Original Research

Environmentally Safe Insect Control: Feeding Deterrent Activity of Alkyl-Substituted γ- and δ-Lactones to Peach Potato Aphid (*Myzus persicae* [Sulz.]) and Colorado Potato Beetle (*Leptinotarsa decemlineata* Say)

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Abstract

The feeding deterrent activity of alkyl-substituted γ- and δ-lactones, including a group of lactones obtained from linalool against peach potato aphid (*Myzus persicae* [Sulz.]) and Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say), was investigated. The deterrent activity was species-specific and developmental-stage-specific (CPB). The strongest antifeedants for *L. decemlineata* larvae and adults were linalool-derived unsaturated lactones (Z) 5-(1.5-Dimethyl-hex-4-enyldiene)-dihydro-furan-2-one and (E) 5-(1.5-Dimethyl-hex-4-enyldiene)-dihydro-furan-2-one, and for CPB larvae – saturated lactone with three alkyl substituents, the 4-Isobutyl-5-isopropyl-5-methyl-dihydro-furan-2-one. The settling of *M. persicae* on plants was strongly deterred by iodolactones: 5-(1-Iodo-ethyl)-4.4-dimethyl-dihydro-furan-2-one, 5-Iodo-4.4.6-trimethyl-tetrahydro-pyran-2-one, 5-Iodomethyl-4-isobutyl-5-isopropyl-dihydro-furan-2-one, and the saturated lactones: 4.4.6-Trimethyl-tetrahydro-pyran-2-one and 4-Isobutyl-5-isopropyl-5-methyl-dihydro-furan-2-one. None of the tested lactones deterred aphid probing, but the probes were significantly shorter as compared to the control. The reduction in probing time may decrease the ability to transmit virus diseases by aphids.

Keywords: lactones, insect antifeedants, *Myzus persicae, Leptinotarsa decemlineata*

Introduction

The discovery and understanding of mechanisms of host plant selection by herbivorous insects opened a wide array of possibilities for the development of environmentally friendly crop protection methods. These include the use of resistant plant varieties and the application of various behaviour-modifying chemicals, often in combination with other methods within integrated pest management strategies [1-3]. The interference with the fixed scheme of insect activities during the process of host plant selection is a promising approach. The disruption of the host plant selection strategy may result in a decline in food consumption. Consequently, the reduced feeding may cause the rejection of a plant, may affect the development and longevity of the insect, or may lead to its death. Aphids

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are very effective vectors of plant virus diseases – approximately 60% of all plant viruses are spread by these insects – and the reduction of their feeding or probing into plant tissues may also protect plants from pathogen infection [4-8]. The peach potato aphid (*Myzus persicae*) is able to live on plants of over 40 families and may transmit over 100 plant viruses [9].

Behaviour-controlling chemicals that negatively affect food consumption by insects are known as feeding deterrents or antifeedants. The best known antifeedants belong to different chemical groups and come from natural sources [10, 11]. However, given the costs and practical aspects, e. g., their low content in plants, natural antifeedants are difficult to apply on a large scale. There is an increasing interest not only in the synthesis of chemical analogues of natural compounds more available for practical use, but also in finding the structural elements that evoke insect antifeedant activity [12, 13].

Terpenoid lactones are widely occurring natural products that exhibit feeding deterrent activity to many insect species. Usually, the lactones isolated from natural sources possess one or more additional functional groups. In the present work we examine the biological activity and discuss possible modes of action of several synthetic alkyl-substituted lactones including a group of lactones obtained from linalool to two economically important agricultural pests: the peach potato aphid *Myzus persicae* and Colorado potato beetle *Leptinotarsa decemlineata*.

