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The effects of new juvenoids on the aphid species *Aphis fabae* (Scop.) and *Myzus persicae* (Sulz.) and on the cotton stainer bug *Dysdercus cingulatus* (F.)([1])([2]).

The increasing resistance of pests to traditional insecticides and the pollution of the environment pose the problem of how to protect field, orchard and other crops from harmful insects. The use of juvenoids seems to be a promising control technique (Williams, 1967).

The search for juvenoids that can disrupt metamorphosis and reproduction, particularly in aphids, has been very extensive (Nasseth, 1980; Hrdý, 1974; Baurenfield, Chapman, 1984) but largely unsuccessful. This is likely ascribible to the poor stability of commercial (sesquiterpene) juvenoids in the environment (Siddal, 1976).

Several derivatives of 2-(4-hydroxybenzyl)-1-cyclohexanone (Sláma, 1985; Kuldová *et al.*, 1990) that have revealed promising biological activity were synthesized at the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences in Prague (Wimmer, Romaňuk, 1981; Wimmer *et al.*, 1985, 1988). The aim of the present study was to test the biological activity of these derivatives and to elucidate the structure-activity relationship.

Materials and methods

Aphis fabae (Scop.) and *Myzus persicae* (Sulz.) were taken from our laboratory stock culture maintained on horse bean sprouts at 19-21°C, 70-80% rh and 16 hr

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([1]) The authors have contributed equally to the data reported in and the preparation of this paper.

([2]) Lavoro accettato il 24 aprile 1991.

photoperiod. The tests were performed with a mixed group of aphid nymph (no less than 30 specimens) for each assay.

Dysdercus cingulatus (F.) individuals were reared in 0.5 l glass jars on cotton seeds and supplied with drinking water. They were kept in 25-28°C and 14 hr photoperiod. The freshly moulted fifth instar larvae (no older than 24 hr) were used for the assays.

The juvenoids used in our study were provided by the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences in Prague (the formulae of the compounds are in figure I).

Application of juvenoids.

Two methods of application were used:

1. Dipping assay: 0.02% solution of tested compound in an emulsion prepared from flax oil, Triton X-100 and water (1:1:100) was applied to the feeding plant. The control was treatment with emulsion alone. The aphids were then transferred to the treated plants.

2. Indirect topical application: the tested juvenoid was dissolved in acetone and applied to the bottom of Petri dishes in doses of 12.7 μg/cm^2 or 1.27 μg/cm^2. Acetone was evaporated by air stream at room temperature. The walls of these Petri dishes were coated with fluon to prevent the tested aphids escaping from the contaminated bottom. The aphids were left to crawl for 3 hr and then transferred to clean horse bean sprouts. All the tested aphids were incubated separately from the stock culture but under the same conditions.

The *D. cingulatus* individuals were treated topically with the acetone solution of a juvenoid in volume 1 μl at concentrations ranging from 10^{-3} to 10^{-10}. Each assay had 15-20 replications.

Evaluation of juvenoid effects.

Aphids were monitored daily and evaluated 6 days after application by comparing mortality in control (assumed as 0%) to the rate in the tested groups. «Ecdysial death» and malformation of wings, genital plate and cauda were also recorded in both aphid species. The effects of juvenoids on *D. cingulatus* were evaluated after Sláma *et al.* (1974).

Results

Both aphid species revealed similar susceptibility to the tested compounds, although *M. persicae* seemed to be more susceptible (compare table 1 to 2). The tables apparently show that dipping is the more effective method of application, but the 12.7 μg/cm^2 dose used with the indirect topical application is about tenfold lower than those used for dipping. Hence it can be inferred that indirect topical application is probably more effective.

The most active of the tested substances on the aphid species were: W-328, W-362, W-376, W-381, W-383, W-420, W-427 and W-488; with the exception of compound W-381, all the others are oxime ethers of 2-hydroxybenzyl-1-cyclohexanone. It seems that the analogues derived from the above base structure would be suitable for control of aphid populations.

