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Juvenile hormone-like activity of (E)- β -farnesene derivatives

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SUMMARY

(E)- β -farnesene, the aphid alarm pheromone, or derivatives were postulated as crop protection agents. Because of their structural similarity to the juvenile hormone precursors their juvenilizing activities were tested on different species. Two groups of derivatives were distinguished : *a*) the compounds with an high juvenilizing activity ; these compounds undergo a reversible reaction and regenerate farnesene ; *b*) the compounds without any juvenilizing activity are stable and do not regenerate farnesene. With these stable derivatives the risk of obtaining supernumerary larvae that could cause more damage to the crops is eliminated.

Additional key words : *Aphid alarm pheromone, (E)- β -farnesene, juvenile hormone analogues, crop protection.*

RÉSUMÉ

Activité mimétique de l'hormone juvénile des dérivés du β -farnesene.

Le (E)- β -farnésène, la phéromone d'alarme des pucerons, et ses dérivés ont été préconisés comme agents pour la protection des cultures. La similitude de structure avec les précurseurs des hormones juvéniles nous a incité à évaluer les propriétés juvénilisantes de ces composés. Deux catégories de substances ont été distinguées : *a*) les composés à forte activité qui sont ceux capables de régénérer le farnésène ; *b*) les composés stables sans activité juvénilisante incapables de régénérer le farnésène. L'utilisation de ces derniers permet d'éliminer le risque d'induire la formation de larves surnuméraires susceptibles de produire plus de dégâts sur les cultures.

Mots clés additionnels : *Phéromone d'alarme des pucerons, (E)- β -farnésène, analogues d'hormones juvéniles, protection des cultures.*

I. INTRODUCTION

Intensive crop production techniques, necessary for high quality and yield, provide favourable conditions for insect pests and lead to extensive use of chemical pesticides. However, pesticide resistance and the risk of environmental pollution require the development of alternative strategies such as use of the defense mechanisms evolved by plant themselves. Although it was originally thought that plants could not respond to attack by phytophagous insects, it is now known that insect-plant relationships are complex and are mediated by substances called allelochemicals (WHITTAKER & FEENY, 1971). Allelochemicals play a major part in the equilibrium between plants and insects and may have contributed to a co-evolution between the chemical defense mechanisms of plants and the adaptive responses of insects (PESSON, 1980). These chemicals include insect-induced chemical signals that regulate secondary plant responses (RYAN, 1983), and others

that are naturally present in plants. Much information about these chemical regulators has been gained over the past 10 years, and has contributed to new concepts of crop protection against insects.

SLAMA (1969) reported that plants can contain material with insect hormone activity. Juvabione, which induces juvenilization of *Pyrrhocoris apterus* L. bugs, was isolated from balsam fir (BOWERS *et al.*, 1966), and this discovery led to the use of chemicals with insect juvenile hormone activity as pesticides (WILLIAMS, 1967). Many other juvenile hormone mimics have now been synthesized and tested as pesticides and some have been developed commercially. Chemicals interfering with feeding behaviour are also of interest. Since this behaviour is controlled by olfactory and gustatory mechanisms, it might be possible to modify chemically the stimuli involved in food choice. If sprayed onto crops such chemical's could keep away insects which would then die of starvation.

Chemicals involved in communication between

members of same species are called pheromones. Insect sex pheromones have received most attention. Many have now been identified, and the synthetic materials are being used in the field as crop protection agents. Alarm pheromones can act as repellents or defense substances, and GIBSON & PICKETT (1983) have identified in glandular hairs of a wild potato the chemical (E)- β -farnesene, which is the main component of the alarm pheromone for most aphid species (BOWERS *et al.*, 1972, PICKETT & GRIFFITHS, 1980). It was demonstrated that the (E)- β -farnesene produced by the hairs induces dispersal of settled aphid colonies and reduces contamination of leaves by wandering apterae. This suggests the possibility of using such chemicals in crop protection strategies.

In order to provide more persistent compounds for field use, numerous derivatives of the aphid alarm pheromone (E)- β -farnesene were synthesized and tested for their effects on settling and transmission of plant viruses (DAWSON *et al.*, 1982). Because of the structural similarity of these derivatives to the juvenile hormones (JH) precursors (BAKER *et al.*, 1983) we have now studied their effects.

