related arthropods to comply with standard "clean-out" requirements within infested dwellings selected for these tests. The chemical was also found to be odorless and nonirritating to the eyes or respiratory system. Preliminary results, from both laboratory and field, indicate that imidazole should prove a valuable tool for many phases of pest control.

Inasmuch as fabric-feeding insects exist largely upon a diet of wool combined with essential contaminants, and because the identity of these requirements has been learned through dietetic studies, it was reasoned that specific antimetabolites might be employed to antagonize, metabolically, the dietary needs of all fabric-feeding insects.

22 proven effective

A total of 114 growth-factor analogs have now been tested with 57 demonstrating varying degrees of metabolic significance. Of this number, 22 have proved to be of sufficient value to "proof" wool satisfactorily against fabric insect attack. Imidazole, one of the most promising, has been currently selected for commercial development based on its effectivenes, economy, and negligible mammalian toxicity. The acute oral toxicity (LD_{50} mg/kg) of pure imidazole for laboratory animals has been established at between 1,000 and 1,500 mg/kg with recent results revealing imidazole satisfactorily to have passed acute inhalation toxicity tests specified for "Hazardous Substances" by the U. S. Food and Drug Administration.

Imidazole is the nucleus of histamine and serves as a pharmaceutical intermediate for the manufacture of certain antihistamines. It is sold commercially as an antimetabolite for histamine. Current experiments with imidazole, plus a few other antihistamines that have compara-

Photomicrograph of a wool fiber containing a few mandibular scars caused by the linear feeding of a young carpet beetle larva. This constitutes the degree of feeding damage to wool impregnated with an antimetabolite. Enough treated wool is ingested to disrupt the insect's metabolism but not enough to damage the fabric from which the fiber was withdrawn.

ble metabolic effects on fabric-feeding insects, indicated that histamine performs a more important biological function within the systems of a number of species of insects than hitherto suspected. Further and more detailed investigations in this area are required. Sufficient experimental evidence has now been accumulated to demonstrate the reversal of the "toxic" symptoms of imidazole by histamine and histamine derivatives.

In testing the effects of imidazole on fabric-feeding insects, it has been shown that 1% of actual imidazole, when applied to wool fabric, will satisfactorily pass all C.S.M.A. (Chemical Specialties Manufacturers Association) standards of evaluation for control of clothes moths, *Tineola bisselliella* (Hummel), and three species of carpet beetles, *Attagenus piceus* (Olivier), *Anthrenus flavipes* Leconte, and *A. verbasci* (L.). It has also been learned that boric acid will synergize imidazole. Boric acid alone applied at 1% to wool fabric has little effect on these insects. Yet fabric saturated with or dipped into a solution of as little as 0.25% of imidazole and 1% boric acid will satisfy the minimum excrement-weight-yield tolerances established under C.S.M.A. standards.

Treated wool

When imidazole was added to wool fabric as a proofing agent and tested under C.S.M.A. standards, its metabolic effects were logged by recording the daily body weight losses and mortality of the insects, as well as the final weight of their total excrement, in contrast to lesser losses of control insects when subjected to complete starvation. Also consistent with standards, was the significant lack of fiber weight loss following the required 14 days of insect exposure. It should be understood that confined carpet beetle larvae must sample minute quantities of imidazole-treated fibers in order to satisfy the need for food. Although fiber losses cannot be observed by the unaided eye, they are sufficient to indicate that enough was ingested to denote symptoms of a metabolic effect—which subsequently dulled the appetite and discouraged feeding. This was further evidenced by the limited amount of pelleted excrement, which, upon microscopic examination, revealed symptoms of catabolic wastes, as was observed by its negative response to the excitations of ultraviolet illumination at 3,650 Angstrom units. Fecal pellets excreted following normal food intake fluoresce brightly.