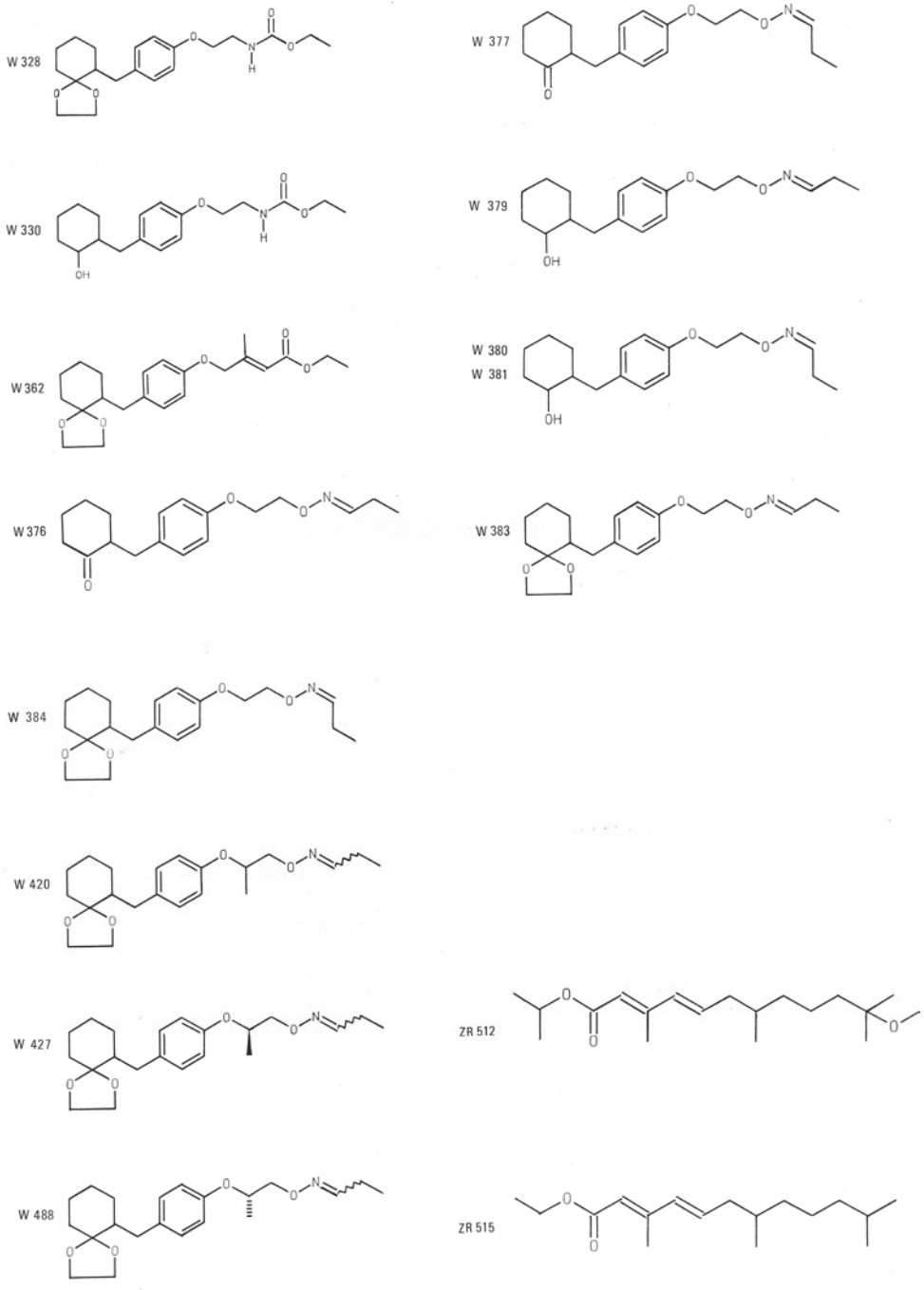

Figure I - Chemical structure of tested juvenoids.

Table 1 - Effects of juvenoids on the aphid *Aphis fabae* expressed as mortality in % of control.

Compound	Treatment and dosage		
	Dipping 0.02%	Indirect application	
		1.27 µg/cm²	12.7 µg/cm²
ZR 512	88.3	26.5	76.1
ZR 515	84.8	39.9	91.0
W 328	75.6	70.2	79.1
W 330	13.8		70.8
W 362	86.4		
W 376	83.1	59.2	73.6
W 377	83.4	50.8	63.6
W 379	69.7		48.3
W 380	61.3		
W 381	90.8	0.0	13.7
W 383	92.6	86.1	
W 384	87.5	98.2	
W 420	85.9	89.8	
W 427	80.9	73.1	95.3
W 488	94.9	88.4	

Table 2 - Effects of juvenoids on the aphid *Myzus persicae* expressed as mortality in % of control.

