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Reproductive phenology and pre-dispersal seed-feeding in *Protium towarens* (Burseraceae), with a description of the first known phytophagous “*Bracon*” species (Hymenoptera: Braconidae: Braconinae)

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Abstract

The reproductive phenology of the cloud forest tree *Protium towarens* (Burseraceae) is analysed for one population in northern Venezuela. Reproductive phenophases were monitored using both long-term (21 years) and detailed short-term (4 years) surveys of flower and fruit set. The reproductive phenology of this tree varies, with periods in which the species behaves as a supra-annual reproducer, and other periods in which it reproduces annually, at the end of the rainy season. Marked spatial variation in reproductive condition was also observed, with subpopulations separated by less than 2 km showing contrasting phenological stages. Larval infestation of seeds by a braconid wasp was observed for a period of 1 year and is described. This wasp, the first obligately phytophagous species of Braconinae, is described as *Bracon phytophagus* Quicke sp. n. Percentage fruit infestation by this wasp was relatively high (50–60%) during the entire period (~10 months) of fruit development. The larval stages are described and illustrated, and compared with those of other phytophagous Ichneumonoidea. DNA sequencing of wasp colour variants provided no indication that multiple species were involved. Two related braconine species described in the genus *Iphiaulax* are transferred to *Bracon*, hence, *B. flavipalpisimus* replacement name (Szépligeti) (= *Iphiaulax flavipalpis* Szépligeti, 1901 not *B. flavipalpis* Thomson, 1892) and *B. glabrescens* (Szépligeti) n. comb. (= *Iphiaulax glabrescens* Szépligeti, 1901). Evolutionary routes to phytophagy in braconid wasps and hypothetical scenarios in which this plant–seed predator interaction can be maintained are discussed.

Keywords: *Bracon*, *Burseraceae*, convergence, larval head capsule, new combination, pre-dispersal seed-feeding, *Protium*, reproductive phenology, seed predation

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Introduction

Our knowledge of the reproductive phenology of Neotropical forests has increased considerably since the beginning of the second half of the 20th century. While the community approach has been intended to determine general patterns in the reproductive process of tropical plants; detailed studies, concentrated on single species, have allowed us to examine the causes of the phenological patterns observed, and the magnitude of variation in these patterns in space and time (Williams-Linera and Meave 2002). An additional gain from these types of studies has been the identification of plant–animal interactions that are tightly coupled with one or several phenophases of the plant. In fact, some of these interactions (e.g. pollination, leaf, flower, and fruit feeding) are considered to play an important role as biotic factors that modulate the population dynamics of the species and the phenological patterns of the plant community (Frankie et al. 1974; Gentry 1974; Stiles 1978; Milton 1991; Aide 1993).

Pre-dispersal seed-feeding by insects, one of the commonest plant–animal interactions, is known from all over the world and especially in the tropics (Janzen 1971; Crawley 1992). Such interactions may be important in regulating recruitment in flowering plant populations (Janzen 1971; DeSteven 1981; Louda 1982; Calvo-Irabién and Islas-Luna 1999) and act as selective factors leading to modification of plant reproductive characters (Ollerton and Lack 1998). Seed loss due to larval feeding can be dramatically high (>90%) in some species (Randall 1986; Crawley 1987; Crawley and Gillman 1989), thus almost completely precluding population recruitment.