The following data illustrate the effects of imidazole on test insects with the reversal effects of histamine and three of its derivatives: Table 1 offers data on the effects of imidazole once it is within the system of the larvae of two species of carpet beetles, *Attagenus piceus* and *Anthrenus flavipes*. The method of testing was in accordance with modified C.S.M.A. standards of evaluation, and included a log of body weight losses. In this study, 25 larvae per test (No. 20 mesh size) were used rather than the 10 larvae customarily employed for these studies. Also included in the tables is a record of the body-weight losses of 25 larvae confined to empty C.S.M.A. cells devoid of any food supply. As carpet beetles are basically scavengers, cast skins of the larvae were removed daily from all cells in order to rule out the factor of sustenance obtained from this readily available by-product source of nutrient supply. All tests were compared with the body-weight gains of standard controls.

High performance

Imidazole's high degree of performance in the systems of fabric-feeding insects led to experiments with this compound on other species of insects, especially those also existing on a unidiet, such as cereals, and comparable dried food products. Owing to imidazole's low mammalian toxicity, it was considered sufficiently safe to be incorporated into foodstuffs on an experimental basis. Imidazole in a 1% aqueous solution was sprayed over spread cereals, allowed to dry, and then exposed to insect attack of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), the drugstore beetle, *Stegobium paniceum* (L.), the confused flour beetle, *Tribolium confusum* Jacquelin du Val, the yellow mealworm, *Tenebrio molitor* L., and the Indian-meal moth, *Plodia interpunctella* (Hubner). Appreciable mortality of the feeding insects, along with minimal feeding damage, was observed in contrast to that of the untreated controls. However, the tests were conducted on an empirical basis with no statistical data attempted as of this writing. The value of imidazole as a mosquito larvacide is promising. In three separate tests it has been shown that imidazole at 1 ppm resulted in 100% control of the larvae of *Culex pipiens fasciatus* Say, and 88% control with 0.5 ppm. According to the U. S. Fish and Wildlife Service, the LD_{50} (lethal dosage causing 50% deaths) of imidazole to small rainbow trout is approximately 12 ppm compared with other insecticides which are toxic in the range of 0.003 to 0.015 ppm. Additional work in this area continues.

The significant metabolic values of imidazole led to the supposition that the compound might perform equally effectively if it could be administered directly into the insect's system. The success of

EFFECTS OF IMIDAZOLE-TREATED TEST FABRIC AT 0.5% AQUEOUS, AND STARVATION, ON THE BODY WEIGHT OF TWO SPECIES OF CARPET BEETLE LARVAE[a]

Category	Weight (mg.)						Weight change (mg.)					
	Imidazole-treated		Starved (check)		Check (untreated)		Imidazole-treated		Starved (check)		Check (untreated)	
	A[b]	B[c]	A	B	A	B	A	B	A	B	A	B
Original weight	41.6	27.6	42.0	27.7	41.6	28.0	..	..	..	..	..	..
Weight after 3 days	39.7	24.1	40.8	23.8	42.1	25.3	−1.9	−3.5	−1.2	−3.9	+0.5	−2.7
Weight after 5 days	38.5	19.4	40.3	21.1	43.0	24.0	−3.1	−8.2	−1.7	−6.6	+1.4	−4.0
Weight after 7 days	35.9	17.9	38.3	18.9	44.7	25.5	−5.7	−9.7	−3.7	−8.8	+3.1	−2.5
Weight after 10 days	34.1	16.9	38.1	17.5	48.8	41.5	−7.5	−10.7	−3.9	−10.2	+7.2	+13.5
Weight after 14 days	28.6	15.2	37.1	16.4	51.0	49.6	−13.0	−12.4	−4.9	−11.3	+9.4	+21.6
Weight after 18 days	25.5	12.3	35.5	14.1	51.1	52.6	−16.1	−15.3	−6.5	−13.6	+10.5	+24.6
Total excrement weight yield (mg.)	2.2	1.3	1.7	2.2	58.8	69.0						
Mortality (%)	12	32	0	4	0	4						
Change from original weight (%)	−38.7[d]	−55.4[d]	−15.5	−49.1[d]	+25.2	+87.9[d]						

[a] A group of 25 larvae (No. 20-mesh size) was weighed and placed on imidazole-treated test fabric confined to standard 2″ × 2″ cells.
[b] A = Attagenus piceus. [c] B = Anthrenus flavipes. [d] Includes weight of dead insects.

such entry would be dependent upon a suitable liquid hydrocarbon carrier having high lipid-soluble characteristics, and whether the compound could be applied as a contact spray. A few of the standard base oil carriers used by pest control operators (deodorized kerosenes) were tested to determine their solubility values. Imidazole is not oil-soluble and requires a mutual solvent, such as oleic acid or isopropyl alcohol, before it can be completely dissolved in an oil carrier.