Compound	Treatment and dosage		
	Dipping 0.02%	Indirect application	
		1.27 µg/cm²	12.7 µg/cm²
ZR 512	75.7	0.0	67.6
ZR 515	62.0	43.4	86.2
W 328	46.2	43.7	61.2
W 330	50.0		45.9
W 362	64.3		
W 376	86.4		49.7
W 377	98.9		74.7
W 379	47.6		34.5
W 380	60.2		
W 381	92.1		85.8
W 383	93.9	93.1	
W 384	92.5	82.5	
W 420	82.9	89.3	85.1
W 427	70.4		82.0
W 488	84.2	84.3	

Table 3 - Effects of juvenoids on *Dysdercus cingulatus* after topical application.

Compound	ID (50) in µg
W 328	100 µg/spec.
W 330	0.00005 µg/spec.
W 376	no effect
W 379	no effect
W 380	0.5 µg/spec.
W 383	no effect
W 384	500 µg/spec.
W 420	no effect
W 427	no effect
W 488	more than 500 µg/spec.
ZR 512	500 µg/spec.
ZR 515	0.5 µg/spec.

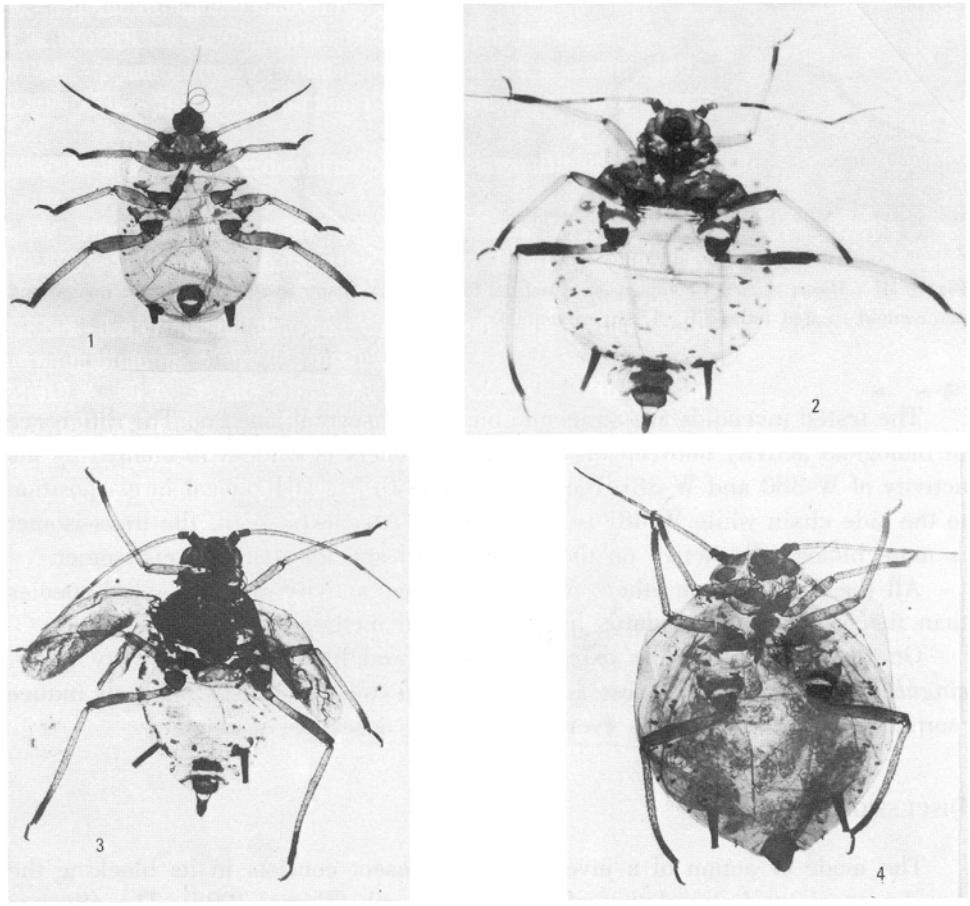

Figure II - *Aphis fabae*: comparison of normal forms (1. IV instar nymph, 2. normal imago) and of juvenoid treated forms (3. wingless supernymph, 4. winged supernymph).

