INSECT GROWTH REGULATORS

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Introduction

The term insect growth regulator (IGR) is used to describe compounds which interfere with insect metabolism in a manner which affects growth. The term is associated with the concept of third generation pesticides (Williams, 1967), implying rationally designed pesticides with highly selective action and having minimum effect on man, wildlife or environment. Most currently used IGR's have juvenile hormone activity or inhibit chitin synthesis. The current paper will consider insect growth regulators only in relation to their use in stored product protection.

Historical aspects

The existence of the juvenile hormone and its source in the corpora allata were known in the 1930's. Farnesal and farnesol were identified from the excreta of Tenebrio molitor Linnaeus in 1961. The compound which later became known as juvenile hormone III was synthesised in 1965 and the structure of juvenile hormone I itself was determined in 1967 (Roller et al., 1967). As is well known these discoveries then stimulated intensive efforts in the search for further compounds with juvenile hormone action (Henrick et al., 1973).

The activity of the benzoyl phenyl ureas in inhibiting chitin synthesis was reported in 1972 and numerous other compounds have since been identified which cause chitin inhibition.

Compounds showing anti-juvenile hormone activity have been reported since the 1970's and they constitute an active field of research recently reviewed by Staal (1986). Their diversity can be illustrated by citing the activity reported for fluoromevalonate (F Mev) (Quistad et al., 1981), imidazoles (Kuwano et al., 1983) and the precocenes.

Structures of representative IGR's are given in Figure 1.

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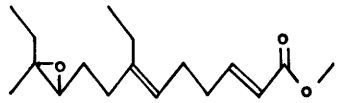
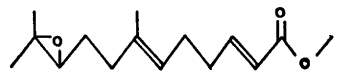
Fig. 1



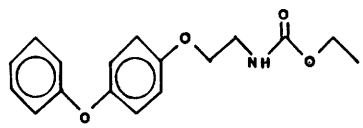
juvenile hormone I

juvenile hormone II

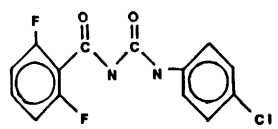


juvenile hormone III

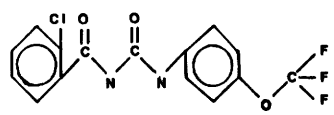
methoprene



fenoxycarb



diflubenzuron



alystin

Mode of Action

Juvenile hormones play a fundamental role in insect development. Perhaps the most obvious is their influence on morphogenesis of the integument and it is well known that exposure of many species to juvenile hormone analogues will result in extra larval, nymphal or pupal forms. These may vary from giant forms to a range of intermediates between immature and adult stages. Metamorphic defects impair sensory function, behaviour, and feeding. Many IGR's also interfere with embryonic development, especially when eggs are exposed at an early stage. In adults they may interfere with mating and other reproductive functions and may cause sterility.

Compounds with a range of structures are known to inhibit chitin synthesis but the most significant are the benzoyl phenyl ureas. Characteristically they act on immature forms particularly during ecdysis. Often the new skin remains attached to the old. Chitin inhibitors are usually also ovicidal.

Antijuvenile hormones accelerate termination of the immature stages. The definitive test for antijuvenile hormone activity is that the candidate compound should negate the action of a juvenile hormone when they are administered together.

Assaying IGR's

Typically, the response of test insects to IGR's is delayed and there is little or no response by the adult insects. Bioassays often require observation of F_1 or even F_2 generations for full expression of the response.

Testing of IGR's has involved the full range of bioassay techniques. Exposure of test insects by topical application or by treated substrate are the most common. Estimation of response has generally involved subjective description of symptoms produced or quantitative estimates of the size of succeeding generations.

Assays with IGR's are necessarily time-consuming and the resultant data tend to be more variable than in conventional assays. High control mortalities are sometimes encountered and use of a full maximum likelihood estimation of control mortality may be more appropriate than Abbott's formula (Finney, 1971). It is worth pointing out that the use of Wadley's Problem statistics (Finney, 1971) is usually appropriate to the analysis of F_1 progeny data.

IGR's are characterised by a high degree of specificity in the response of different species. Therefore, there seems to be no alternative but to evaluate the response of each important species. Current data have indicated the potential for resistance to IGR's, so the testing of several strains to represent the genetic background of each species is desirable.

Finally, since the future of IGR's is likely to be in integrated pest management systems it will be important that they be evaluated in actual storage systems.

