BIOLOGICAL AND ECOLOGICAL CONSEQUENCES OF *DIOLCOGASTER* SP. (HYMENOPTERA: BRACONIDAE) PARASITIZING *AGARAEA MINUTA* (LEPIDOPTERA: ARCTIIDAE) AND THE EFFECTS ON TWO *COSTUS* (COSTACEAE) PLANT SPECIES IN BRAZIL

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Abstract

Costus spicatus (Jacq.) Sw. and *Costus spiralis* (Jacq.) Roscoe var. *spiralis* (Costaceae) are economically important plants due to their pharmacological and medicinal properties and ornamental value. These plants are natives from the Brazilian Atlantic Rainforest and are fed upon by *Agaraea minuta* Schaus, 1892 (Lepidoptera: Arctiidae). This study describes the damage done by *A. minuta* on *C. spicatus* and *C. spiralis* and the biological and ecological aspects of parasitism of *A. minuta* by *Diolcogaster* sp. (Hymenoptera: Braconidae). Twenty stems of *C. spicatus* and *C. spiralis* with 100 last-instar caterpillars of *A. minuta*, were collected per plant in each of 2 years. The stem heights (F, $P > 0.05$), leaf lengths (F, $P > 0.05$), leaf widths (F, $P > 0.05$) and the number of leaves per stem (F, $P > 0.05$) of both plant species; number of pupae obtained from caterpillars of *A. minuta* (F, $P > 0.05$), adult emergence of this defoliator (F, $P > 0.05$) and of *Diolcogaster* sp. (F, $P > 0.05$) were similar during the 2 study periods. *Agaraea minuta* fed on *C. spicatus* and *C. spiralis*, and *Diolcogaster* sp. was shown to be a parasitoid suppressor of populations of this defoliator.

Key Words: Arctiidae, biological control, Costaceae, defoliation, parasitoids

Resumo

Costus spicatus (Jacq.) Sw. e *Costus spiralis* (Jacq.) Roscoe var. *spiralis* (Costaceae) são plantas economicamente importantes devido às suas propriedades farmacológicas e medicinais e valor ornamental. Essas plantas são nativas da Mata Atlântica brasileira e alimento para *Agaraea minuta* Schaus, 1892 (Lepidoptera: Arctiidae). Esse estudo descreve o dano por *A. minuta* sobre *C. spicatus* e *C. spiralis* e aspectos biológicos e ecológicos de seu parasitismo por *Diolcogaster* sp. (Hymenoptera: Braconidae). Vinte hastes de *C. spicatus* e *C. spiralis*, com 100 lagartas de último estádio de *A. minuta*, foram coletadas por planta em cada um de dois anos. A altura das hastes (F, $P > 0,05$), comprimento das folhas (F, $P > 0,05$), largura das folhas (F, $P > 0,05$) e o número de folhas por haste (F, $P > 0,05$) de ambas as espécies de plantas; número de pupas obtidas de lagartas de *A. minuta* (F, $P > 0,05$), emergência de adultos desse desfolhador (F, $P > 0,05$) e de *Diolcogaster* sp. (F, $P > 0,05$) foram semelhantes durante os dois períodos de estudo. *Agaraea minuta* pode se alimentar de plantas de *C. spicatus* e *C. spiralis*, e *Diolcogaster* sp. mostrou ser um parasitóide supressor de populações desse desfolhador.

Palavras Chave: Arctiidae, controle biológico, Costaceae, desfolha, parasitóides

The ginger plants, *Costus spicatus* (Jacq.) Sw. and *Costus spiralis* (Jacq.) Roscoe var. *spiralis* (Zingiberales: Costaceae), are plants of economic importance because of their pharmacological and medicinal properties as well as their ornamental value. They are native to the Atlantic Rainforest biome of Brazil. *Costus spicatus* is an herbaceous species with a hard stem, alternate, invaginate, dark green, hairy leaves and yellow flowers with crimson-colored bracts (Campos et al. 2008). Its rhizomes, leaves, bark, and stems have pharmacological and medical properties, such as diuretic, anti-fever and weight loss (Silva et al. 2008; Quintans et al. 2010). *Costus spiralis* has tortuous and branched stems, spirally arranged dark green leaves and red, odorless bracts and flowers (Viel et al. 1999; Antunes et al. 2000). This plant is used to manufacture pharmacological and herbal drugs and has been used in antidiabetic, antirheumatic, diuretic, sudorific and tonic drugs (Da Silva & Parente 2004; Britto et al. 2011).

