

COMPARATIVE EFFECTIVENESS OF INSECT GROWTH REGULATORS WITH JUVENILE HORMONE, ANTI-JUVENILE HORMONE AND CHITIN SYNTHESIS INHIBITING ACTIVITY AGAINST SEVERAL STORED FOOD INSECT PESTS.

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Abstract - Several IGRs, which disrupt important functions in insects, were tested for their insecticidal activities on stored food insects. We have applied JH-analogues (methoprene and KA1488), anti-JH-analogues (β -asarone, azadirachtin and J2710) and a chitin synthesis inhibitor (buprofezin, to several stored food insect species; Tribolium confusum, T. castaneum, Ephestia kuehniella, Sitophilus granarius, S. oryzae, S. zeamais and Oryzaephilus surinamensis. Concentrations ranging from 0.1 to 1000 ppm (except for β -asarone where 10 to 300 μ l/L volume was used) of the products were admixed with appropriate amounts of food medium to which the insects were exposed. Assessment of treatment was based on the effects of the IGRs on the P- and F1-progeny. 3 compounds affected the parental adults. All the compounds, except for methoprene (S. zeamais and S. granarius) and buprofezin (S. granarius), acted on the F1 progeny. KA1488 and azadirachtin were found to be the most effective compounds. KA1488 inhibited completely the development of the F1 progeny (no larvae, pupae or adults) at a concentration as low as 0.1 ppm for O. surinamensis, 5 ppm for T. confusum (the same result was obtained for azadirachtin) and S. granarius. No F1 progeny was found at an azadirachtin concentration of 1 ppm, 50 ppm for S. zeamais and 100 ppm for T. castaneum.

INTRODUCTION

The use of fumigants and conventional organic insecticides to control insect pests of stored products has given rise to problems of residual toxicity and development of resistant strains of insects. These problems have enhanced the need to develop more effective and relatively safer insecticides. Insect growth regulators (IGRs) have several characteristics that make them potentially successful alternatives to standard pesticides. In addition to their rapid degradation in the environment (Quistad *et al*, 1974; Staal, 1975; Zurflueh, 1976) and generally low toxicity to nontarget species (Siddall and Slade, 1971; Staal, 1975; Hoppe, 1976; Loschiavo, 1976; Siddall, 1976; Oberlander *et al*, 1978), IGRs are effective against some strains resistant to conventional insecticides (Amos *et al*, 1974; Silhacek *et al*, 1976). Some have a very high biological activity (Slama *et al*, 1974) and may potentially be integrated in other pest management techniques (IPM) such as biological control (Wright and Spades, 1972).

Substantial data are available in the literature which support the claim of effectiveness of these compounds against a variety of stored product insects. We only mention those of the last 5 years (Bengston, 1987; Burkholder, 1987; Cogburn, 1988; Edwards and Abraham, 1985; Jilani et al, 1988; Kramer et al, 1985; Mkhize, 1986a,b; Pisarev, 1987; Risha, 1986; Shaaya and Pisarev, 1986; Smet et al, 1986; 1987a,b; 1989; Snelson, 1987; Srinivas and Rao, 1986; Vick et al, 1985; White, 1987).

IGRs include compounds that may affect moulting and metamorphosis by mimicking (juvenile hormone analogues) or antagonizing (anti-juvenile hormone compounds) JH-activity or by interfering with cuticle formation (chitin synthesis inhibitors).

The aim of this study was to assess the effectiveness of 6 IGRs with JH (methoprene and KA1488), anti-JH (azadirachtin, β -asarone and J2710) and chitin synthesis inhibiting activity (buprofezin) against 7 species of economically important stored product insects. To determine the potential of these compounds as pest control agents, tests were carried out either by exposing the insects to treated food (for methoprene, KA1488, azadirachtin, J2710 and buprofezin) or, where the possible use as a fumigant had to be evaluated, like for β -asarone, insects were exposed to treated air.