Juvenile Hormone Analogues

Many juvenile hormone analogues have been evaluated against storage insects e.g. Thomas and Bhatnagar-Thomas (1968), Metwally and Landa (1972), Dyte (1972), Henrick *et al.*, (1973), Bhatnagar-Thomas (1973), Strong and Diekman (1973), Hoppe (1974), Staal (1975), Loschiavo (1975), McGregor and Kramer (1975), Marzke *et al.*, (1977), Silhacek *et al.*, (1976), Amos and Williams (1977), Kramer and McGregor (1978), Nickle (1979), Kramer et al., (1981), Mkhize and Gupta (1983) and Rup and Chopra (1984). These data will not be reported in detail but the discussion will focus on specific aspects which appear relevant to their future development and use.

Firstly, the more active compounds exhibit a potency comparable with that of conventional pesticides. They are active at application rates from 0.1 to 10 ppm. In general they are relatively ineffective against Sitophilus spp. and this is a major factor restricting their widespread use. These properties are well illustrated by considering

methoprene which is currently the most highly developed of the juvenile hormone analogues. A summary of data on its activity against Sitophilus spp. and Rhyzopertha dominica (Fabricius) is given in Table I.

TABLE I. REDUCTION IN F_1 PROGENY BY METHOPRENE APPLIED TO WHEAT

Author			Rhyzopertha	
	S. granarius			
Loschiavo 1976	10 ppm	100%	1 ppm	99-100%
Amos and Williams 1977	20 ppm	14%	1 ppm	99%
Edwards and Short 1984	100 ppm	nil		
	S. oryzae			
Strong and Diekman 1973	50 ppm	92%	5 ppm	100%
McGregor and Kramer 1975	10 ppm	13%	2 ppm	>99%
Loschiavo 1976	10 ppm	nil		
Amos and Williams 1977	20 ppm	12%		
Mian and Mulla 1982a	5 ppm	38%		
Mian and Mulla 1982b	10 ppm	81%	1 ppm	100%
Bengston (unpublished)	25 ppm	nil	0.125 ppm	100%
	S. zeamais			
Strong and Diekman 1973	50 ppm	46%		

Despite some discrepencies in the data it is clear that its use against Sitophilus spp. would require doses which are impracticably high. On the other hand it is highly potent in suppressing progeny of R. dominica. As will be discussed later, this is significant since it complements the activity of conventional organophosphorus compounds against other members of the pest complex typical of cereal grains. Data on the activity of methoprene against other species are not presented in detail but progeny production in the major pest species is suppressed at 10 ppm or less.

Other juvenile hormone analogues are more active against Sitophilus spp. For example, Kramer et al., (1981) and Edwards and Short (1984) tested S. granarius (Linnaeus), S. oryzae (Linnaeus) and S. zeamais (Motschulsky) and despite some variation in the data, an application rate of 10 ppm fenoxycarb gave at least 95% reduction in progeny. Nevertheless this seems unlikely to be a sufficient level of activity to permit its use against Sitophilus spp. in situations where complete control is required.

In some experiments juvenile hormone analogues were effective against Sitophilus spp. when incorporated into reconstituted wheat pellets (Kramer and McGregor, 1978). This suggests that the low

activity could be chiefly due to lack of penetration of the IGR's to the internal of the grain kernels. This is supported by reports of enhanced activity when surfactants are incorporated into standard formulations.

Chitin Inhibitors

Chitin inhibitors have also been studied against several storage insects e.g. Carter (1975), McGregor and Kramer (1976), Mian and Mulla (1982a). Several appear more active than juvenile hormone analogues against Sitophilus spp.

Carter (1975) reported that diflubenzuron 10 ppm on wheat completely reduced progeny production by Tribolium castaneum (Herbst) and Oryzaephilus surinamensis (Linnaeus) and gave 98% reduction in F_1 progeny of the most tolerant strains of S. granarius and S. oryzae. McGregor and Kramer (1976) obtained parallel results and extended them to demonstrate complete reduction in progeny for Tribolium confusum (Jacquelin du Val) and almost complete reduction for S. zeamais. Mian and Mulla (1982a) reported that both alystin and diflubenzuron were effective against eggs and early instars of O. surinamensis, T. castaneum and R. dominica and 5 ppm on wheat completely prevented the emergence of F_1 progeny of S. oryzae. In a further study Mian and Mulla (1982b) reported that 1 ppm of either compound completely prevented F_1 progeny of R. dominica for a year, while 5 ppm diflubenzuron gave 100% and 5 ppm alystin gave 95 to 99% reduction in F_1 progeny of S. oryzae. Other data suggest 20 ppm may be necessary to achieve this result with Australian strains of S. oryzae (Bengston unpublished). Determination of the minimum effective dose of these compounds against typical strains remains an important task.