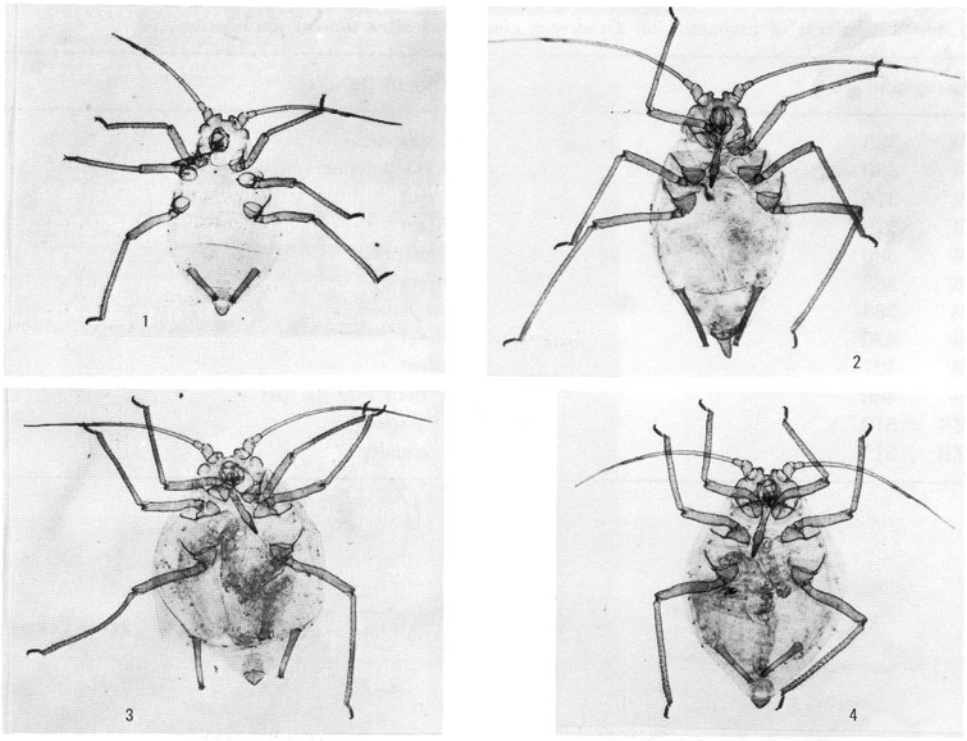

Figure III - *Myzus persicae*: comparison of normal forms (1. IV instar nymph, 2. normal imago) and of juvenoid treated forms (3., 4. supernymphs).

The tested juvenoids are a racemic mixture of several isomers. The difference in biological activity between cis- and trans-isomers is evident in comparing the activity of W-380 and W-381: the former (W-380) has OH-radical in cis-position to the side chain while W-381 is trans-isomer. As can be seen, the trans-isomer is more biologically active on the tested aphid species than the cis-isomer.

All the tested oxime ethers possessed higher activity on both aphid species than the commercial standards hydroprene and methoprene (table 1, 2).

On the other hand, the oxime ethers showed but very low activity on *D. cingulatus* (table 3). The most active substance was W-330, which can induce morphogenetic abnormalities even in picogram doses per specimen.

Discussion

The mode of action of a juvenoid on an insect consists in its blocking the morphogenesis and stimulation of isometric growth (Sláma, 1985). The effect is manifested in wing malformation, development of supernumerary nymph and decline of reproduction (Applebaum *et al.*, 1975; Mittler *et al.*, 1979). Similar patterns