Experimental Procedures

The feeding deterrent activity of alkyl-substituted γ- and δ-lactones was investigated using apterous viviparous females of peach potato aphid (*Myzus persicae*) and adults and larvae of Colorado potato beetle (CPB) (*Leptinotarsa decemlineata*). These insects differ in their way of feeding: the beetles and larvae of *L. decemlineata* have a chewing feeding apparatus, while the aphids have sucking-piercing mouthparts and collect their food directly from phloem vessels. Moreover, aphids do not possess external contact chemoreceptors [14], and the acceptance of the host plant requires the ingestion of plant sap [15]. Therefore, different methods of evaluation were applied to reveal antifeedant activity of the studied compounds to aphids and the coleopterans. For aphids, the behavioural experiments were designed – we studied their behaviour during settling (see "aphid behaviour" below) on plants and the durability of the deterrent effect (see "aphid settling" below). For the Colorado potato beetle, we applied standard procedures of choice- and non-choice feeding assays.

Tested Compounds

Syntheses of lactones studied were performed from known **1 (E) 3.3-Dimethyl-hex-4-enoic acid** [16, 17], **11 (Z/E) 5.9-Dimethyl-deca-4.8-dienoic acid** [18, 19], and new (**8**) γ,δ-unsaturated acids. Acid (**8**) **3-Isobutyl-5-methyl-4-methylene-hexanoic acid** was synthesized in a four step synthesis from 3-methylbutanal. Aldehyde was subjected to aldol condensation in a base condition and next reduced with $NaBH_4$. Unsaturated alcohol obtained was transformed into acid *via* the Claisen-orthoacetate rearrangement followed by hydrolysis of ester. Lactones (**4-7**) were obtained from acid **1** in two step synthesis *via* the iodolactonization process (Fig. 1). γ and δ-iodolactones (**2**) **5-(1-Iodo-ethyl)-4.4-dimethyl-dihydro-furan-2-one** and (**3**) **5-Iodo-4.4.6-trimethyl-tetrahydro-pyran-2-one** [20] were separated by column chromatography on silica gel using the mixture of hexane-diethyl ether, 6:1 as eluent. Saturated lactones (**4**) **5-Ethyl-4.4-dimethyl-dihydro-furan-2-one** and (**6**) **4.4.6-Trimethyl-tetrahydro-pyran-2-one** were the products of reduction of corresponding iodolactone (**2**) and (**3**) with tributyltin hydride respectively. Dehydrohalogenation of iodolactone (**2**) with 1.8-diazabicyclo[5.4.0]undec-7-ene (DBU) carried out in methylene chloride afforded unsaturated lactone (**5**) **5-Ethylidene-4.4-dimethyl-dihydro-furan-2-one**. Under similar conditions γ-iodo-δ-lactone (**3**) was transformed *via* the 1.3-elimination-cyclization process into bicyclic lactone with gem-dimethylcyclopropane system **(7) 4.6.6-Trimethyl-3-oxa-bicyclo[3.1.0]hexan-2-one**.

Acid **(8)** in the reaction of iodolactonization with I_2/KJ solution in basic condition ($NaHCO_3$) gave the δ-iodo-γ-lactone **(9) 5-Iodomethyl-4-isobutyl-5-isopropyl-dihydro-furan-2-one** as the only product in 80% yield (Fig. 2). Lactone (**10**) **4-Isobutyl-5-isopropyl-5-methyl-dihydro-furan-2-one** was obtained by reduction of iodolactone (**9**) with tributyltin hydride. Its structure as well as lactone **(9)** was confirmed by spectroscopic methods [21].

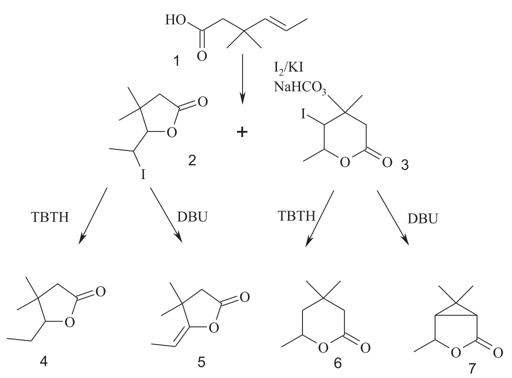

Fig. 1. Scheme of synthesis of lactones **2-7** from acid **1**.

