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Developmental distribution of insect juvenile hormone III in the sedge, *Cyperus iria* L.

J.C. Bede[a], W.G. Goodman[b], S.S. Tobe[a,*]

[a]*Department of Zoology, University of Toronto, Toronto, Ontario, Canada*
[b]*Department of Entomology, University of Wisconsin, Madison, WI, USA*

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Abstract

In insects, juvenile hormones (JHs) regulate physiological processes such as metamorphosis and reproduction but their presence in plants is poorly understood. The temporal and tissue distribution of insect juvenile hormone III (JH III), methyl-10*R*,11-epoxy-3,7,11-trimethyl 2*E*,6*E*-dodecadienoate, was determined in sedge (*Cyperus iria* L.) over an eight month developmental time course, from the seedling to the senescent plant. Juvenile hormone III levels increased in immature plants until flowering, at which time cessation of plant growth and a transient decrease in JH III content was observed. In mature plants, JH III levels in all plant tissues again increased. In older plants, JH III levels in aerial tissues declined as they became senescent. This decrease was not observed in root tissues which remained viable over the duration of the time course. Moreover, 85% of the total JH III in the plant was present in the roots, suggesting that they are a site of synthesis and/or storage.

Keywords: Cyperus iria; Cyperaceae; Juvenile hormone III; Insects; Sedge; Developmental distribution

1. Introduction

Insect juvenile hormones (JHs) are structurally-related sesquiterpenoids that regulate developmental processes such as metamorphosis and reproduction (Gilbert, Rybczynski & Tobe, 1996). The insect hormone ecdysone and its metabolites are responsible for induction of the moulting process, whereas the titer of JH in the insect haemolymph prior to the moult determines the nature of the moult (Gilbert et al., 1996). In adult females of most insect species, JHs are also involved in the regulation of reproduction (Tobe & Stay, 1985).

One defensive strategy of plants against insect herbivory is the production of secondary metabolites that interfere with insect physiological functions (Rosenthal & Berenbaum, 1991; Rosenthal & Janzen, 1979). Plants may contain compounds that either mimic JH activity, such as juvabione isolated from balsam fir, *Abies balsamea* (L.) Miller (Bowers, Fales, Thomson & Uebel, 1966) or that act as antagonists by inhibiting JH biosynthesis, such as the precocenes found in *Ageratum houstonianum* Miller (Bowers, Ohta, Cleere & Marsella, 1976). In 1988, insect juvenile hormone III (JH III), methyl-10*R*,11-epoxy-3,7,11-trimethyl 2*E*,6*E*-dodecadienoate (Fig. 1), and its metabolic precursor in insects, methyl farnesoate, were first reported in the sedges *Cyperus iria* L. and *C. aromaticus* (Ridley) Mattf and Kük (Toong, Schooley & Baker, 1988).

Cyperus iria is a tufted annual sedge native to Eurasia (Holm, Plucknett, Pancho & Herberger, 1977), with red fibrous roots and a long lower bract. In the present study, the distribution of JH III in the sedge

* Corresponding author. Tel.: +1-416-978-3517; fax: +1-416-978-3522.

E-mail address: tobe@utcc.utoronto.ca (S.S. Tobe).

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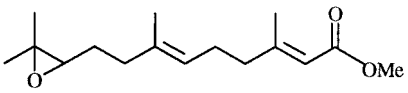

Fig. 1. Insect juvenile hormone III, methyl-10*R*-epoxy-3,7,11-trimethyl 2*E*,6*E*-dodecadienoate.

C. iria was investigated over an eight month period using a radioimmunoassay (RIA). This RIA was originally developed for quantification of JH in insect haemolymph (Goodman, Orth, Toong, Ebersohl, Hiruma & Granger, 1995) and has been recently optimized for the analysis of plant tissue (Bede, Goodman & Tobe, 1999). This technique allowed measurement of JH III in small amounts of plant material (ca. 10 mg) permitting separate analysis of root, culm (stem), leaf, bract, inflorescence and nutlet (fruit) tissues. Investigation of the distribution of JH III in these plant tissues may lead to a better understanding of the role that this compound plays in the plant.