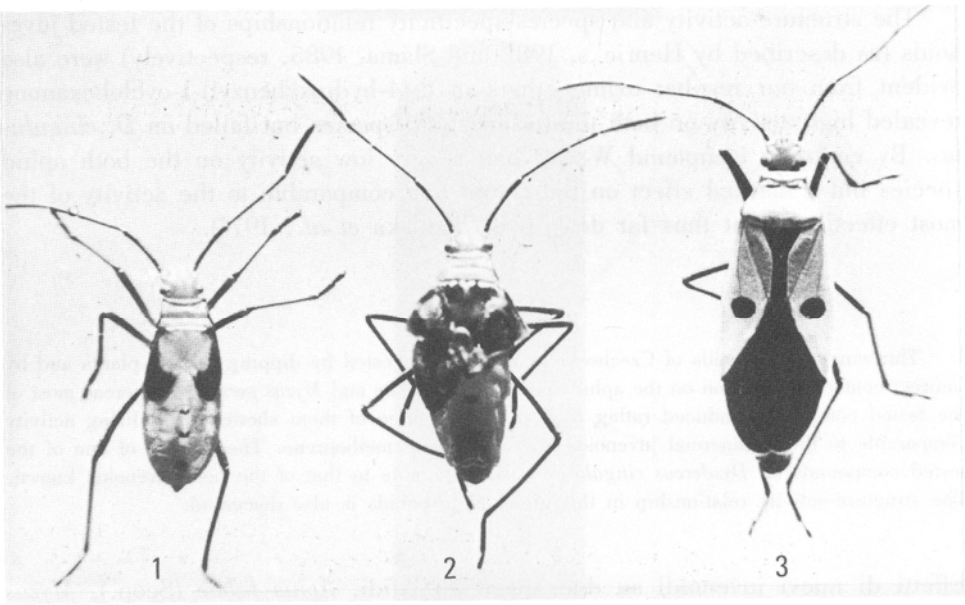

Figure IV - *Dysdercus cingulatus*: effect of juvenoids on metamorphosis. 1. V instar normal nymph, 2. adultoid, 3. normal imago.

were found in our assays (figures II, III, IV). Increased mortality was another effect of juvenoids on aphids. It was probably caused by ecdysial failure of the recipient insects (Sehnal, 1976). The old cuticle remained attached at the tip of their legs, mouth appendages and abdomen and they were unable to cast it off probably as a result of the disruption of their ecdysial behaviour (Sláma *et al.*, 1974). Hence, they were trapped in their old cuticle and died. Some of the polycyclic juvenoids also have side-effects, including mortality, as reported by Masner *et al.*, (1981).

Kuldová *et al.* (1990) tested similar derivatives of 2-(4-hydroxybenzyl)-1-cyclohexanone on *D. cingulatus*, *Galleria mellonella*, *Musca domestica* and the aphid *Acyrtosiphon pisum*. They found that the new juvenoids are as active as the commercial juvenoids hydroprene and methoprene (tested by Hrdý and Zelený, 1973). Because of their greater stability in the environment the new juvenoids would be better growth and reproduction regulators than the commercial standards.

Another factor influencing the biological activity of juvenile hormone mimetics is their geometrical isomery. Wigglesworth (1969) and Sláma *et al.* (1974) reported that the trans-isomers of a juvenoid are usually more active than the cis-isomers. This is evident in a comparison of the compound W-380 (cis-isomer) and W-381 (trans-isomer) data: the activity of the latter exceeded the activity of the cis-isomer in both tested aphid species, irrespective of the method of application (table 1, 2).

The structure-activity and species-specificity relationships of the tested juvenoids (as described by Henricks, 1982 and Sláma, 1985, respectively) were also evident from our results: oxime ethers of 2-(4-hydroxybenzyl)-1-cyclohexanone revealed high activity on both the tested aphid species but failed on *D. cingulatus*. By contrast, compound W-330 had a very low activity on the both aphid species but a marked effect on the cotton bug comparable to the activity of the most effective agent thus far described (Poduska *et al.*, 1971).

SUMMARY

Thirteen new juvenoids of Czechoslovak origin were tested by dipping feeding plants and by indirect contact application on the aphid species *Aphis fabae* and *Myzus persicae*. Whereas most of the tested compounds induced rather high mortality, three of them showed juvenilizing activity comparable to the commercial juvenoids hydroprene and methoprene. The activity of one of the tested compounds on *Dysdercus cingulatus* was comparable to that of the best juvenoids known. The structure-activity relationship in this group of juvenoids is also discussed.

Effetti di nuovi juvenoidi su due specie di Afidi, *Aphis fabae* (Scop.), *Myzus persicae* (Sulz.) e sul Pirrocoride *Dysdercus cingulatus* (F.)

RIASSUNTO

È stato valutato, con prove di laboratorio, l'effetto di tredici nuovi juvenoidi, sintetizzati in Cecoslovacchia, nei confronti di due specie di Afidi, *Aphis fabae* e *Myzus persicae*. L'applicazione dei prodotti è stata eseguita in modo indiretto: attraverso il trattamento delle piante nutrici e di superfici vetrose (fondo di capsule Petri) entro cui sono stati mantenuti gli insetti per un certo periodo. Molte delle sostanze ormono-simili saggiate hanno provocato un'elevata mortalità. Tra queste tre hanno manifestato un'attività juvenilizzante comparabile a quella di prodotti commerciali, idroprene e methoprene. I composti di nuova sintesi sono stati saggiati anche nei confronti del Pirrocoride *Dysdercus cingulatus* mediante applicazioni topiche. In queste prove solo una delle sostanze utilizzate ha presentato effetti simili a quelli indotti dai migliori juvenoidi conosciuti. Infine, poi, viene discussa la relazione esistente fra la struttura e l'attività di questi prodotti.

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