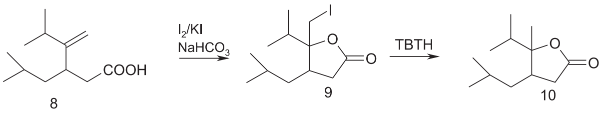

Fig. 2. Scheme of synthesis of lactones **9-10** from γ, δ-unsaturated acid **8.**

The unsaturated γ-lactones (**14-16)** were obtained also in two step synthesis from mixture (E/Z) of acid (**11)** (Fig. 3). The iodolactonization of acid (**11**) gave the mixture δ-iodo-γ-lactone (**12**) **5-(1-Iodo-1.5-dimethyl-hex-4-enyl)-dihydro-furan-2-one** (73%) and γ-iodo-δ-lactone (**13**) **5-Iodo-6-methyl-6-(4-methyl-pent-3-enyl)-tetrahydro-pyran-2-one** (27%). Pure iodolactone (**12**) (diastereoisomeric mixture) was separated by column chromatography on silica gel, using a mixture of hexane-diethyl ether 10:1 and next 2:1 as eluent. The reduction of this iodolactone with tributyltin hydride afforded saturated lactone (**16**) **5-(1.5-Dimethyl-hex-4-enyl)-dihydro-furan-2-one** in 72% yield. The reaction of iodolactone (**12**) with DBU gave a mixture of two isomeric (E/Z) unsaturated lactones: (**14**) **(Z) 5-(1.5-Dimethyl-hex-4-enyldiene)-dihydro-furan-2-one** (50%) and (**15**) **(E) 5-(1.5-Dimethyl-hex-4-enyl-diene)-dihydro-furan-2-one** (50%). They were separated by column chromatography on silica gel with the application of hexane-diethyl ether 10:1 mixture, next 2:1 as eluent (Fig. 3). It is worth noticing that lactones (**14**) and (**15**) are characterized by fresh mushroom odour [22, 23].

Aphids

Aphids (*Myzus persicae*) and plants (Chinese cabbage *Brassica pekinensis*) were reared in laboratory at 20°C, 65% r.h., and L16:8D photoperiod. All experiments were carried out under the same conditions.

The compounds were applied to adaxial surface of a leaf as 0.1% ethanolic solutions, 0.01ml/cm^2 of the leaf according to a method described by Polonsky *et al.* [24]. All biological tests were performed 1 hour after the application of the compounds to allow the evaporation of the solvent.

Aphid settling was assessed using the half-leaf choice-test: compounds were applied on one half of the leaf; the other side of the midrib was treated with ethanol and acted as a control. Aphids had a choice between equal areas of treated and control surfaces. Aphids that settled on each side of the midrib were counted at 15', 30', 1h, 2h, and 24h intervals after access to the leaf (8 replicates, 20 viviparous apterous females/replicate). The data were analyzed using one way ANOVA. If aphids showed clear preference to the half of the leaf treated with the tested compound (P<0.05), the compound was described as having attractant properties. If aphids settled mainly on the control half of the leaf (P<0.05), the compound tested in the respective choice-test was determined to be a feeding deterrent.

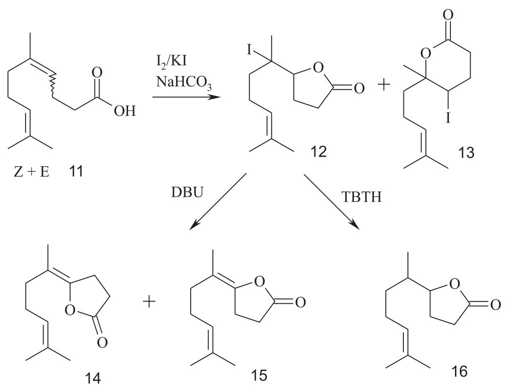

Fig. 3. Scheme of synthesis of lactones **14-15** from acid **11**.