Kramer and McGregor (1979) evaluated a range of benzamid chitin inhibitors and established that the most active material, N-[4-(4-nitrophenoxy)-3,5 dichlorophenylaminocarbonyl]-2-chlorbenzamide was generally as effective as diflubenzuron against Coleoptera. It was much more active against Ephestia cautella (Walker), Plodia interpunctella (Hubner), and Sitotroga cerealella (Olivier).

Some data e.g. Mian and Mulla (1982a,b) suggest that the efficacy of chitin inhibitors applied to grain may increase somewhat with time of storage, possibly due to redistribution of residues.

Current Application of IGR's in Stored Product Protection

The most significant current use of IGR's in stored product protection is the application of methoprene 10 mg kg^{-1} for protection of stored tobacco chiefly against Lasioderma serricorne (Fabricius) and Ephestia elutella (Hubner). The treatment is effective for two years storage provided that even distribution of the methoprene is achieved using minimum quantity of liquid and this generally necessitates special spray equipment (Manzelli, 1979). The treatment is registered or cleared for use in 29 countries and has replaced phosphine fumigation in some areas.

In USA, Bulgaria and UK the use of methoprene is agreed as a residual spray in food and feed handling establishments. In USA it is

agreed for use on stored agricultural commodities against major pest species but not including Sitophilus spp. Temporary tolerances for residues have been established on cereal grains, beans (dry), nuts, apples, apricots, raisins, peaches, pears, cocoa, coffee, spices, peas (dried), corn grits, hominy, macaroni and prunes.

In Australia provisional maximum residue limits have been agreed for cereal grains and derived products to permit pilot usage on 10 000t of stored grain.

Internationally, maximum residue limits appropriate for use of methoprene on cereal grains have been agreed by the Codex Alimentarius Commission at Step III of its procedure.

Combinations of IGR's with Conventional Pesticides

Extensive studies are nearing completion in Australia on the use of combinations of IGR's with conventional organophosphorus pesticides for the protection of stored cereals. The programme is carried out under the auspices of the Australian Wheat Board's Working Party on Grain Protectants. It involves researchers from the State and Federal Departments of Agriculture and CSIRO and work is undertaken in the storage facilities of the state Grain Handling Authorities. Specifically the programme has involved the combination of methoprene 1 ppm with fenitrothion 12 ppm on wheat stored in unaerated silos for 9 months. The combination has provided effective protection against representative strains of the major pest species i.e. S. oryzae, S. granarius, R. dominica, T. castaneum, T. confusum, O. surinamensis and E. cautella.

Evaluation of a similar combination is planned under the auspices of the Australian Centre of International Agricultural Research (ACIAR) in cooperation with researchers in Malaysia, Philippines and Thailand. This programme will involve rice and maize.

Residues of IGR's

The behaviour of residues of methoprene are the best documented of the IGR's. The compound is rapidly degraded by sunlight and in plants, animals and soils but in storage methoprene is highly persistent. Mian and Mulla (1983) determined losses from 61 to 66% of methoprene residues on wheat of 13.5% moisture at 27^{0}C stored over a year. One contrasting result was obtained using radiolabelled methoprene and indicated a half life of six weeks at 20^{0}C on wheat of 12% moisture content (Rowlands, 1976). Possibly vapour losses or losses to the container were significant with the small sample of maize.

Several studies have resulted in residues which were unexpectedly low (50% of intended value) when determined analytically immediately after treatment. It is not clear whether this is due to losses during application or during subsequent sampling or analysis. Nevertheless the evidence suggests considerable persistence. Unpublished data on residues sampled from a 500 t silo of treated wheat in Australia are given in Table II (Bengston et al., unpublished).

TABLE II. RESIDUES OF METHOPRENE (PPM) IN WHEAT STORED IN UNAERATED VERTICAL CONCRETE BINS PRIOR TO ASSAY

Nominal Treatment	Site and Storage Conditions	Calculated Application Rate	Laboratory	Approximate time after spray application (months)					
				0	1½	3	4½	6	9
Methoprene 1 ppm	Queensland								
	Malu, 12% moisture, 31 to 21°C	1.1	a	0.61	0.57	0.57	0.54	0.52	0.61
			b	0.74	0.58	0.44	0.45	0.53	0.40
Methoprene 1 ppm	New South Wales								
	Gooloogong, 11%, 28 to 23°C	1.0	a	1.0	1.0	1.0	1.0	0.78	-
			b	1.2	1.1	0.74	0.72	-	-

(a) Agricultural Chemistry Branch, Queensland Department of Primary Industries

(b) Australian Wheat Board

Data on resulting milling and baking fractions are given in Table III.