MATERIALS AND METHODS

Insects

T. confusum and T. castaneum were reared on a culture medium composed of 91% whole wheat flour and 9% dried beer yeast. E. kuehniella was raised on a culture medium composed of whole wheat flour, powdered yeast and glycerine in the ratio (w/w) of 10/1/2. O. surinamensis beetles were reared on rolled oats, whole wheat flour and dried beer yeast (5/5/1, w/w). S. granarius and S. oryzae were reared on whole wheat and S. zeamais on whole corn. T. confusum, S. oryzae and S. zeamais were held at 30°C \pm 2 and 40 r.h. \pm 5%. T. castaneum, S. granarius, E. kuehniella and O. surinamensis were kept at 25°C \pm 2 and 40 r.h. \pm 5%.

All the cultures originated from laboratory cultures and were maintained in 1-litre glass jars with gauze.

Products

The compounds used were :

Methoprene (isopropyl-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), 90% pure, supplied by Zoecon Corporation, Palo Alto, California, U.S.A.

KA1488 (CGA045128 : N-ethylcarbamic acid 2-(4-phenoxy) ethylester) supplied by Ciba-Geigy, Basel, Switzerland
 β -asarone (2,4,5-trimethoxy-1-propenylbenzene), 70% cis- β -form, 30% trans- α -form, was purchased from Janssen Pharmaceutica N.V., Beerse, Belgium.

Azadirachtin, 85% pure, was extracted by Trifolio-M GmbH, Lahnau, West-Germany.

J2710 (5-methoxy-6-(1-(4-methoxyphenyl)ethyl)-1,3-benzo-dioxole was synthesized by Dr. Jurd, U.S.D.A, Berkely, California, U.S.A.

Buprofezin (2-tert-butylimino-3-isopropyl-5-phenyl,3,4,5,6 tetra-hydro-2-thiadiazine-4-one, Applaud, FD4076) was provided by Ir. Van de Veire (Fac. of Agriculture Sciences, R.U.Gent, Gent, Belgium) under form of a 25% Wettable Powder.

Treatments

Stock solutions of methoprene, KA1488, azadirachtin and J2710 were prepared in acetone. Buprofezin (25% WP) dilutions were made with water. Appropriate amounts of the compounds to be tested were mixed with the food medium of the target insect so that concentrations ranging from 0.1 to 1000 ppm were obtained. Food treated with acetone and water (for buprofezin) was used as a control. The food was left to dry to remove the acetone and the water. Care was taken that all acetone and water had evaporated before insects were added to the treated food. Table I shows a list of which products have been used against which insect and in what conditions. In general, 20, 30 or 50 newly emerged, unsexed adults or 1 week old larvae (P-generation) of each species were collected at random and placed on the media of 25g, 100g or 200g. After a period of 2 or 3 weeks in which the insects were allowed to oviposit their eggs, the adult parental insects were removed. After an incubation period, emerging F1 adults (first-generation) were removed and counted twice a week until emergence was complete. The course of larval development was followed for *T. confusum*, *T. castaneum*, *E. kuehniella* and *O. surinamensis*. Since the larvae of *S. granarius*, *S. oryzae* and *S. zeamais* develop inside grain kernels only the number of live adults found externally was recorded.

To test the effects of β -asarone vapours on the mortality of *T. confusum*, *S. oryzae* and *E. kuehniella* the product was applied on paper strips (concentrations of 0, 10, 100 and 300 μ l/L volume) (4 replicates/concentration) which were suspended under the covers of glass jars in which the test insects remained. Mortality was monitored daily and the day at which 50% mortality occurred was recorded.

For monitoring effects on reproduction, newly emerged *S. oryzae*, *E. kuehniella* and *T. confusum* adults (P-generation) were transferred to jars with the suitable culture media. β -asarone was applied on strips of paper (concentrations of 0, 10, 100, 200 and 300 μ l/L volume) which were suspended in glass jars, where target insects were introduced. Half of the jars were closed with a glass lid and rubber fittings. The other half was only covered with a glass lid for providing enough air. The development of the 1st generation was subsequently followed.