Caterpillars of *Agaraea minuta* Schaus, 1892 (Lepidoptera: Arctiidae) (Suppl. Figs. 1A and 1B), which feed on *C. spicatus* and *C. spiralis*, are hairy and active during the d. Its adults are small and light brown in color (Suppl. Figs. 2A, 2B, 2C and 2D) (Watson & Goodger 1986). Two males and 1 female, and 1 male and 1 female of this insect were originally collected in Tabasco (Mexico) and in Las Mercedes (Guatemala), respectively (Hampson 1901). In Brazil *A. minuta* has attracted little attention, probably because until recently it was regarded only as a pest of ornamental plants of minor economic importance.

Species of *Diolcogaster* (Hymenoptera: Braconidae) (Suppl. Figs. 3B, 3C, 3D and 3E) are solitary or gregarious endoparasitoids of Macrolepidoptera (Noctuidae, Geometridae and Pyraloidea) and of microlepidopteran leafminer larvae in the native forest of Rio Grande do Sul State, Brazil (Restello & Penteado-Dias 2006). In agricultural settings worldwide they were recorded from caterpillars of Arctiidae, Geometridae, Lasiocampidae, Limacodidae, Lymantriidae, Noctuidae, Notodontidae, Plutellidae, Pyralidae, Tenthredinidae, and Thaumetopoeidae (Whitfield et al. 2009; Fernández-Triana 2010; Zeng et al. 2011). Color photographs of these organisms and damage to *Costus* spp. are available online at http://purl.fcla.edu/fcla/entomologist/browse and each figure is designated herein as a Suppl. Fig.

Because of recent interest in the cultivation of *Costus* spp. there is concern about their pests and how they might be managed. This study describes: 1) the damage that *A. minuta* caterpillars caused to *C. spiralis* and *C. spicatus* plants over 2 consecutive yr, and 2) mortality inflicted by *Diolcogaster* sp. on *A. minuta*.

Materials and Methods

Experimental Site

We studied one clump of *C. spicatus* and one clump of *C. spiralis*, both of which were approximately, 5 yr old and located in the herbarium of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil (S 20°45' W 42°51' at 651 m asl). The distance between the clumps was 72.4 m. The herbarium was shaded and humid throughout the whole yr; contained fertile soil covered with litter and grew native shrubs and trees of the Brazilian Atlantic Rainforest (Tavares et al. 2011a, 2011b).

Evaluating Clumps of *Costus*

The occurrence of lepidopteran defoliators was monitored on the clumps of *C. spicatus* and *C. spiralis* (Suppl. Figs. 1A, 1B, 1C, 1D, 1E, 1F, 1G and 1H) in May 2010 and 2011 because of their prior appearance during this month in 2009. The height of the stems, number of leaves per stem and the length and width of the leaves of 20 stems of each species were evaluated in May 2010 and 2011. The experimental design was a randomized block with each stem considered as a replicate (T1- 20 stems of *C. spicatus* collected in 2010; T2- 20 stems of *C. spiralis* collected in 2010; T3- 20 stems of *C. spicatus* collected in 2011 and T4- 20 stems of *C. spiralis* collected in 2011). The data were submitted to variance analysis (ANOVA) and the means for each species compared between the 2 yr periods using Tukey's test at the 5% significance level with the computer program SAEG (2007) (Supplier: UFV).

