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Antifeedant and growth inhibitory effects of extracts and drimanes of *Drimys winteri* stem bark against *Spodoptera littoralis* (Lep., Noctuidae)

Nelson Zapata[1], Flor Budia, Elisa Viñuela, Pilar Medina*

Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Politécnica de Madrid, Ciudad Universitaria, E-28040 Madrid, Spain

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ABSTRACT

Antifeedant and toxic activity of crude plant extracts [n-hexane, acetone, methanol:water (80:20)], and the drimanes (drimendiol, isodrimeninol, isotadeonal and polygodial) isolated from the stem bark of *Drimys winteri* J.R. Forster et G. Forster (Winteraceae) were investigated in the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lep., Noctuidae) using different bioassays. n-Hexane extract at 5000 ppm and the drimane polygodial at 1000 ppm exhibited the strongest antifeedant activity against sixth instar larvae when applied on leaf discs under choice and no-choice conditions (feeding deterrence index: 75.5% and 94.7% for n-hexane and polygodial, respectively, on the choice test). Nutritional indices were calculated after exposure of L6 to the drimanes over a 12 h period, resulting in reduced feeding and growth rates, being significantly higher with polygodial. To distinguish between antifeedant and toxic effect, growth efficiency, calculated from the values of relative consumption and relative growth rates after exposure to non-treated leaf discs for 12 h more to the same larvae which had been exposed on the previous bioassay. From the results, it is concluded that only polygodial and isodrimeninol exert their toxic effects at physiological level. Polygodial was the most potent feeding and growth inhibitor for *S. littoralis* (DC_{50} = 708 ppm and EC_{50} = 198 ppm, respectively).

1. Introduction

Despite all the efforts exerted in protecting crops from noxious pests all over the world, losses due to these causes can annually reach 10–20% (Ferry et al., 2004), still remaining a challenge to be resolved. Currently, synthetic insecticides are the most used mechanisms to control pests. However, concerns over the development of resistance, toxicity and environmental pollution associated with conventional synthetic insecticides compel us to look for new compounds (Coscollá, 2004). Attention is being directed towards plants, that can be an alternative to synthetic insecticides because they have evolved together with herbivorous insects, developing mechanisms to interact and defend themselves (Wink, 2003). In this respect, plants are able to synthetize a broad range of different chemical compounds called secondary metabolites (Howe and Jander, 2008); many of them providing new sources of natural pesticides (Schoonhoven et al., 1998; Isman, 2002, 2006). Furthermore, among the main advantages reported by the use of natural compounds, their narrower toxicity spectrum and fewer environmental impacts stand out (Jermy, 1990; Morimoto and Komai, 2000; Harborne, 2001). These compounds, when biosynthetized, are easily degraded following enzymatic pathways, without any case of bioamplification being reported (Regnault-Roger, 2003).

The most studied secondary metabolites against insects are those with antifeedant activity. It is believed that most of the plants that remain unattacked by insects probably contain antifeedant compounds. Although it is possible to find plant compounds with antifeedant properties belonging to all chemical groups, it does not seem possible to find a sole compound with antifeedant activity for all the insects (Schoonhoven, 1982). The ingestion of secondary metabolites can affect the insect nutrition (Raubenheimer and Simpson, 1992), that can be measured using different nutritional indices such as the relative consumption and growth rates, and growth efficiency (Scriber and Slansky, 1981; Bowers et al., 1991). The study of nutritional indices after the ingestion of secondary metabolites can help to determine whether a chemical compound has not only antifeedant activity but also any toxic post-ingestive effect (Berenbaum, 1986; Liu et al., 1990).

Drimys winteri J.R. Forster et G. Forster (Winteraceae), is a native tree (up to 30 m height) in southern Chile, commonly found in humid and even marshy lands (Hoffmann et al., 1992). It is estimated that there are about 650,000 ha of forests in Chile where *D. winteri* is the main species. Many of these timber forests are commercially cultivated to obtain high value wood (INFOR, 2004). Currently, the Chilean government promotes their exploitation under sustainable management to impel its renovation and

* *Corresponding author*. Tel.: +34 91 336 57 73; fax: +34 91 543 48 79.
E-mail address: pilar.medina@upm.es (P. Medina).
[1] Present address: Facultad de Agronomía, Universidad de Concepción, Avenida Vicente Méndez 595, Chillán, Chile.

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conservation. The forest exploitation of this species produces many non-profitable residues (bark, leaves), that could be employed as raw material for the chemical industry.

D. winteri is one of the sacred plants of the Mapuche Indians who use its aerial parts for the treatment of several diseases in cattle or human beings (Muñoz et al., 1999). Tannins, ascorbic acid and several flavonoids have been isolated from the bark of this species (Muñoz et al., 1999). It is also known that their leaves and bark contain important amounts of essential oils (Barrero et al., 2000). Moreover, several sesquiterpenoids (drimane group) have been isolated from its bark (Cortés et al., 1982; Brown, 1994; Rodríguez et al., 2005). It is supposed that its high content of drimane sesquiterpenoids and monoterpenoids protect this species quite well from herbivorous insects. Nevertheless, the insecticidal activity of *D. winteri* is not well-documented. Drimane derivatives are compounds existing on vegetal species from the families Canellaceae, Winteraceae and Polygonaceae. Sesquiterpenoids of drimane type possess a wide variety of biological activities, including antibacterial, antifungal, cytotoxic, plant growth regulatory, antifeedant and phytotoxic properties (Jansen and Groot, 2004).