Predispersal seed-feeding by insects is predominantly associated with three orders, Diptera, Coleoptera, and Lepidoptera, whereas despite its size, the Hymenoptera provides far fewer examples. Although there have been many transitions to secondary phytophagy through gall-formation or inquilinism in the parasitic Hymenoptera, these have not been randomly distributed. Apart from in the cynipid gall-wasps, nearly all other transitions to phytophagy have occurred in the Chalcidoidea. The only other superfamily known to include phytophagous species is the Ichneumonoidea but this biology is far less common in these. Among the ichneumonids there are no known obligately phytophagous species, but there is an instance of partial phytophagy in a species of groteine Labeninae which is parasitic on bee larvae but after consuming them appears to continue to feed on the bee's pollen store. A probable instance of obligate partial phytophagy occurs in another labenine genus, *Poecilocryptus* Cameron which is associated with various gall-forming insects, and appears to complete development by feeding on the gall tissue itself (Short 1978; Gauld and Holloway 1986; Gauld and Wahl 2000). In contrast, among the Braconidae, phytophagy in association with gall formation (cecidogenesis) appears to have evolved on at least three occasions and some evidence suggests that it might have arisen as many as six times. Interestingly, apart from the Australian genus *Mesostoa* van Achterberg (Mesostoinae) which forms galls on *Banksia* stems (Proteaceae), all the other confirmed instances of gall formation in the Braconidae involve members of a single subfamily (Doryctinae, treated here as including *Monitoriella* Hedqvist on the basis of molecular evidence; see Zaldivar-Riverón and Quicke forthcoming) and all are exclusively Neotropical. Surprisingly, phytophagy among braconid wasps was not known (or suspected) until 1989 when Macêdo and Monteiro showed conclusively that a Neotropical species of the doryctine genus *Allorhogas* Gahan feeds on the seeds of the legume *Pithecellobium*, though the behaviour was initially referred to as “seed predation”. That gall tissue was induced was subsequently demonstrated by Macêdo et al. (1998). Seed-associated gall formation has now been demonstrated for two other *Allorhogas* species

and there is reasonable evidence that several other species may do the same with some being associated with Bignoniaceae and Melastomataceae (P. Hanson, personal communication). However, various other *Allorhogas* species are known definitely to be idiobiont parasitoids of gall-forming cecidomyiid flies in the neotropics. The next definitive case of phytophagy involved the enigmatic genus *Monitoriella* Hedqvist which has been shown to be a primary gall-former on *Philodendron* (Araceae) (Wharton 1993; Infante et al. 1995). Ramirez and Marsh (1996) report that some *Psenobolus* species are inquiline in fig syconia and they probably consume principally galled flowers. There is reasonable evidence that at least some species in several other genera of New World Doryctinae may be phytophagous (P. Hanson, personal communication).

Here we describe the reproductive phenology and pre-dispersal seed-feeding process in a cloud forest tree from Venezuela, *Protium towarens* Pittier (Burseraceae), and illustrate larval and adult stages of a new species of “*Bracon*” Fabricius which is obligately phytophagous within the fruit of this tree, and comment on its relationships to other “*Bracon*” species.

Terminology follows van Achterberg (1979, 1988). Descriptions of sculpture follow Harris (1979).

Materials and methods

Study site

The study site is located along the northern ocean-facing flanks of the summit of an internal branch of the “Cordillera de la Costa”, in northern Venezuela. The specific location is known as Altos de Pipe (10°20'N, 66°55'W), at 1747 m above sea level, 11 km from Caracas along the Panamerican Road. The annual mean precipitation is 1200 mm, with a dry period between December and March and a rainy period between May and November. Mean annual temperature is 19°C. The soils of the study site are clay loam and acidic, with a low cation exchange capacity and a high aluminium content (García-Miragaya and Herrera 1971). The dominant vegetation in the location can be characterized as cloud forest composed of three strata, the emergent stratum is 25–30 m in height and is dominated by *Aspidosperma fendleri* Woodson and *Podocarpus pittieri* Buchh and Gray. The canopy (second stratum) is approximately 15 m in height and includes a large variety of species, including *Richeria grandis* Vahl, *Gaffneridia latifolia* Naud, *Protium towarens* Pittier, *Byrsonima reticulata* K.L. and Karst, and *Tetrorchidium rubrivervium* Poepp and Endl. The understorey stratum contains species from the Melastomataceae and Rubiaceae families, including *Miconia dodecandra* Deror and *Palicourea fendleri* Standl.