Aphid behaviour was studied by direct observation of the freely moving aphids on a leaf treated with the tested compounds, using a video camera. The experiment was carried out for 15 min (16 aphids/compound). The duration of probing was recorded based on the relationship between antennal and body movements and penetration of the stylets as described by Hardie *et al.* [6]. The position of antennae parallel to the abdomen and the cessation of body movements were associated with stylet penetration. The total time spent by aphids on leaves, total probing time, number of probes, and mean probing time was determined from this experiment. These parameters reflect the suitability of a plant as a host for the aphids. In the case of the artificially applied chemicals to the surface of the leaf, the shorter time that aphids spent on the leaf during the initial 15 minutes in comparison to the control may indiciate the repellent properties of the given compound. In the same way, the reduced mean probing time may reflect its deterrent character.

The data were analyzed using one way ANOVA followed by the Tukey test.

Colorado Potato Beetle

In the experiments with *L. decemlineata*, newly ecdysed third instar larvae selected from laboratory colony and adults collected from an unsprayed potato field were used. For the feeding assays 0.1% alcohol solutions of compounds were prepared. Assays were conducted in feeding arenas made of glass Petri dishes (150x25mm) lined with moistened filter paper.

Leaf discs (40mm in diameter) were cut from potato leaves, dipped in the solution of lactone or alcohol (control) and left to air-dry. After the solvent evaporated the leaf discs were placed into the feeding arenas. In each arena, two treated and two control discs were placed at alternate corners, equidistant from each other, and 10 larvae uniform in size or 6 adults (3 pairs) in the center (choice-test). In no-choice test only three control or only three treated discs were placed in the dishes. In each of the four replicates, the insects were allowed to feed *ad lib.* for 24h at 24°C under a 16:8 light-dark photoperiod (climate chamber). After completion of the experiments, the remaining uneaten area of each potato leaf disc was measured using a scanner (Microtec Scan Maker 3800) and special software programmed by A. Zienkiewicz (Department of Biophysic Nicolaus Copernicus University, Toruń, Poland). The amount of ingested food in all variants was calculated as the difference between the surfaces before and after feeding. From the data thus obtained the coefficients of deterrence (relative R and absolute A) were calculated using the formulae according to Nawrot *et al.* [25].

$$R = (C\text{-}T/C\text{+}T) \times 100 \quad \text{(choice test)}$$

$$A = (CC\text{-}TT (CC\text{+}TT) \times 100 \quad \text{(no-choice test)}$$

C, CC – the consumed area from the control discs
T, TT – the consumed area from the discs treated with tested compound

The measure of the deterrent activity of a given compound is its total coefficient

$$T = A\text{+}R$$

The data were statistically analyzed using one-way analysis of variance (ANOVA) [26]. Compounds whose T-values range from 151 to 200, are very good deterrents, those with coefficient values 101-150 are good deterrents, medium active T range from 51-100. Compounds whose T-values are lower than 50 are weak deterrents. Negative T-values point to attractant properties of the compound.

Results and Discussion

Myzus persicae

Aphids settle on a plant only when they accept it as a food source [15]. Therefore, the number of aphids that settle and feed on a given substrate is a good indicator of its suitability.

The 24-hour assay showed that the studied lactones had various effects on aphid settling. The iodolactones **2, 3, 9,** and the saturated lactones **6**, **10** deterred aphid settling from the onset of the experiment until its termination. Their activity was highly significant as indicated by the P values of the statistical analysis (Table 1). The activity of compounds **4, 5,** and **15** was less durable: no activity was found within the 24 hours of the experiment. The values of coefficient R were lower than for the aforementioned compounds. The unsaturated lactone **14** was not active at all and the bicyclic lactone **7** had attractant properties and that effect lasted for 2 hours.