Table I : Conditions in which the experiments were performed.

Explanation of the terms used; **mort**: mortality; **fert**: fertility; **P-generation**: amount of insects from the parental generation (adults or larvae) which were used per jar or petri-dish; **Food**: amount of food used per jar or petri-dish; **P-time**: time the parental generation was exposed to the treated medium; **Replicates**: how many times the same concentration was tested; **+**: closed with rubber fittings; **-**: ventilated; **Conditions**: environment (temperature and relative humidity) in which the insects were held in during the experiment; **Concentrations**: different concentrations which were used.

Insect Product	T. confusum							
	β-asarone (mort)	β-asarone (fert)	azadirachtin	methoprene	KA1488	J2710	J2710	
P-generation	20 adults	20 adults	20 adults	20 adults	20 adults	20 adults	20 adults	40 larvae
Food	None	25 g	25 g	25 g	25 g	25 g	25 g	25 g
P-time	Continuous	Continuous	2 weeks	2 weeks	2 weeks	2 weeks	2 weeks	Continuous
Replicates	4 times	3 times (3+/3-)	1 time	4 times	4 times	3 times	3 times	3 times
Conditions	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5
Concentrations	10/100/300 µl/L	10/100/200/ 300 µl/L	0.1/1/5/10/ 50/100 ppm	0.1/1/5/10/50/1 00/1000 ppm	0.1/1/5/10/ 50/100 ppm	10/100/ 1000 ppm	10/100/ 1000 ppm	10/100/ 1000 ppm
Insect Product	H. haemulidella				S. granarius			
	β-asarone (mort)	β-asarone (fert)	methoprene	KA1488	azadirachtin	methoprene	KA1488	buprofezin
P-generation	20 adults	20 adults	20 adults	20 adults	20 adults	50 adults	50 adults	20 adults
Food	None	100 g	200 g	200 g	200 g	200 g	200 g	200 g
P-time	Continuous	Continuous	2 weeks	2 weeks	3 weeks	3 weeks	3 weeks	3 weeks
Replicates	4 times	4 times (4+)	4 times	4 times	4 times	4 times	4 times	4 times
Conditions	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	30°C ± 2/65% ± 5	30°C ± 2/65% ± 5	30°C ± 2/65% ± 5	30°C ± 2/65% ± 5
Concentrations	10/100/300 µl/L	10/100/300 µl/L	0.1/1/5/10/ 50/100 ppm	0.1/1/5/10/ 50/100 ppm	0.1/1/5/10 ppm	0.1/1/5/10/ 50/100 ppm	0.1/1/5/10/ 50/100 ppm	0.1/1/5/10/ 50/100 ppm
Insect Product	S. oryzae		S. zeamais		T. castaneum		O. surinamensis	
	β-asarone (mort)	β-asarone (fert)	methoprene	KA1488	methoprene	KA1488	methoprene	KA1488
P-generation	20 adults	30 adults	50 adults	50 adults	20 adults	20 adults	20 adults	20 adults
Food	None	25 g	200 g	200 g	25 g	25 g	100 g	100 g
P-time	Continuous	Continuous	3 weeks	3 weeks	2 weeks	2 weeks	3 weeks	2 weeks
Replicates	4 times	3 times (3+/3-)	4 times	4 times	4 times	4 times	4 times	2 times
Conditions	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	30°C ± 2/65% ± 5	30°C ± 2/65% ± 5	25°C ± 2/50% ± 5	25°C ± 2/50% ± 5	30°C ± 2/65% ± 5	30°C ± 2/65% ± 5
Concentrations	10/100/300 µl/L	10/100/200/ 300 µl/L	0.1/1/5/10/ 50/100 ppm	0.1/1/5/10/ 50/100 ppm	0.1/1/5/10/ 50/100 ppm	0.1/1/5/10/ 50/100 ppm	0.1/1/5/10/ 50/100 ppm	0.1/1/5/10/ 50/100 ppm

RESULTS

Table II shows the external morphology of the P- or F₁-generation exposed to IGR-treated food or atmosphere (in different concentrations) and the mean number of adults obtained at the end of the F₁-generation or mean number of days at which 50% mortality occurred for β -asarone.