II. MATERIALS AND METHODS

A. Insects

1. *Dysdercus fasciatus* L. (Heteroptera)

Milkweed bugs were reared on cotton seeds and ad libitum water, at 27 °C, 75 % relative humidity (RH), and 16h daylength. Insect development was synchronized by collecting the eggs daily and the larvae at hatching and at each moult. Juvenile hormone activity was tested with 24 h old 5th instar larvae.

2. *Pieris brassicae* L. (Lepidoptera) and *Manduca sexta* L. (Lepidoptera)

Large cabbage white larvae were grown on fresh cabbage leaves at 20 °C, 75 % RH and 16h daylength. Larvae were synchronized at the beginning of the last larval instar. Six h old larvae, weighing less than 200 mg (MAUCHAMP, 1979), were used for activity evaluation.

Tobacco hornworm larvae were reared on an artificial diet at 26 °C, 76 % RH and 16 h daylength. The larvae were collected as synchronous batches at the beginning of each instar.

3. *Aphis fabae* Scop. (Homoptera)

Aphids were grown on faba bean leaves at 20 °C, 70 % RH and 8 h daylength. Neonate larvae were reared on leaves infested with large numbers of aphids which produced many alatae. Synchronization was not as easy as with other species, but it was possible to detect the 1st signs of wing development on the mesothorax during the 3rd larval instar. Treated larvae were put on leaf discs deposited on agar in small circular boxes (2 cm in diameter). Thirty to fifty larvae were brought together in each box after treat-

ment. The boxes were stored at 20 °C, under long day conditions.

B. Tested chemicals

The compounds tested during these experiments were (E)- β -farnesene derivatives. They are listed with their structures in figure 1. They were synthesized according to the procedure published by DAWSON *et al.* (1982) in which (E)- β -farnesene prepared from nerolidol was derivatised by the Diels-Alder's reaction (1,4 cycloaddition reaction).

JH III, which is thought to be the natural juvenile hormone of bugs, was used as a standard ; JH I was used for Lepidoptera.

Figure 1

Chemical structure of synthetic compounds tested.

Structure chimique des composés de synthèse testés.

C. Treatment of insects

Compounds were tested by topical application. They were dissolved in acetone and 1 μ l of solution was applied to the dorsal surface of the larva. With aphids, dilution in methanol allowed applications of smaller volumes (0.1 or 0.5 μ l). Control insects were treated similarly with acetone or methanol.

The activity of the compounds against lepidopteran larvae was also evaluated by injection. Compounds were dissolved in peanut oil and 5 μ l was injected into larvae anaesthetized by CO₂. After recovery from anaesthesia insects were transferred to controlled temperature chambers.

D. Evaluation of effects

To make the bioassay reproducible and sensitive, we used insects (bugs and Lepidoptera larvae) of precise age *i.e.* shortly after the moult to the 5th larval instar. Effects were evaluated after the following moulting.

1. *D. fasciatus*

Different grades of activity may be considered. We noticed that the relationship between concentration and the juvenilizing effect was less easy to establish than the relationship which exists between concentration and the mortality for insecticidal activity. With the milkweed bugs, which are highly sensitive to JH mimics, several ways of evaluation were possible. JH activity was initially evaluated as the ability of the compounds to prevent the formation of adults, or to cause morphological changes. It was expressed as a percentage of supernumerary larvae produced or of morphological changes induced by the treatment. To establish the relationships between dose and response, the exact type of morphological change must be defined. To compare the effects of the new compounds with those of JH III, we used a scoring system described by SLAMA *et al.* (1974) where results were expressed as a percentage of morphological change.

Intermediate morphs were classified as follows :

(4) Active supernumerary larvae ; these larvae were able to moult into giant adults (+ + + +).

(3) Supernumerary larvae which died at the end of the instar (+ + +).

(2) Larvae whose wings had some adults characteristics (+ +).

(1) Adults whose wings had some larval characteristics (+).

(0) Normal adults without any morphological changes (-).

At the lowest doses we monitored local effects by scanning electron microscopy. Abdominal sternites were fixed in Carnoy's fixative and prepared according to the critical point procedure. They were then observed with a Cameca scanner.