All compounds influenced aphid behaviour during the initial 15 minutes of the contact with the treated substrate (Fig. 4). Of all studied compounds, only one of the derivatives of linalool (**15**), and linalool itself, reduced the total time spent on the leaf, which might reflect repellent properties of that lactone. Unsaturated lactone **15** reduced also the total and mean probing time, which might be a very desirable activity of a potential antifeedant to aphids because it might decrease the rate of virus transmission by these insects. This would be especially important in the case of circulative (persistent) viruses, as long probes reaching to the phloem vessels are needed for the acquisition of the virus particles. *Myzus persicae* needs at least 10 minutes to reach the phloem vessels (Gabrys, unpublished). Probes of shorter than ca. 2 min. duration do not reach beyond the epidermis layer [27]. During that time the acquisition and inoculation of even non-persistent viruses is very low [7]. The average time of a probe was 8.5 min on control plants. This time is sufficient for the transmission of both types of viruses [7, 8]. The significant reduction of that time was caused by all tested compounds, but compounds **4**, **10**, and **15** had the strongest effect. Mean probing time was on average four times shorter than in control leaves. The time of probing was reduced also by lactones **2**, **3**, **5**, **6**, **9**, and **14**: mean probing time was ca. twice shorter than in control leaves. However, the duration of these probes was 3-5 min on average, which means that aphids might have reached inner tissues (e.g., mesophyll) with their stylets.

In general, the studied compounds did not deter aphids from probing (with the exception of lactone **15**). However, all compounds caused the sooner termination of probes as compared to control.

Leptinotarsa decemlineata

The list of deterrence coefficient values in Table 2 indicates a differential activity of the studied compounds. The iodolactones (**2**, **3**) were characterized by very low values of coefficient T and did not significantly affect CPB feeding, and lactone **2** even stimulated feeding in adult individuals. The removal of the iodine atom from the molecule of either of those compounds caused a change in their activity. The saturated lactones (**4, 6**) thus obtained restrained the insects´ feeding to a higher degree than their precursors. The differences, hovewer, were statistically insignificant (with the exception of the activity of lactones **3** and **6** against larvae). Similarly, iodolactone **9** with three alkyl substituents was a moderate deterrent to larvae and a poor one to beetles, but the removal of the iodine atom likewise caused increased activity of the compound thus obtained **10**, particularly to *L. decemlineata* adults. Our earlier studies have shown that dehalogenation of the iodolactone molecule affects the resulting saturated lactones in different ways. The removal of the iodine atom from iodolactone leading to the bicyclic lactone molecule with dimethyl cyclohexane system **7** affected the antifeedant properties of the compound to CPB larvae and beetles only to a small extent, it caused, however, a decrease in its activity against the lesser mealworm, *Alphitobius diaperinus* Panzer larvae [28]. Dehydrohalogenation of γ-iodolactone **2** did not induce a change in the activity of the obtained unsaturated lactone **5** to larvae but increased the activity of that compound on adult individuals. In case of structural changes in δ-iodolactone **3** the reverse was observed. The removal of hydrogen iodide markedly increased the deterrent activity of bicyclic lactone **7** to larvae but it showed attractant properties to the beetles in the choice test.

Table 1. Settling deterrent activity of alkyl-substituted γ- and δ-lactones against *Myzus persicae*. Numbers for "test" and "control" represent mean number of aphids settled on test or control half of the leaf (choice-test). P – significance level (ANOVA) – significant differences between the number of aphids settled on either half of the leaf are underlined.

Compound		Time after access to the plants				
		15 min	30 min	1 hour	2 hours	24 hours
2	test	3	3	3	3	2
	control	9	8	8	8	4
	P	0.0000	0.0002	0.0009	0.0025	0.0243
3	test	3	3	2	3	1
	control	10	11	12	12	10
	P	0.0000	0.0000	0.0000	0.0000	0.0002
4	test	6	6	5	5	3
	control	7	8	8	9	6
	P	0.1249	0.1643	0.0206	0.0205	0.1136
5	test	3	4	4	3	5
	control	10	11	12	12	8
	P	0.0003	0.0002	0.0001	0.0000	0.2286
	R	54	45	51	57	20
6	test	4	4	3	3	0
	control	10	11	11	12	9
	P	0.0001	0.0000	0.0000	0.0000	0.0000
7	test	9	10	10	10	5
	control	5	6	5	5	7
	P	0.0419	0.0366	0.0029	0.0033	0.4040
9	test	3	2	2	2	1
	control	11	12	12	12	13
	P	0.0000	0.0000	0.0000	0.0001	0.0000
10	test	3	3	2	2	1
	control	10	10	11	11	9
	P	0.0000	0.0001	0.0000	0.0000	0.0001
14	test	5	4	3	3	0
	control	4	3	4	4	1
	P	0.2769	0.8638	0.6969	0.2831	0.5307
15	test	4	3	3	4	4
	control	6	7	7	7	4
	P	0.0779	0.0074	0.0077	0.0458	0.5036
Linalool	test	3	3	2	2	1
	control	8	11	9	9	7
	P	0.0002	0.0000	0.0011	0.0005	0.0017