KA1488, azadirachtin and β -asarone showed acute toxicity to the parental adults of 4 different insect species. There was a significant difference ($P < 5\%$, t-test) between the parental toxicity of the control and 10 ppm azadirachtin or higher on S. granarius, and 5 ppm KA1488 or higher on S. granarius. There was also a significant difference (2 way analysis of variance, $P < 0.1\%$) between the parental mortality of the control and 10 $\mu\text{l/L}$ of β -asarone or higher on T. confusum, E. kuehniella and S. oryzae.

There were pronounced differences among the tested compounds on the F₁-generation of the different species :

Tribolium :

The JH-analogues, KA1488 and methoprene, have a stronger insecticidal effect on T. confusum than on T. castaneum. No F₁ T. confusum generation was found at a concentration of 5 ppm of KA1488 and higher. T. castaneum was only controlled by KA1488 at a concentration \geq or equal to 100 ppm. Methoprene could not prevent, unlike KA1488, the production of an F₁ larval generation.

E. kuehniella :

Methoprene and KA1488 suppressed adult emergence of E. kuehniella from a concentration of 50 ppm and 10 ppm respectively on. None of the compounds (methoprene, KA1488 and β -asarone) were able to prevent the development of the larval F₁-generation. Permanent larvae were observed which started to spin a cocoon but never pupated. When these larvae were transferred to untreated food medium, they developed further into adults still able to reproduce. So, there is not much hope for these IGRs to be used against E. kuehniella.

Sitophilus :

The genus Sitophilus is especially important to test because, unlike the other insects, it is less susceptible to IGR treatment (Mkhize, 1986a;b). S. granarius, S. oryzae and S. zeamais were used in the tests. From 5 ppm of KA1488 and 1 ppm of azadirachtin on, no first generation of S. granarius adults were observed. S. zeamais was controlled by KA1488 at 50 ppm and higher. Methoprene and buprofezin exerted no effect whatsoever on S. granarius and S. zeamais. β -asarone could not prevent F₁ adult emergence of S. oryzae but there was a significant difference ($P < 5\%$, t-test) between the adult emergence of the control and the other concentrations tested.

Oryzaephilus :

Methoprene only prevented F₁ development at a concentration \geq or equal to 50 ppm, where KA1488 was able to control surinamensis from 0.1 ppm on.

The effects obtained with the IGRs, KA1488, methoprene and azadirachtin on T. confusum, E. kuehniella, T. castaneum and O. surinamensis are qualitative not substantially different from those obtained with other IGRs : supernumerary larvae,

Table II : External morphology of larvae, pupae, adults, adultoids and numbers of adults of the different P- or F₁-generations exposed to food treated with different concentrations of IGRs.

Explanation of the abbreviations; **fert** : fertility; **lpa** : morphology of l(arvae), p(upae) and a(dults) completely normal. The numbers between brackets represent the mean number of adult insects obtained at the end of the F₁-generation; **L** : only supernumerary larvae were found; **A** : abnormalities, this means pupal-adult intermediates or adultoids : anterior part of the body have adult shape and pigmentation with crumpled and greatly diverging fore- and hindwings, whereas the abdomen was typically pupal and unpigmented; **D** : dead, black larvae; **Y** : yellow, dried larvae; **P** : dead pupae; **0** : no first generation offspring; ***** : there is a significant difference between the number of first generation adults from the given concentration and that of the control group; **□** : there is a significant difference between the day at which 50% mortality of the parental adults occurred from the given concentration and that of the control group; **closed** : closed with rubber fittings.