Influence of time of application was also evaluated for compounds that revealed biological activity. These experiments were performed with doses giving scores (3) or (4).

2. *A. fabae*

Compounds were tested on presumptive alate aphids ; their effects were assessed on the prevention of alatiform formation, delayed development or mortality.

3. *M. sexta* and *P. brassicae*

A dose-response curve was more difficult to determine than in the case of exopterygote insects. Also these curves were not established for each compound. In *M. sexta*, juvenile hormone mimic activity was apparent by its influence on the pigmentation in the cuticle. STAAL (1977) also reported that on the integument of young larvae treated with juvenoids, black spots on the dorsal part of each segment disappear. When we applied large quantities at the beginning of

the last larval instar, we obtained supernumerary larvae but exuviations were rarely complete. The size of the last larval instar of *M. sexta*, required the use of large quantities of the test compounds, therefore the experiments on supernumerary larvae induction employed *P. brassicae*.

III. RESULTS

A. Effects on *D. fasciatus*

1. Treatment at the beginning of the 5th larval instar

To detect compounds with juvenilizing properties we carried out a 1st screening by application of 20 μ g of each per larva. Results are listed in table 1. Compounds which showed juvenilizing activity were tested at a range of different concentration. Results were compared with those from JH III (fig. 2). At high

TABLE 1

Juvenilizing effect of synthetic compounds compared with JH III and (E)- β -farnesene on studied species.

Effets comparés des composés de synthèse avec la JH III et le (E)- β -farnesene sur les espèces étudiées.

Chemicals tested	Score	Chemicals tested	Score
JH III	++++	7 A	-
E- β -farnesene	++++	8 A	-
1 A	++++	15 A	-
2 A	++++	18 A	-
3 A	++++	20 A	-
4 A	+++	25 A	-
5 A	-

Figure 2

Log dose/activity of the four alarm pheromone analogs showing juvenilizing activity of *D. fasciatus*. Dose was expressed in ng. The juvenilizing effect was compared to that of (E)- β -farnesene, the natural aphid alarm pheromone and to that of JH III, the putative natural juvenile hormone of *D. fasciatus*.

Log. dose/activité des 4 analogues de la phéromone d'alarme montrant une activité juvénilisante chez *D. fasciatus*. Les doses sont exprimées en ng. L'effet juvénilisant est comparé avec celui du (E)- β -farnesene, phéromone d'alarme naturelle du puceron et celui de la JH III, hormone juvénile supposée de *D. fasciatus*.

Plate 1

Scanning electron microscopy of cuticular structures :

Abdominal sculpturing of (1) 5th larval instar, (2) 6th supernumerary larval instar and (3) adult.

Pronotum sculpturing of (4) 5th larval instar, (5) 6th supernumerary larval instar and (6) adult.

Bar represents 5 µm.

Microscopie à balayage des structures cuticulaires :

Sculptures abdominales du (1) cinquième stade larvaire, (2) du sixième stade larvaire surnuméraire et de l'adulte.

Sculptures du pronotum de (4) larve de 5^e stade, (5) de sixième stade surnuméraire et (6) d'adulte.

L'échelle représente 5 µm.

concentrations (more than 2 µg per larva for compounds 1A, and more than 5 µg for compound 4A) the larvae moulted into a 6th supernumerary larval instar that was able to survive. These supernumerary larvae had perfect larval characteristics with respect to wing buds, tarsus number and abdominal microstructure (plate 1). Nearly all these larvae grew until the pharate adult stage but they had difficulty in moulting. Higher mortality occurred during exuviation and when we obtained complete adults they died soon after. At lower concentrations we obtained a gradation of effects. Perfect supernumerary larvae were able to moult into viable adults that could mate and the females could lay eggs. We noticed that the membrane of the wings did not have the same venation as the control. This is important because the arrangement and the number of the veins serve to separate the different families (fig. 3). The wide variety of intermediate effects between supernumerary larvae and adults is illustrated in plate 2A. Gradation of effects was easily observed in the wing development. Intermediate forms showed brown parts corresponding to the clavus and the corium, but the clavus remained joined with the scutellum ; furthermore, the axillary sclerites were not differentiated, thus preventing any movement. The membrane had a larval aspect. The juvenile hormone mimics also had an effect of the duration of the instar (table 2). When scores (4) or (3) were obtained,

larvae moulted after 6.5 to 7 days. In control animals, moult occurs at day 9. Intermediate durations were obtained according to concentrations.