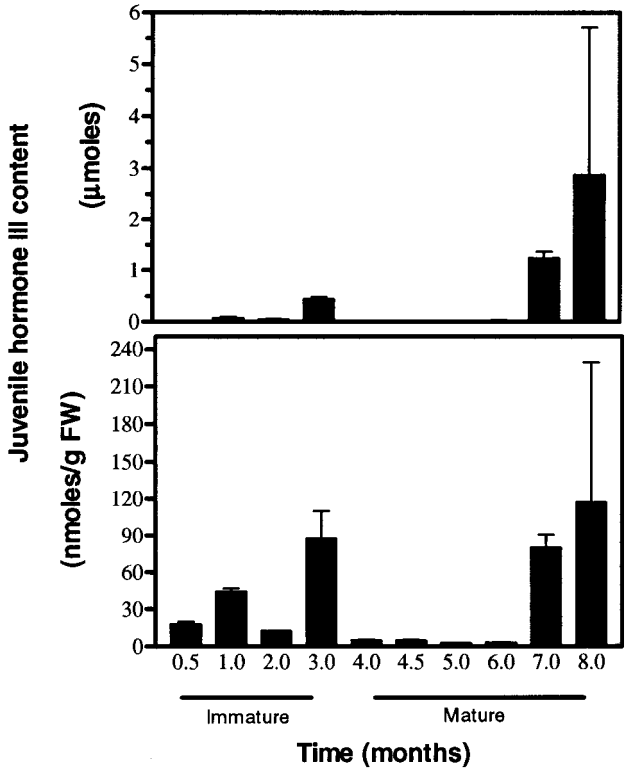

Fig. 2. *Time course of juvenile hormone III accumulation in Cyperus iria plants*. The developmental distribution of JH III was measured by radioimmunoassay using antiserum 31867 (1 : 1750) and [^{3}H-methyl]-10*R*, 11-JH III as the radiotracer. Four individuals were sampled over an eight month period, except immature 0.5 month-old plants (n = 8) and mature 5, 6 and 8 month-old plants (n = 3). Bars represent the mean ± standard error. *Top panel: Amount of juvenile hormone III. Bottom panel: Juvenile hormone III levels.* The amounts of JH III were determined per gram of plant tissue (FW).

2. Results and discussion

2.1. Juvenile hormone III levels in whole plants of C. iria

Juvenile hormone III levels increased in immature plants of *C. iria* from 17.6 nmol/g fresh weight (FW) in two week old plants to 87.8 nmol/g FW over the first three months. At flowering, a dramatic decrease of JH III in all plant tissues was observed (Fig. 2). One possible explanation for this is that at maturation, the plant directs its resources into developing reproductive tissue rather than into vegetative growth and the biosynthesis of this secondary metabolite. Juvenile hormone III levels subsequently increased over the next three months to approximately 1.24 µmol in the mature plant. In 8 month-old plants, levels of JH III were highly variable between plants, which may reflect their state of senescence.

2.2. Juvenile hormone III levels in aerial tissues

Fig. 3(a) and (b) depict JH III levels in the aerial tissues. In immature plants, the aerial tissues consisted of culm and leaf which were not separated. After flowering, plants were divided into culm (stem), leaf, bract and inflorescence tissues. In 4–4.5 month-old plants, inflorescence tissue also included the fruit; in older plants (5–8 months), JH III content of nutlets was measured separately.

Juvenile hormone III levels in aerial tissues of immature plants did not significantly change until flowering (Fig. 3(a)) (one-factor ANOVA (Analysis of Variance), $p = 0.355$), although the total amount of JH III increased from 3.0 to 8.6 nmol (one-factor ANOVA, $p = 0.006$). As plants matured, JH III levels in these tissues declined, then increased again over the next three months.

Further analysis of plant tissues revealed that JH III levels remained low in culm and leaf tissues until seven months, at which time a significant increase in levels was observed (Fig. 3(a)) (one factor ANOVA, $p = 0.004$ (culm); $p < 0.001$ (leaf)). Juvenile hormone III levels decreased in both tissues as aerial parts became senescent.

A similar developmental profile was observed in bracts and inflorescences (Fig. 3(b)). In mature plants, the JH III levels increased to their highest in 7 month-old plants and then declined in senescent tissue. There was no distinguishable difference in JH III levels between the bract and inflorescence (paired t-tests, $p = 0.928$ (4 months), $p = 0.138$ (4.5 months), $p = 0.060$ (5 months), $p = 0.337$ (6 months), $p = 0.666$