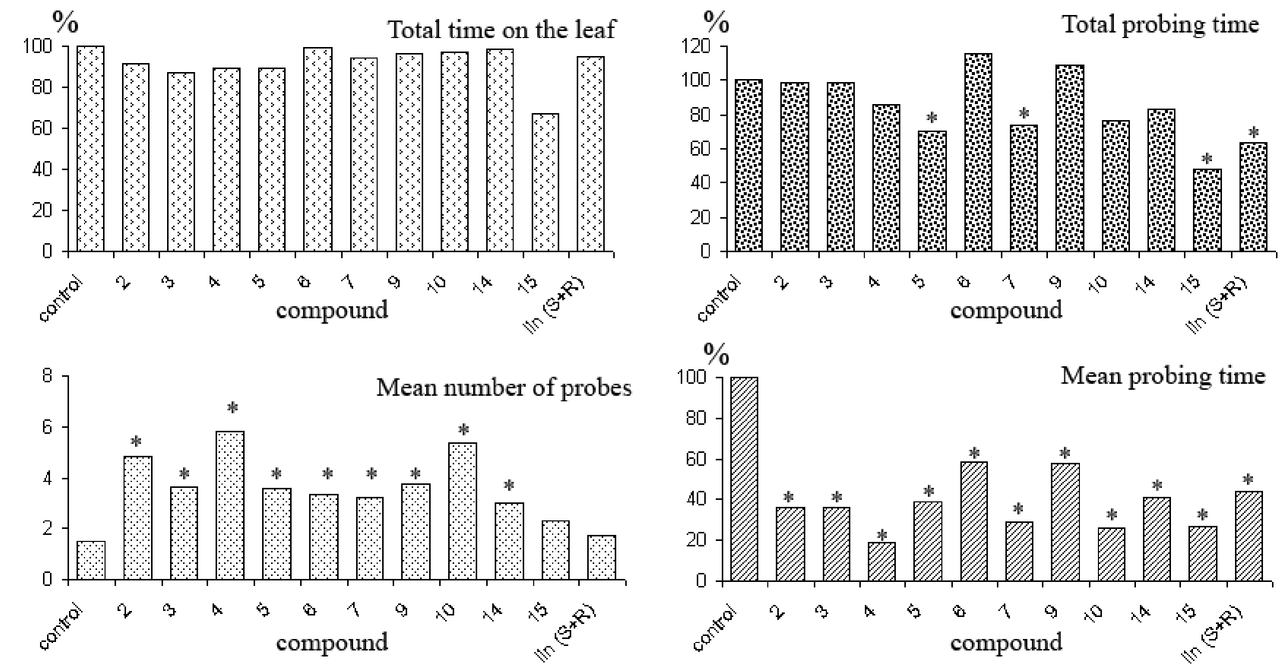

Fig. 4. Behavioural effects of alkyl-substituted γ- and δ-lactones on the peach potato aphid *Myzus persicae*. Astersisks indicate statistically significant differences (ANOVA) as compared to control.

Table 2. Feeding deterrent activity of alkyl-substituted γ- and δ-lactones against *Leptinotarsa decemlineata* Say. A – absolute coefficient of deterrence, R – relative coefficient of deterrence, T – total coefficient of deterrence. Values followed by the same letter within a column are not significantly different at the 0.05 level.