Plate 2

Biological effects of synthetic compound tested.

A — Effects on *D. fasciatus*. Treatment was at the beginning of the 5th larval instar (b). With inefficient compounds larvae moult to give the adult (a). With the most efficient compounds larvae moult into a perfect 6th supernumerary instar (c). In some cases these supernumerary larvae were able to moult into adults which then died (d) or viable adults (e).

According to the doses used for treatment it was possible to get intermediates between a perfect 6th supernumerary larval instar and our normal adult (f — k).

B — Effect of the active compounds on *M. sexta* pigmentation. Treated animal (a) control (b).

Effets biologiques des composés de synthèse.

A — Effets sur *D. fasciatus*. Le traitement a été réalisé au début du cinquième stade larvaire (b). Avec les composés inactifs les larves muent en adulte (a). Les composés les plus actifs donnent des larves surnuméraires (6^e stade) parfaites (c). Dans certains cas les larves surnuméraires sont capables de muer en adultes qui meurent (d) ou qui survivent (e).

Selon les doses utilisées il est possible d'obtenir des formes intermédiaires entre des larves surnuméraires parfaites et des adultes normaux (f — k).

B — Effets des composés actifs sur la pigmentation de *M. sexta*. Chenilles traitées (c), témoins (b).

(A)

a

b

c

d

e

f

g

h

i

j

k

(B)

a

b

Figure 3

Mesothoracic wings of control adult (a) and adult obtained from a 6th supernumerary instar (e). Allometric growth was observed and a change in the venation of the membrane. The increase in vein numbers may be the result of the increase in cell numbers during the pharate adult development. Cor. : corium; mem. : membrane; cl. : clavus; cls. : claval suture; v. : veins.

Ailes mésothoraciques d'adultes témoin (a) et d'adultes obtenus à partir d'un sixième stade larvaire (e). Une croissance allométrique est observée ainsi qu'une modification de la nervation dans la membrane. L'augmentation du nombre de nervures peut être le résultat d'une augmentation du nombre de cellules pendant la phase pharate adulte. Cor. : corie; mem. : membrane; cl. : clavus; cls. : suture clavaire; v. : nervures.

TABLE 2

Effects of treatment on duration (days) of the 5th larval instar of *D. fasciatus* (100 larvae per test) ($\pm$ standard deviation).

Effets du traitement sur la durée (jours) du cinquième stade larvaire de *D. fasciatus* (100 larves traitées par essai) ($\pm$ standard deviation).

Doses (ng)	Compounds			
	JH III	Farnesene	1 A	3 A
1	8,5 $\pm$ 0,5	9,0 $\pm$ 0,2		
2	7,5 $\pm$ 0,3	8,1 $\pm$ 0,3		
10	7,0 $\pm$ 0,2	7,5 $\pm$ 0,1	9,2 $\pm$ 0,2	
50	—	—	—	9,1 $\pm$ 0,3
100	6,5 $\pm$ 0,2	6,5 $\pm$ 0,2	7,0 $\pm$ 0,1	8,5 $\pm$ 0,2
500	—	—	—	8,1 $\pm$ 0,3
1 000	—	—	6,5 $\pm$ 0,1	7,0 $\pm$ 0,1

Quote control figure i.e. 9 days.

2. Treatment at the beginning of the 3rd or 4th larval instar

12 h old larvae of the 3rd or 4th instar were treated topically with 1 μ g of 4 biologically active compounds (1A, 2A, 3A and 4A). No effects were detected after the 3rd or 4th moult; larvae had normal size and behaviour and the length of respective instars was similar to that of the untreated insects. 5th instar larvae were similar in size, but after 7 days (instead of 9 in the untreated insects) they moulted to produce a supernumerary instar. Sixty per cent of these giant nymphs were able to moult into giant adults similar to

those obtained after treatment during the 5th instar. The wings of these adults were perfectly differentiated and showed additional veins in the membrane portion of the front wing and in the hind wing. Such adults could survive over one month and were able to mate. Mating was observed only between giant males and giant females, producing fertilized eggs.