In the present paper, we report on the antifeedant and growth inhibitory effects of stem bark of *D. winteri* extracts, as well as the drimanes drimendiol, isodrimendiol, isotadeonal and polygodial against larvae of the generalist herbivore, *Spodoptera littoralis*, a major pest on cotton and different horticultural crops (Gómez de Aizpúrua and Arroyo, 1994). The fact that the insect infests more than 87 host plants belonging to 40 plant families makes it a model of serious polyphagous pests (Sadek, 2003).

2. Materials and methods

2.1. Plant material

The stem bark of *D. winteri* adult trees was collected at the Ñuble province (Chile), in January 2003. A voucher specimen is deposited in the Herbarium of the Faculty of Agronomy, University of Concepción, Chile. Fresh bark was carefully washed with distilled water in abundance to remove any residue. Afterwards, it was dried in an oven (D-6450, Heraeus, Germany) at 35 °C to a constant weight. Once dried, it was stored in hermetic plastic containers at 4 °C.

2.2. Extract preparation

Stem bark was ground to less than 2.0 mm in a hand mill (Micro Hammer Mill, Culatti, Switzerland). Dried ground bark of *D. winteri* (20 g) were extracted sequentially at room temperature for 48 h with n-hexane (2 × 200 ml), 24 h with acetone (2 × 200 ml) and 48 h with methanol:water (80:20) (2 × 200 ml). Each extract was dried down in an evaporator at 35 °C and a pressure of 300 mbar (Rotavapor RE-114 and vacuum pump B-169, Büchi, Switzerland).

2.3. Drimanes isolation

Dried and powdered stem barks of *D. winteri* (120 g) were extracted with light petroleum (2 × 700 ml) for 48 h at room temperature. Filtration followed by evaporation of the solvent under reduced pressure (300 mbar) and low temperature (30 °C) gave a yellowish oily residue (39.13 g, 32.6% on plant material). Part of this extract (17.4 g) was subjected to flash column chromatography [silica gel, 230–400 mesh (570 g), light petroleum–EtOAc mixtures as eluents]. The fractions eluted with 4:1 light petroleum–EtOAc yielded isotadeonal (1.67 g, 3.13% on dry plant material), and elution with 3:1 light petroleum–EtOAc successively gave polygodial (4.35 g, 8.15%) and isodrimeninol (400 mg, 0.75%). Finally, the fractions eluted with 1:1 light petroleum–EtOAc provided small amounts of impure warburganal (25.2 mg, 0.047%) and drimendiol (504 mg, 0.94%). Attempts at obtaining pure warburganal were unsuccessful. The isolated drimanes showed spectroscopic (^{1}H and ^{13}C NMR and mass spectra) data identical with those reported previously for these compounds.

2.4. Insects rearing

S. littoralis larvae were selected from established colonies never exposed to insecticides and maintained for more than 10 generations under laboratory conditions. Larvae were reared on an artificial diet, slightly modified from those of Poitout and Bues (1974). All colonies and experiments were reared in a climatic chamber at 25 ± 2 °C, 75 ± 5% R.H. and 16:8 (L:D) light:dark photoperiod.

2.5. Bioassays

Newly six instar *S. littoralis* larvae (less than 12 h from the last moulting) and homogeneous weight (228 ± 18 mg) were selected in the morning and placed into clean Petri dishes without food. Larvae were starved 4–5 h prior to each bioassay. Fresh leaf discs were cut from sweet pepper (*Capsicum annuum* L.) cv. Largo de Reus leaves grown in the lab, using a cork borer (1.5 cm diameter). Each experimental unit consisted of a plastic Petri dish (9 cm diameter; 1.5 cm height) containing 20 ml of an agar solution (1.5%) to prevent desiccation (Escoubas et al., 1993). Eight circular sections of agar (1.5 cm diameter) were cut and substituted by the leaf discs. Afterwards, every disc was treated with the corresponding carrier solvent, extract or drimane derivative. Once the solvent had evaporated and after 4–5 h of starvation, one larvae was deposited into the centre of each disc using forceps and allowed to feed.

Data of leaf discs consumed and larval weight increase were expressed as dry weight (mg). After each experiment, larvae were frozen and, then, dried in an oven at 60 °C. The estimation of initial dry weight of larvae was done using the following linear regression: Y(dry weight, mg) = 0.1212X(fresh weight, mg) − 0.788; R^2 = 0.923; N = 143. The remnants of leaf discs were collected and dried to determine the amount consumed and refer all data to dry weight. Initial dry weight was estimated at the beginning of each bioassay by employing a conversion factor calculated when 5 groups of 8 leaf discs were dried.

2.5.1. Antifeedant activity

Antifeedant activity of test substances was assayed by using leaf disc choice and no-choice test as follows:

Control leaf discs were painted on one side with 20 μl of the carrier solvent and test leaf discs with the same amount of the test substances, n-hexane, acetone and methanol:water (80:20) extracts at a solution of 5000 ppm and drimane derivatives at 1000 ppm. Ethanol was the carrier solvent used in case of drimane derivatives. In choice test, only alternating leaf discs were treated with the test substances (C = control leaf disc; T = treated leaf disc), whereas all leaf discs were treated in the no-choice test (T = treated leaf disc). A control variant (Cv = control leaf disc), with only the carrier solvent was established in every bioassay as a reference to stop the experiment. When approximately 50% of the control leaf discs in choice test and 75% in no-choice test had been eaten, larvae were removed from the Petri dishes. 10 replicates per treatment and control variant were used in every test.

From choice test data, it could be calculated: feeding deterrence index (FDI) = 100[($C - T$)/($C + T$)], where C and T are the control and treated leaf weight consumed by the insect (Sadek, 2003). This