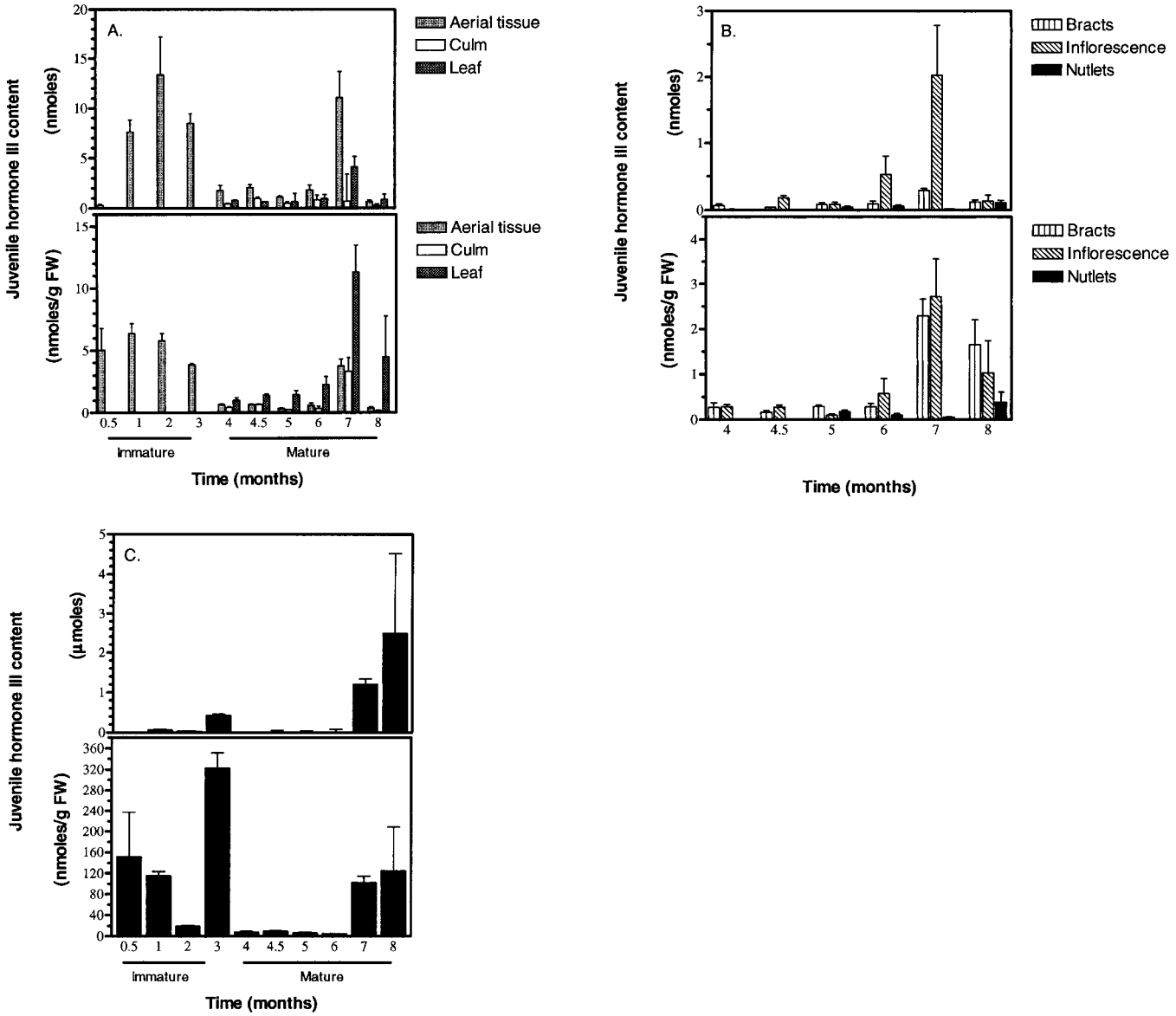

Fig. 3. *Time course of juvenile hormone III distribution in tissues of Cyperus iria*. The developmental distribution of JH III was measured by radioimmunoassay using antiserum 31867 (1 : 1750) and [^{3}H-methyl]-10*R*,11-JH III as the radiotracer. (a) *Distribution of juvenile hormone III in culm, leaf and total aerial tissues*. In immature plants (before flowering), there was insufficient material to allow separate analysis of culm and leaf tissues. *Top panel: Amount of juvenile hormone III*. Bars represent the mean ± standard error of three to five individuals. *Bottom panel: Juvenile hormone III levels*. The amounts of JH III were determined per gram of plant tissue (FW). Bars represent the mean ± standard error of three to five individuals. (b) *Distribution of juvenile hormone III in bracts, inflorescence and seeds*. In mature plants, the amounts of JH III were determined in flowering tissues which were divided into the bracts (modified leaves surrounding inflorescence), inflorescence (flowers, pedicels and peduncles) and nutlets. Due to insufficient material, prior to five months, nutlets were not analyzed separately from the inflorescence. *Top panel: Amount of juvenile hormone III*. Bars represent the mean ± standard error of three to five individuals. *Bottom panel: Juvenile hormone III levels*. The amounts of JH III were determined per gram of plant tissue (FW). Bars represent the mean ± standard error of three to five individuals. (c) *Distribution of juvenile hormone III in subterranean tissues of C. iria. Top panel: Amount of juvenile hormone III*. Juvenile hormone III amounts were measured in the root tissue. Bars represent the mean ± standard error of three to four individuals. *Bottom panel: Juvenile hormone III levels*. The amounts of JH III were determined per gram of plant tissue (FW). Bars represent the mean ± standard error of three to four individuals.

(7 months), $p = 0.516$ (8 months)). However, the amount of JH III in these tissues was low and represented less than 20% of that detected in the aerial parts. Juvenile hormone III levels in nutlets did not significantly change over the period of study (one factor ANOVA, $p = 0.226$) (Fig. 3(b)).

2.3. Juvenile hormone III levels in subterranean tissues

Most of the total JH III in the plant (>85%) was sequestered in the fibrous roots. In immature plants, JH III levels increased until flowering (Fig. 3(c)). Again, it was observed that JH III levels dropped in

root tissues as the plant matured and then increased over the next three months. Juvenile hormone III levels in root tissues did not change significantly in mature 7 and 8 month-old plants (Fig. 3(c)) (unpaired t-test, $p = 0.799$ (concentration); $p = 0.557$ (amount)). This differs from the observation in aerial tissues, in which a significant decrease in JH III levels was measured at this time (Fig. 3(a) and (b)). Aerial tissues become senescent after seed production in these plants. Therefore, we believe that there is an inhibition of JH III biosynthesis and/or transport of the compound out of these senescent tissues. However, since vegetative propagation in these plants also occurs through tillers, the root tissue remained viable and contained high levels of JH III over the time course.