Study species

Protium towarens (Burseraceae) is a 20–25 m tall evergreen tree, occurring mostly in undisturbed forest and predominantly in areas with a pronounced slope. It has alternate leaves, small androdioecious flowers arranged in inflorescences of wide range of sizes (2–47 flowers per inflorescence, mean=17.6), with white corolla and usually three to five green sepals; with 6–10 stamens, superior ovary, the fruit is a dehiscent green capsule with an oily resin on the surface, and one to five seeds surrounded by a fleshy white aril. Mature flowers live for 1–3 days, starting anthesis between 07:00 and 08:00 h. Flowers produce small

amounts ($<5\ \mu\text{l}$) of nectar and have an intense resin-like fragrance. The nectar is produced in a disk-like nectar gland at the base of the ovary. Open flowers are visited, and presumably pollinated, by flies (Diptera). These insects are the most frequent visitors (≤ 8 visits per day). Social wasps also were observed visiting flowers, but at a lower frequency (≤ 2 visits per day). The fruits develop very slowly, remaining attached to the branches of the plant for as long as 10 months. The seeds are mainly dispersed by birds. A substantial proportion of the seeds falls to the ground, below the tree. Many of these seeds show evidence of pre-dispersal feeding by braconid wasps, the subject of this study. In the study location, individuals are scattered over the forest, well separated from each other. For this study, we selected 20 reproductive trees distributed in two fragments (F1 and F2) of forest separated by about 1 km.

Reproductive phenology

Two separate studies of reproductive phenology were conducted on the species. The long-term study comprised 21 years of uninterrupted observations (1983–2004). Ten individuals of fragment F1 were labelled and monitored for presence/absence of flowers and fruits every month. The short-term study comprised 4 years of uninterrupted detailed phenological observations (April 2000 to April 2004). Twenty individuals were selected and labelled (12 from F1 and eight from F2). Each month, we recorded the percentage of occurrence of four reproductive phenophases: immature flowers, mature flowers, immature fruits, and mature fruits. We also estimated the relative intensity of each phenophase, as the number of flowers or fruits produced by an individual divided by the maximum number of flowers or fruits recorded for that individual during the whole study period. This index of phenophase intensity varies between 0 and 1, with 1 being the maximum intensity possible. With these data, we obtained temporal patterns of flowering and fruiting for both the long- and short-term study.

Survival and population structure

Two cohorts of seedlings under reproductive trees in the study site were selected to estimate survival percentage. The 1989 cohort included 55 seedlings, the 1994 cohort included 65 seedlings. All seedlings were numbered with aluminium foil tags. Both cohorts were monitored for survival every year until 2003. The age structure of the population was examined using three $25 \times 25\ \text{m}$ plots randomly chosen in the study site in 2003. Within each plot, we determined presence of individuals of *P. towarensis*, density, and height, using a scale stick in the case of trees taller than 2 m. With this information we constructed the age structure of the population.

Fruit infection and seed-feeding

Floral and fruit (young immature fruit) visits were recorded in order to identify the reproductive unit at which the braconid infestation occurs. We recorded over 7 days the identity of the insects visiting the flowers and their frequency of visitation, from 08:00 to 18:00 h for 10 min every hour, on a variable number of flowers per inflorescence. In addition, we filmed 8 h of floral visitation, between 07:30 and 16:30 h, using a Sony Handycam DCR-HC40 NTSC, positioned in front of an inflorescence at approximately 10 m from the camera and set in automatic mode. Twenty-two 1-month-old

infructescences were labelled and monitored for fruit visitors during 15 days, every other day, from 08:00 to 18:00 h for 10 min every hour.