Insect	Methoprene						J2710 (adults)	J2710 (larvae)
	<i>T. confusum</i>	<i>E. kuehniella</i>	<i>S. granarius</i>	<i>S. zeamais</i>	<i>T. castaneum</i>	<i>O. surinamensis</i>	<i>T. confusum</i>	<i>T. confusum</i>
control	lpa(224)	lpa(1174)	819	353	lpa(325)	lpa(1233)	lpa(159)	lpa(36)
0.1 ppm	lpa(246)	lpa(970*)	719	341	lpaA(149*)	lpPa(729*)		
1 ppm	lpAa(259)	lYLpa(616*)	728	370	lLPpA	lpPaA(250*)		
5 ppm	lpAa(5*)	lDYlpa(128*)	1220	456	L	lP	lpa(164)	lpa(34)
10 ppm	lpA	lYLpa(9*)	1017	424	L	L		
50 ppm	L	DYL	1035	372	L	0	lpa(180)	lpa(36)
100 ppm	L	YL	769	370	L	0		
1000 ppm	L						lpa(22*)	lpa(26*)
Product Insect	KA1488						azadirachtin	<i>T. confusum</i>
	<i>T. confusum</i>	<i>E. kuehniella</i>	<i>S. granarius</i>	<i>S. zeamais</i>	<i>T. castaneum</i>	<i>O. surinamensis</i>	<i>S. granarius</i>	<i>T. confusum</i>
control	lpa(137)	lpa(1145)	527	508	lpa(410)	lpa(76)	79	lpa(72)
0.1 ppm	lpA	lDPpa(307*)	237*	288	lLPpA(6*)	0	54	lpaA(87)
1 ppm	L	lDPpa(150*)	32*	81*	L	0	0	lLPa(20)
5 ppm	0	lDYpPa(4*)	0	28*	L	0	0	0
10 ppm	0	DY	0	6*	L	0	0	0
50 ppm	0	DYL	0	0	L	0	0	0
100 ppm	0	DYL	0	0	0	0	0	0
Product Insect	β-asarone (mort)			β-asarone (fert)			buprofezin	
	<i>T. confusum</i>	<i>E. kuehniella</i>	<i>S. oryzae</i>	<i>T. confusum</i>	<i>E. kuehniella</i>	<i>S. oryzae</i>	<i>S. granarius</i>	
control	50% mortality	50% mortality	50% mortality	closed	ventilated	closed	closed	ventilated
10 µL	day 21.7	day 6.8	day 6.5	lp(0)	lpa(675)	lpa(15)	0	476
100 µL	day 18.3□	day 5.6□	day 3.5□	lpa(466*)	lpa(463)	lpa(118*)	44*	60*
200 µL	day 17.6□	day 4.5□	day 3.3□	lpa(116*)	lpa(140*)	lpa(122*)	35*	10*
300 µL	day 16.2□	day 3.7□	day 2.4□	lpa(115*)	lpa(140*)		13*	25*
				lpa(191*)	lpa(139*)	lpa(97*)	86*	35*
Concentrations							control	239
							0.1 ppm	126
							1 ppm	267
							5 ppm	408
							10 ppm	405
							50 ppm	254
							100 ppm	368

adultoids (pupal-adult intermediates) or inhibition of embryonic development and/or eclosion.

A special mention should be made about the effect of β -asarone on T. confusum, E. kuehniella and S. oryzae fertility. In the hermetically closed jars of the control we got, as expected, a drastic reduction of the F₁ adult generation, whereas to our surprise in the β -asarone treated hermetically closed jars we did not.

CONCLUSIONS

Our results show that development of economically important pests of stored products can be affected to some extent by most of the IGRs used in our study.

E. kuehniella can't be controlled efficiently by any of the used IGRs.

The efficacy as control agent of methoprene, buprofezin, J2710 and β -asarone is substantially lower than that of KA1488 and azadirachtin.

Methoprene prevented larval development in only one insect, namely O. surinamensis and this at a concentration \geq or equal to 50 ppm. In S. granarius and S. zeamais it could not prevent F₁ adult emergence. In the other tested species methoprene prolonged larval life by inhibiting the transition of the pupal stage.

Buprofezin had no effect whatsoever on S. granarius.

J2710 only exerted an effect at an economically unimportant dose \geq or equal to 1000 ppm.