Compound	Coefficients of deterrence					
	larvae			adults		
	A	R	T	A	R	T
2	8.57 ab	16.03 b	24.6 b	32.4 abc	-43.3 a	-10.9 a
3	31.53 d	-21.01 a	10.53 a	11.79 ab	20.46 b	32.25 ab
4	21.22 d	36.72 bc	57.95 bc	30.99 abc	-12.27 a	18.27 ab
5	9.21 ab	17.92 b	27.13 b	29.50 abc	14.73 bcd	44.23 b
6	17.92 bc	23.71 b	41.63 bc	30.0 abc	3.54 abc	33.55 ab
7	20.65 d	43.29 bcd	63.94 bc	19.97 ab	-25.99 a	-6.02 a
9	13.09 ab	42.16 bcd	56.22 bc	16.21 ab	13.20 bcd	29.41 ab
10	36.49 d	76.82 cd	113.31 d	20.84 ab	43.60 cd	64.44 c
14	15.19 bc	66.19 bcd	81.38b cd	39.62 bc	62.18 de	101.80 cd
15	9.92 ab	91.72 d	101.64 d	45.53 c	100.0 e	145.53 d
Linalool	-7.14 a	27.24 bc	20.1 b	2.6 a	-11.05 a	-8.45 a

Unsaturated lactones **14** and **15** are derivatives of linalool, which is a common compound found in floral fragrances [29]. The compound also occurs in potato plants and is an attractant for the CPB [30]. To other insect species, such as *Thrips tabaci* [31], linalool shows deterrent properties, and to *Acanthoscelides obtectus* Say and *Callosobruchus maculatus* Fabr. [32, 33] it acts as a repellent. According to Bolter *et al.* [34], the amount of released linalool, small in intact potato plants, increases dramatically after 2h of feeding by herbivores. The results of our studies also point to the attractant properties of linalool to both larvae and beetles of CPB. A change in the structure of that monoterpene and joining the lactone moiety to isoprenoid chain considerably increased the activity of the resultant isomeric lactones **14** and **15** (the differences are statistically significant)**.** Those compounds were better deterrents against beetles than against larvae. Particularly good antifeedant properties to beetles were found in the lactone with E configuration of the double bond. In the choice test, it completely inhibited feeding, in the no-choice test the beetles consumed 60% less food compared to the control. It was also a good deterrent to larvae but this activity was apparent only in the choice test. In the no-choice situation, the treated food was accepted by the larvae, and the feeding level only slightly differed from control.

Conclusions

The deterrent activity of alkyl-substituted γ- and δ-lactones was species-specific and, in the case of the Colorado potato beetle, also developmental-stage-specific. The structural changes in the molecule affected the activity in a different way in relation to the tested insects. While the Colorado potato beetle feeding was not or only slightly affected by the iodolactones **2**, **3,** the same lactones significantly deterred the peach potato aphid and the effect was of long duration (at least 24 hours). The removal of iodine from the molecule of lactone **2** caused an increase in the deterrent activity of the compounds against adults and larvae of *L. decemlineata* but decreased the antifeedant properties against *M. persicae* (lactones **4** and **5),** which ceased within 24 hours after application. The removal of iodine from lactone **3 i**ncreased its activity against CPB larvae and peach potato aphid and did not have any effect on CPB adults (lactone **6**). However, the transformation of iodolactone **3** into a bicyclic lactone with gem-dimethylcyclopropane system (lactone **7)** caused drastic changes: this compound became an attractant to *M. persicae*, a weak attractant to adults of *L. decemlineata*, and a much stronger antifeedant for larvae of *L. decemlineata* compared to the precursor. The removal of iodine from lactone **9** increased the antifeedant properties for CPB and had no effect for aphids: both, the iodolactone **9** and lactone **10** were strong antifeedants of comparable potency. The saturated lactones **14** and **15** had only little effect on *M. persicae* and a strong deterrent effect on both, the larvae and adults of *L. decemlineata*. In the case of these compounds, the lactonization strongly affected their activity in comparison to the linalool precursor. Linalool was an attractant to CPB and a strong deterrent to aphids.

The described features of the studied compounds allow their consideration as environmentally safe agents for insect pest control. They are highly selective (species- and instar- specific) and their isoprenoid structure ensures their easy biodegradation.

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