The cellular site of JH III accumulation in the sedge is unknown. In plants, lipophilic secondary metabolites are often sequestered in specialized cells or resin ducts, or covalently modified by the addition of sugars or phosphate groups for storage in the vacuole (Fahn, 1979; Grebenok, Venkatachari & Adler, 1994; Hösel, 1981). Secretory oil cells have been characterized in some taxa in the family Cyperaceae (Kern, 1972–76; Metcalfe, 1971). However, the presence of JH III in these cells in the sedge *C. iria* has not yet been demonstrated.

2.4. Juvenile hormone III levels in a plant cluster

The above profiles represent the developmental distribution of JH III in individual *C. iria* plants. However, tillers may be present as early as 2 months after the germination of primary plants. These secondary plants were not included in the above analyses. In general, the amounts and JH III levels in these plantlets reflected the developmental profile observed in individual plants in which accumulation of this secondary metabolite increased in the tissue over time and was more prevalent in inflorescence and leaf tissues than in the culm (data not shown).

2.5. Conclusions

In the original report identifying JH in a plant, JH III was isolated by steam distillation and identified, following chemical derivitization, by electron impact mass spectroscopy (Toong et al., 1988). The authors determined that the plant contained 151 μg JH III/g FW. This is comparable to levels found in a subsequent study in which 193 and 143 μg JH III/g FW were detected in leaves of 1 and 2 month-old plants, respectively (Schwartz, Paskewitz, Orth, Tesch, Toong & Goodman, 1998). These values are approximately seven times the levels detected in the present investigation, though plant analyses in both studies were performed using a RIA which was able to detect pg quantities of JH III. Discrepancies in JH III levels are attributed to different cultivars and environmental conditions under which the plants were grown.

It is tempting to speculate that the high level of JH III in *C. iria* may be involved in the protection of the plant against insect herbivory. Juvenile hormone titer is precisely regulated during insect development. During the final larval stadium, there is a drop in haemolymph titer prior to the moult to an adult (Szibbo, Rotin, Feyerisen & Tobe, 1982) and topical application of JH or synthetic analogues at this stage results in the inappropriate retention of juvenile characteristics at the next moult (Sehnal, 1983). Also, application of these compounds to insect eggs can disrupt embryonic development (Riddiford, 1972) through mechanisms ranging from immediate ovicidal effects to delayed developmental effects. The levels of JH III observed in the plant are significantly higher than the amounts found in insects. In the female cockroach, *Diploptera punctata*, JH III concentrations reached their maximum 4 to 5 days after mating. In whole body extracts, the level of JH III is approximately 504 ng/g (day five) (Tobe, Ruegg, Stay, Baker, Miller & Schooley, 1985). By comparison, 7 month-old *C. iria* plants contain on average a total of 327.7 μg JH III which is approximately 21 μg/g FW, over 40 times the level found in day five, mated female cockroaches.