Collecting *Agaraea minuta* Caterpillars

One hundred last-instar caterpillars of *A. minuta* (Suppl. Figs. 2G and 2H) per clump of *C. spicatus* and *C. spiralis* were collected with a brush in May 2010 and 2011. Caterpillars were placed in plastic pots (20 caterpillars per pot) with 2 leaves of the host plants as food and substrate for pupation and these were changed daily. The petioles of the leaves were moistened to avoid desiccation, and the pots were kept in the Laboratory of Biological Control of Insects (LCBI) of UFV at 25 ± 1 °C at 12:12 h L:D and 70 ± 10% RH. Previous studies showed that *Diolcogaster* sp. parasitize early larval stages of *A. minuta*.

Biological Aspects of Parasitism of *Agaraea minuta* by *Diolcogaster* sp.

The number of pupae and the percentage of adult emergence of *A. minuta* and the parasitoids were recorded in a randomized design (T1-

100 caterpillars of *A. minuta* collected in 2010 on *C. spiralis*; T2- 100 caterpillars of *A. minuta* collected in 2010 on *C. spicatus*; T3- 100 caterpillars of *A. minuta* collected in 2011 on *C. spiralis* and T4- 100 caterpillars of *A. minuta* collected in 2011 on *C. spicatus*). The pupae obtained were individually put into 50 mL plastic cups separately per treatment. Each pupa was considered as a replicate. The data were submitted to ANOVA and the means for each plant species and comparisons between the 2 yr periods were analyzed using Tukey's test at the 5% significance level.

Damage on Clumps of *Costus*

Stem heights, leaf lengths, leaf widths, and number of leaves per stem for *C. spicatus* and *C. spiralis* were measured and the damage (parts fed, aspects after feeding, and viability of stems) done to the clumps by the caterpillars were visually observed during the collection of the caterpillars in the herbarium (May 2010 and 2011) and in the following month (June 2010 and 2011) in order to quantify the effect of *A. minuta* caterpillars on the plants. The caterpillars were removed after the collections to quantify the parasitism rate in the laboratory, which can affect the damage.

Insect Identification

Adults of *A. minuta* were mounted with entomological pins in polystyrene supports and some were sent to Dr. Vitor Osmar Becker, Uiraçu Institute in Camacan, Bahia State, Brazil, for identification. Individuals of the parasitoid, *Diolcogaster* sp. (Suppl. Figs. 3B, 3C, 3D and 3E), that had emerged from *A. minuta* pupa, were transferred to 70% ethanol following identification by M.Sc. Geraldo Salgado-Neto. Voucher specimens were stored at the Regional Entomological Museum of the Federal University of Viçosa (UFVB) and at the Entomological Museum of the Federal University of Santa Maria (UFSM).

Results

Parameters of Clumps of *Costus*

A comparison of *C. spicatus* and *C. spiralis* plants showed that the stem heights, number of leaves per stem and leaf widths and lengths (F, $P > 0.05$) were similar between treatments (Table 1).

Pupae and Adults of *Agaraea minuta* and Larval Parasitism by *Diolcogaster* sp.

The number of pupae (Suppl. Figs. 2E and 2F) obtained from caterpillars collected from plants of *C. spicatus* and *C. spiralis* and the adult emergence of *A. minuta* and *Diolcogaster* sp. (F, $P > 0.05$) were similar in both yr (Table 2).

Damage on *Costus spicatus* and *Costus spiralis*

Caterpillars of *A. minuta* fed on the apical buds (Suppl. Figs. 1C and 1D) and on the leaves (Suppl. Figs. 1E and 1F) of *C. spicatus* and *C. spiralis*, but left the midribs, scarifying them but leaving the epidermis intact. These apical buds and leaves dried quickly becoming light brown and nonviable (Suppl. Figs. 1G and 1H). However, some stems remained green and developed new shoots at the ground level or the apical bud began to grow again. This enabled the plant to survive.

Parasitism on *Agaraea minuta* Larvae by *Diolcogaster* sp.