Contact effect

Imidazole at 1.5% in base oil was tested and found to have a definite contact effect on a number of species of insects and related arthropods. In order to quicken its knockdown time and improve effectiveness, a number of synergists were tried. Three revealed definite activity, although detracting somewhat from the safety of imidazole alone. These synergists are formaldehyde, phenol, and 2-Aminopyridine, the latter having some contact value of its own. Recently it has been learned that pyrethrins at 0.10% of actual, or as little as 0.03% when synergized with 0.15% of piperonyl butoxide, will, when added to imidazole, improve knockdown, while maintaining a low mammalian toxicity for the total formulation. New and more effective synergists, classified under patent continuations, are currently being employed to improve imidazole performance.

Base oil

Base oil is known to readily penetrate the lipid of an insect cuticle but lacks the high degree of solubility expected of it under these circumstances. Two adjuvants will contribute toward the efficacy of any insecticide designed for use in oil. The first of these is a hydrophilic surfactant which, when added to oil, noticeably improved its total effectiveness. Although the beneficial effect of a hydrophilic surfactant on oil has not been fully explained in a physical and/or chemical basis, it has been demonstrated in a physiological sense. Hydrophilic surfactants will not "wet" oil or recognizably improve upon its penetrating capabilities. Yet, once the lipid layer of an insect cuticle has been penetrated by the surfactant-treated oil, there is biological indication that the surfactant present will instantly combine with the first encountered aqueous content of the cuticle and then carry the active toxicant in all directions into and throughout the insect body.

Imidazole, 1.5%, synergized with 0.5% 2-Aminopyridine and combined with base oil, has been tested both with and without surfactants. Tests were conducted on adults of the German cockroach. The technique for this testing was to apply a single free drop of each formulation from the tip of a No. 30 hypodermic needle onto the folded wing covers of each cockroach at a point between the meso- and metathorax. The drop would then eventually work its way through the wings and into the thorax proper. Thirty cockroaches, 15 of each sex, were treated per test, with time recorded for knockdown.

Effectiveness

To date, imidazole when properly formulated has been found effective on an experimental basis against a long list of insects and related arthropods, including silverfish, cockroaches, termites, carabid beetles, bedbugs, fleas, flies, mosquitoes, ticks, spiders, and phytophagous mites. Included also are all fabric-feeding and many stored-products insects. Synergized imidazole at 0.75% aqueous with 0.5% agricultural spray oil added has killed many of the common plant insects including aphids, cicadas, mealybugs, scale insects (both soft and armored), thrips, including Cuban Laural thrips, and whiteflies. With very little phytotoxicity being observed at this early stage, considerable investigation lies ahead to understand fully the effectiveness of imidazole when specifically formulated for plant insects with careful consideration given to plant tolerances.

Residual effects

Imidazole lacks a long-lasting residual effect because it tends to evaporate into the atmosphere. It is therefore necessary that it be deposited in sufficient quantity to prolong its activity. The following information has been obtained from residual data accumulated as of this writing. Test procedure was to dissolve imidazole in acetone, allowing 2.5 ml. of the liquid to flow freely over glass surfaces, which are then set aside to dry. The actual imidazole quickly became plated over the surfaces in long crystals, at which time it was subjected to longevity studies to determine the lethal effects of the deposits on immature cockroaches that were forced to crawl over the treated surfaces. The tests indicated that 1% imidazole was active for 6 days; 2% imidazole, active for 9 days; 3% imidazole, active for 14 days; and 4% imidazole was active for 17 to 24 days.

The tendency of imidazole to lose its identity in the atmosphere is noted only when it is deposited onto hard surfaces. Once it has been integrated with wool fibers, its effectiveness is long-lasting. Bioassays of three-month-old imidazole-treated test fabric indicate that imidazole may remain active in wool for 11 months or longer. Detailed studies of the longevity of imidazole in wool are currently underway.

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