During 1 year (January to December 2001), we monitored fruit infection and seed-feeding processes at monthly intervals in five trees that produced fruits that year. Observations began at the first stage of the immature fruits. Each month between 15 and 134 (mean=49.75) fruits were dissected and checked for the presence of braconid larvae. The percentage of infected, aborted, and healthy fruits were recorded. The fruit and embryo volume were estimated assuming the volume of an ellipsoid, which reasonably matches the form of these structures. Adult braconid wasps that emerged from the seeds of *P. towarens* were collected and preserved in ethanol. Pearson correlation analyses were used to test for association between the length of the larva and the lengths of the embryo and fruit during fruit development.

Molecular protocol

DNA was extracted from absolute alcohol-preserved specimens of braconid wasps. A middle or fore leg was removed from each individual and left to dry for 15–20 min on filter paper. Samples were then homogenized, placed into 50 µl of 5% Chelex (Bio-Rad) containing 12 µg ml⁻¹ proteinase K, digested for 100 min at 56°C, then heated to 96°C (2 min) to inactivate enzymes and coagulate proteins, and subsequently stored at -20°C without separating the supernatant. Template DNA was taken from the supernatant.

Polymerase chain reactions (PCR) were carried out in a volume of 25 µl using *pureTaq* ready-to-go PCR beads (Amersham Biosciences) (2.5 U pure *Taq* DNA polymerase and reaction buffer, 200 µM dNTPs in 10 mM Tris-HCl, 50 mM KCl, and 1.5 mM MgCl₂), 22 µl of H₂O, and 2 µl of DNA template. The following primers were employed (Porter and Collins 1991): ITS2 forward: 5'-TGT GAA CTG CAG GAC ACA TG-3'; ITS2 reverse: 5'-ATG CTT AAA TTT AGG GGG T-3'. The PCR programme for all the amplifications had an initial 3 min denaturation at 80°C, followed by 40 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. A 10 min extension followed the final cycle. The PCR products were cleaned with the *Wizard SV* gel and PCR clean-up system (Promega) and then sequenced using the dideoxy terminator cycle sequencing (Applied Biosystems) and an ABI 6400 automated DNA sequencer.

28S rDNA D2-D3 sequences from one of the braconid wasp adults and one of the larvae extracted from within a seed were generated as follows. PCR conditions were 30 cycles of 98°C denaturation (15 s), 48°C annealing (30 s), and 72°C extension (40 s) with an initial denaturation of 3 min at 93°C and a final extension of 3 min. Primers used for amplifying the D2-D3 28S rDNA fragment were from Belshaw et al. (2001) (forward 5'-AGA GAG AGT TCA AGA GTA CGT G-3' and reverse 5'-TAG TTC ACC ATC TTT CGG GTC-3').

Results

Reproductive phenology

Long-term reproductive phenology. *Protium towarens* presents a variable and complex reproductive pattern (Figure 1), including time periods in which flowering and fruiting are supra-annual (1983–1997) and other periods in which it is an annual reproducer (1991–1993, 1997–2003). Time intervals without reproduction can be up to 3 years long,

Figure 1. Long-term flowering and fruiting phenology of *Protium towarens* in northern Venezuela, including periodicity of El Niño and La Niña events.

though runs of several contiguous reproductive years have also been observed. During reproductive years, flowering occurs only once per year. Normally, the flowering period is shorter (3–4 months) than the fruiting period (6–12 months). Unripe fruits can remain on the plants for up to 12 months. The flowering season can vary substantially among years. In eight out of the 13 flowering periods observed, flowering occurred during the rainy season (May to November), the other five during the dry season (December to April). Mature fruits are mainly found during the dry season. No apparent association was observed between the reproductive activity of *P. towarens* and El Niño/La Niña phenomena; however, two of the longest time intervals without reproductive activity (1988–1989 and 1995–1996) coincided with La Niña events (El Niño/La Niña data obtained from www.pmel.noaa.gov/tao).