There have been few investigations on the interactions between *C. iria* and associated insects. In laboratory studies, third stadium grasshopper nymphs, *Melanoplus sanguinipes*, were fed a diet of wheat seedlings or *C. iria*; no difference in growth was observed (Toong et al., 1988). However, following the moult to an adult, 90% of those fed exclusively *C. iria* showed deformed wings and other morphological changes consistent with development in the presence of excess JH, and the adult female grasshoppers were infertile. Leaves of *C. iria* exhibited a larvicidal effect when added to water containing larvae of the mosquito, *Aedes aegypti* (Schwartz et al., 1998). In field studies, eggs of the dipteran leafminer, *Hydrellia* sp., did not hatch if laid on leaves of *C. iria* (Meneses & Garciá de la Osa, 1988). It is assumed that these above effects were related to the JH III content of the plant. However, in other reports, *C. iria* had no effect on insect development (Dela Cruz, 1986; Naresh & Smith, 1984). It is unclear whether these insects were able to avoid adverse developmental effects by metabolizing or sequestering the ingested JH or by avoiding tissues that contained high levels of the compound. However, other biological functions of JH III, such as its potential role as an allelopathic agent in plant–plant interactions or in plant defense against nematode, fungal or bacterial attack, should also be recognized.

3. Experimental

3.1. Plants

Sedges were grown in the greenhouse (Department of Botany, University of Toronto, January–October 1997, photoperiod ranged from 9.00 : 15.00 (January) to 10.29 : 13.31 (October) (light : dark)) from seeds obtained from Y. Toong (Penang, Malaysia). Plants were grown in individual pots in organic potting mix (Home Gardener) and kept continuously moist by maintaining the plants in 2–5 cm water. Greenhouse temperatures ranged from approximately 22°C in the winter months to ambient during the summer. Voucher specimens of *C. iria* have been deposited at the Royal Ontario Herbarium, Canada (TRT).

3.2. Plant analysis

Immature plants were assayed at 0.5, 1, 2 and 3 months (1 month = 4 weeks) post-germination. At approximately 4 months, the bracts opened, exposing the inflorescence. Plants at this stage were defined as mature and assayed at 4, 4.5, 5, 6, 7 and 8 months. Immature plants were divided into aerial tissues and subterranean tissues (roots). After flowering, mature plants were divided into root, culm (stem), leaf, bract (modified leaves which subtend the inflorescence) and the inflorescence (comprised of compound multiple spikes, flowers and nutlets (fruits)) tissues. A month after flowering, nutlets were analyzed separately from the rest of the inflorescence. Amounts of JH III were compared in the different plant tissues during development. In this paper, the level of JH III in the plant refers to the amount of JH III extracted per gram of plant tissue (FW).

3.3. Extraction

Plant tissues were ground in acid-washed sand (Sigma) and liquid nitrogen. After maceration, between 0.1 and 0.4 g of plant material was weighed and extracted overnight in 2 ml pentane (HPLC grade, Burdick and Jackson) at RT (22–24°C). In most cases, sufficient tissue was available for triplicate samples. After incubation, the mixture was vortexed and centrifuged (450 $g \times 5$ min) to pellet the insoluble plant material. The organic phase was removed and the extraction repeated twice. Combined supernatants were evaporated under a stream of nitrogen. Samples were resuspended in toluene (ACS, BDH) for analysis by RIA.

3.4. Radioimmunoassay

Juvenile hormone III was quantified by RIA as previously described in Ref. (Bede et al., 1999). From the standard curve (four parameter logistic curve, Graphpad Prism, version 1.0), concentrations of unknown samples were interpolated. Assays were performed in triplicate.

3.5. Statistics

Analyses were performed using the statistical package SPSS 7.5. One-factor ANOVA tests were conducted to evaluate the relationship between JH III levels in plant tissue over the developmental time course. Unpaired student's *t*-tests determined JH III differences between two time points.

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