B. Effects on lepidopteran larvae (*P. brassicae* and *M. sexta*)

Only compounds 1A, 2A, 3A and 4A gave juvenilizing effects on larvae treated during the last instar. 20 μ g of each compound applied at the beginning of the last larval instar of *P. brassicae* induced the formation of supernumerary larvae. They had larval pigmentation, larval mouthparts and larval claws on the prolegs and also undifferentiated imaginal wing discs outside the body wall. This had also been observed after treatment with JH1 (MAUCHAMP, 1979). In *M. sexta*, in spite of the use of higher doses (100 μ g), we did not obtain exuviated supernumerary larvae. Under the 5th instar larval cuticle we noticed that the epidermal cells differentiated into a new larval integument instead of a pupal integument. The larvae were unable to moult and died in the old cuticle. The juvenilizing effect of the compounds was observed when treatment occurred at the beginning of the 3rd or the 4th larval instar. Pigmentation of the larvae was also affected. Treated larvae lacked the black spots observed on control insects (plate 2B) and the larvae had completely white legs and brown-colored spiracles. One μ g of farnesene and compounds 1A to 4A affected the pigmentation change during the 3rd larval instar. These treated larvae were able to moult and we obtained normal pupae and adults.

C. Effects on aphids

Results were very heterogeneous and not conclusive. We noticed a mortality in individual treated aphids and in mass treated colonies. In many cases, the moult aborted; several morphs were obtained but alate aphids showed crumpled wings. With (E)- β -farnesene and compound 1A additional ecdyses occurred.

IV. DISCUSSION AND CONCLUSION

(E)- β -farnesene, the aphid alarm pheromone, is very effective in controlling the behaviour of these insects (BOWERS *et al.*, 1972). Because it is highly volatile and quickly oxidized, it cannot be used directly as an aphid repellent in the field. Derivatives which were persistent and would maintain activity over a long period could be used to protect plants against aphids and to reduce virus transmission (DAWSON *et al.*, 1982; BRIGGS *et al.*, 1982; GIBSON & PICKETT, 1983).

It was demonstrated that farnesol, a precursor in juvenile hormone biosynthesis, has juvenilizing

effects. Thus it is not surprising that we obtained such effects with (E)- β -farnesene and some of its derivatives which are structurally similar to farnesol. The activity of the juvenile hormone analogs depended on the carbon number of the aliphatic chain ; molecules containing between 10 to 20 carbons had higher activity (BOWERS, 1969 ; SCHWARZ *et al.*, 1970 ; BRIEGER, 1971 ; OHTAKI *et al.*, 1972). The influence of the carbon number on the juvenilizing properties of the farnesene derivatives was investigated.

The juvenilizing effect was not only correlated with the size of the molecule : only the compounds that could regenerate farnesene were active on different species. These compounds (1A to 4A) could undergo the reversible reaction (BRIGGS *et al.*, 1983) shown here :

Differences in activity between compounds 1A to 4A may be due to differences in penetration or effectiveness of transformation. For all these juvenoids, activity depended on time of application. The most marked effects on *D. fasciatus* were obtained if treatment occurred at the beginning of the last larval instar. However, if treatment took place during the penultimate instar we also obtained supernumerary larvae and giant adults. The latter had normal behaviour and were able to mate and lay eggs. This was not observed when penultimate instar larvae were treated with natural juvenile hormone. In Lepidoptera the compounds 1A to 4A showed also juvenilizing

activity. These derivatives could therefore be considered as projuvenoids, *i.e.* substances that need to be metabolized to show juvenilizing activity.

The compounds obtained by the reactions shown here :

are stable and do not generate (E)- β -farnesene and have no juvenile hormone mimic activity. Biological activity with regard to aphid colonization or virus transmission was evaluated by BRIGGS *et al.* (1983) and showed that activity increased from 1A through 2A, 3A, 4A to 15A, the most active compound. However we demonstrated that 15A had no juvenile hormone mimic activity. Thus, preparation of these stable derivatives increased activity against aphid-mediated damage and eliminated with others species the risk of obtaining supernumerary larvae or giant insects that could cause more damage to the crops. These experiments show that the compounds described here could prove a valuable lead for new types of crop protection agents.

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