TABLE III. RESIDUES (PPM) OF METHOPRENE IN MILLED PRODUCTS IN QUEENSLAND FROM WHEAT TREATED AT 1 PPM 9 MONTHS PRIOR TO PROCESSING

Sample Analysed	Methoprene Residue[a]	Methoprene (moisture free basis)
Wheat	0.61	0.69
Bran	1.35	1.53
Pollard	2.33	2.59
Wholemeal Flour	0.53	0.60
White flour	0.17	0.19
Wholemeal Bread	0.34	0.51
White Bread	0.11	0.16

a - Agricultural Chemistry Branch, Department of Primary Industries.

As with many conventional pesticides, the residues are concentrated in the bran and are reduced in the flour. When residues are considered on a moisture free basis it is apparent there was little if any loss during the baking of bread.

Few detailed data are available for other compounds but available evidence suggests they are likely to be persistent. This has been established analytically for alystin and diflubenzuron on wheat (Mian and Mulla, 1983) and is inferred from prolonged biological activity of several of the other compounds e.g. fenoxycarb (Kramer et al., 1981). They are also likely to concentrate in the bran fraction.

Toxicology of IGR's

Many IGR compounds have low acute toxicity. This is often the major factor favouring their development as pesticides for stored commodities. Data on some of the more prominent compounds are summarised in Table IV.

However, most health authorities (including the Codex Alimentarius Commission) require long term feeding studies (2 years) before maximum residue limits are set for major dietary commodities. Completion and evaluation of the results of such studies are still in progress.

TABLE IV. ACUTE TOXICITY OF IGR'S

Compound	LD_{50}[a]
alystin	> 5 000
diflubenzuron	4 640
fenoxycarb	16 800
hydroprene	34 000
methoprene	>50 000

[a] Acute oral toxicity for rats.

Resistance to IGR's

The idea that insects would not develop resistance to insect hormones (Williams 1967) was quickly refuted. A low level of cross-resistance to JHI was shown in a strain of T. castaneum with resistance to numerous pesticides including organophosphorus compounds (Dyte, 1972) and similar results were obtained for JHII (Silhacek et al., 1976). In T. confusum, selection for 11 generations induced 4X resistance to methoprene (Brown et al., 1978). For P. interpunctella, a malathion resistant strain was cross-resistant to JHII (Silhacek et al., 1976). No cross-resistance was shown in Australian strains of T. castaneum tested against 11 juvenile hormone analogues (Amos et al., 1974, 1977) and Hoppe (1976) reported both positive and negative cross-resistance.

In regard to chitin inhibitors, Carter (1975) reported negative cross-resistance for diflubenzuron to organophosphorus resistant strains in S. oryzae and positive and negative cross resistance in T. castaneum. Selection of T. confusum for 8 generations resulted in 2X resistance to diflubenzuron (Brown et al., 1978).

In summary the current data indicate the potential for the development of strains resistant to IGR's, but given the limited use to date, there have been no reports of field failures.

Future of IGR's

Use of IGR's in stored product protection is constrained by the specificity in response of different pest species and by the survival of adults. Nevertheless their favourable toxicology and their mode of action independent of conventional pesticides are major advantages.

Current uses of IGR's will increase somewhat as the suitability of juvenile hormone analogues for particular purposes is increased. Current uses will also expand as further detailed information on both the residue levels for commodities under different conditions and on

basic toxicology of the compounds become available and are accepted by appropriate authorities.

However, major expansion of use of IGR's will require an integrated pest management approach to complement the strengths and weaknesses in the action of IGR's. Use of current juvenile hormone analogues alone will be restricted to storage situations in which Sitophilus spp. are not important. The combination of juvenile hormone analogues with conventional organophosphorus compounds will be developed for use in general storage situations and especially for stored cereal grains. The properties of chitin inhibitors will be further explored and their suitability for the control of Sitophilus spp. will be determined. In situations where the continued presence of live adults is unacceptable e.g. cereal grain exported from Australia, the use of IGR's will be integrated with appropriate stock management practices and with use of fumigation or other rapid disinfestation techniques.

The speed at which IGR's are introduced will depend on the development of resistance to conventional pesticides and on the acceptability of their residues and on competitive pricing. Given the inevitable delays in the acceptance and implementation of new systems it would be irresponsible for researchers not to develop and demonstrate such systems forthwith.

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