Agaraea minuta caterpillars that had been parasitized produced a cocoon made of white colored silk (Suppl. Fig. 3A). Pupation occurred on the abaxial surfaces of the leaves of *C. spicatus* and *C. spiralis* as well as on the covers of the plastic cups. The silk was wrapped around the leaves causing them to fold and become cone-shaped, which might reduce the photosynthesis rate. On the other hand, this process may lead to a reduction in caterpillar predation. Only one species of parasitoid was found and only a single individual emerged per *A. minuta* pupa. The caterpillars of *A. minuta* were of different ages, suggesting that

Table 1. Stems evaluated, stem heights (m), leaves per stem, largest width and greater length (cm) (Mean ± SE) of *Costus spicatus* and *Costus spiralis* (costaceae) in Apr 2010 and 2011.

	Costus spicatus		*Costus spiralis*	
Parameters	2010	2011	2010	2011
Stems evaluated	20	20	20	20
Stem heights	1.73 ± 0.28	1.68 ± 0.26	1.44 ± 0.35	1.42 ± 0.33
Leaves/Stem	14.0 ± 3.0	12.0 ± 2.0	22.0 ± 6.0	20.0 ± 5.0
Largest width	18.3 ± 2.7	18.2 ± 2.6	18.3 ± 3.3	18.1 ± 3.1
Greater length	45.6 ± 5.4	45.4 ± 5.3	15.3 ± 2.5	15.1 ± 2.4

Means between the years 2010 and 2011, within each row per plant species did not differ by Tukey's test at the 5% significance level.

Table 2. Number of pupae from *Agaraea minuta* (Lepidoptera: Arctiidae) caterpillars collected on *Costus spicatus* and *Costus spiralis* (Costaceae) plants and percent of adult emergence of this defoliator and of *Diolcogaster* sp. (Hymenoptera: Braconidae).

	Costus spicatus		*Costus spiralis*	
Parameters	2010	2011	2010	2011
Caterpillars evaluated	100	100	100	100
Pupae number	97	98	96	98
Parasitoids - # emerged	89	90	91	93
Adults moths - # emerged	11	10	09	07

Means between the years 2010 and 2011, within each row per plant species did not differ by Tukey's test at the 5% significance level.

they had originated from eggs laid at different times, although only the last-instar larvae were collected. At least 2 generations of *A. minuta* were observed per yr in the field.

The caterpillars of *A. minuta* showed gregarious feeding habits and caused greater damage to the clumps of *C. spicatus* (Suppl. Fig. 1H) than to those of *C. spiralis* (Suppl. Fig. 1G), because green tissues remained in some leaves of the latter species. Egg masses of *A. minuta* were found deposited at the tops of the plants where the caterpillars began feeding after hatching. Some feces of these caterpillars remained on damaged leaves but some fell to the soil. All leaves and apical buds of *C. spicatus* plants were damaged by caterpillars after each infestation in the 2 yr study.

Discussion

Agarea minuta appears to be unaffected by the putative pharmacologically active substances of *Costus* spp. The roots of *Costus* spp. are rich in saponins (heterosides from plant secondary metabolism), which are known insect deterrents (Da Silva et al. 1999), but extracts of *Costus* spp. leaves (without saponins) showed no deterrent effect on insects, for example, after leaves had been offered to *Paraponera clavata* F., 1775 (Hymenoptera: Formicidae) (Dyer et al. 2003).

Damage by *A. minuta* was sufficiently severe to reduce leaf yield, which is used in medical and pharmacological products (Da Silva & Parente 2004; Silva et al. 2008; Quintans et al. 2010; Britto et al. 2011). *Costus* spp. used by the folk medicine are generally cultivated without synthetic pesticides. Fresh feces on the leaves or on the soil indicate the presence of caterpillars in the plants. To reduce caterpillar feeding, there is a need to use alternative methods of control such as diversification and crop rotation, trap plants, alternative insecticides, places of refuge for natural enemies, etc.

Agaraea minuta has potential to defoliate *C. spicatus* and *C. spiralis* plants, although it damages the former more severely than the latter. The parasitoid, *Diolcogaster* sp., could suppress populations of *A. minuta*, which could result in increased plant biomass.

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