β -asarone vapours affected the reproduction and mortality of Tribolium, Ephestia and Sitophilus but not in a satisfactory way. According to a recent article of Streyloke et al (1989) the insecticidal activity on stored-product pests of the gaseous phase of β -asarone is strongly temperature-dependent. At 25°C (used in our experiments) and at a concentration of 10 μ l/400 ml Risha (1986) found a very low mortality (6%) for Sitophilus. At 30°C the mortality was raised to 79%. The insecticidal activity suddenly drops when working conditions change from 30°C to 25°C. If we would have performed our experiments at 30°C we probably would have encountered more positive results.

Two articles from Abel (1986;1987) describe the chromosome damaging effects of β -asarone on human lymphocytes. So, using β -asarone as a fumigant in food commodities would become very hazardous and any further research with β -asarone would therefore be inconvenient.

In our opinion KA1488 is a good candidate for control of T. confusum, S. granarius and O. surinamensis. KA1488 inhibited completely the development of the F₁-generation (no larvae, pupae or adults) at a concentration of 5 ppm for T. confusum and S. granarius and 0.1 ppm for O. surinamensis. Azadirachtin controlled S. granarius and T. confusum at a concentration of 5 ppm and 10 ppm respectively and therefore also has a potential as a grain protectant.

From the physiological point of view, the observation that β -asarone seems to counteract the damaging effects of hermetically closing the glass jars in which the insects were raised, is very interesting.

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**EFFICACITE COMPAREE DE REGULATEURS DE CROISSANCE DES INSECTES
(HORMONE JUVENILE, HORMONE ANTI-JUVENILE ET INHIBITEUR DE LA
CHITINE) SUR PLUSIEURS INSECTES DES DENREES STOCKEES**

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RESUME

Il existe un besoin urgent de moyens alternatifs de lutte contre les insectes ravageurs attaquant les denrées stockées, besoin causé par la multiplication des problèmes de résistance aux pesticides et des restrictions limitant le taux des résidus insecticides dans la nourriture. La très basse toxicité des régulateurs de croissance (IGRs) sur les vertébrés rend leur emploi particulièrement intéressant contre les insectes ravageurs de produits stockés, tout spécialement s'il s'agit de denrées destinées à la consommation humaine.

Plusieurs IGRs, interrompant certaines fonctions importantes chez l'insecte, ont été étudiés du point de vue de leur action insecticide. Nous avons appliqué des analogues l'hormone juvénile (méthoprène et KA 1488), des analogues anti-HJ (β -asarone, azadirachtine et J2710) à un inhibiteur de la synthèse de la chitine (buprofézine) à différentes espèces d'insectes : *Tribolium confusum*, *T. castaneum*, *Ephestia kuehniella*, *Sitophilus granarius*, *S. zeamais* et *Oryzaephilus surinamensis*. Les concentrations allant de 0,1 à 1.000 ppm (sauf pour la β -asarone dont on a utilisé des concentrations de 10 à 300 μ l/L), ont été mélangées aux aliments du milieu dans lequel vit l'insecte. La mesure de l'efficacité du traitement est basée sur la toxicité immédiate et retardée des IGRs, ainsi que sur leur action sur la génération F1. Aucun composé n'a affecté les adultes. Tous, sauf le méthoprène, (*S. zeamais* et *Sitophilus granarius*) le buprofézine (*Sitophilus granarius*) et la β -arazon (*S. orysae*) ont agi sur la génération F1. KA1488 et l'azadirachtine se sont avérés les composés les plus efficaces. KA1488 a complètement inhibé le développement de la descendance F1 (aucune larve ni nymphe ni adulte) à la concentration de 0,1 ppm pour *O. surinamensis*, 5 ppm pour *T. confusum* (le même résultat a été obtenu avec l'azadirachtine) et *S. granarius* (aucune descendance F1 à la concentration de 1 ppm d'azadirachtine et plus), 50 ppm pour *S. zeamais* et 100 ppm pour *T. castaneum*.