Short-term reproductive phenology. With the exception of one individual from fragment F2 during 1 year, all reproductive trees observed between April 2000 and April 2004 belonged to fragment F1. Only 55% ($N=20$) of the subpopulations sampled produced flowers during that time interval. From the floral traits observed in these individuals we determined that 40% of them produced hermaphroditic flowers, 15% produced male flowers, and 45% were of indeterminate sex, because they never reproduced. The number of flowering months per individual varied from 1 to 5 during the 4 years monitored, with male individuals having lower numbers (1–2 months) of flowering months than hermaphroditic ones (2–5 months). Floral bud production was relatively cyclical during the 4 years (Figure 2). Four main peaks of bud production were observed, all between October and January, which corresponds to the end of the rainy season and beginning of the dry season, respectively (Figure 1). There was an unusually extended period of bud production in

Figure 2. Short-term flowering and fruiting phenology of *Protium towarens* including phenophase occurrence (a) and intensity (b). See text for explanation.

2002, an El Niño year, compared with other years. Even though the occurrence of mature flowers overlapped with the main peaks of flower bud production during the 4 years of observations, availability of mature flowers was relatively more restricted in time than flower buds in terms of both phenophase occurrence and intensity. The most intensive flowering period (flowering intensity = 0.53 ± 0.14 SE) occurred at the end of 2000. After that year, the surveyed trees showed relatively low reproductive activity. Flowering intensity between 2001 and 2004 was comparatively lower (0.09 – 0.13) during the reproductive months.

Time intervals of fruiting were relatively more extended than flowering intervals during the 4 years of observations (Figure 2). Immature fruits were found during 7 consecutive months in the first part of 2001 and 7 months again in the first part of 2002. Shorter intervals with immature fruits occurred at the end of 2002 (2 months), the beginning of 2003 (2 months), and during the last 5 months of observation. The highest numbers of immature fruits (0.43 ± 0.2 SE) were found between February and June 2001, and again (0.36 ± 0.12 SE) between February and April 2003. Whereas immature fruits developed slowly over a period of several months, mature fruit were present for a relatively more restricted period of time. Ripe fruits reached their highest densities between July and November 2001, followed by two lower peaks in 2002. Although all reproductive phenophases occurred during the 4 years monitored, the majority of individuals sampled did not reproduce, and no trees from the F2 fragment reproduced. More than 60% of the

trees from the F1 fragment did not reproduce during any of the 4 study years. The highest reproductive intensities for the different phenophases occurred in a relatively short period of time (<5 months) in these 4 years.

Seedling survival and population structure

Substantial seedling mortality (54–57%) occurred during the first year of observations in the two cohorts monitored (Figure 3). During the following 3 years, survival decreased at a lower rate in both cohorts, from 45.0% to 27.2%. After the fourth year, percentage survival decreased slowly and stabilized after the sixth year. Only 12% of individuals from the 1994 cohort survived to the ninth year. For the 1989 cohort, percentage survival after 14 years was considerably lower at only 3.6%. Seedlings monitored for 14 years reached a size of 0.32 m.

For the age structure census, no seedlings (up to 0.12 m) were detected in the plots examined (Table I). The most abundant size class observed corresponded to 0.58–0.99 m. Plants of this size averaged 5.7 ± 0.41 individuals per plot, which represents a density of 90 individuals per hectare. These plants are non-reproductive and could be more than 15 years old. Sexually mature individuals (>9.0 m tall) had a relatively low density, below eight trees per hectare.

Figure 3. Percentages of survival of two cohorts of seedlings of *Protium towarens* monitored during 9 and 14 years.

Table I. Age structure of *Protium towarens* as indicated by height in three 25 m² plots in a cloud forest in northern Venezuela.

Size class (m)	Plot 1	Plot 2	Plot 3	Mean ± SE
0.00–0.12	0	0	0	0.00 ± 0
0.13–0.17	2	2	0	1.33 ± 0.41
0.18–0.57	5	4	1	3.33 ± 0.74
0.58–0.99	7	5	5	5.67 ± 0.41
1.0–3.0	2	3	4	3.00 ± 0.35
3.1–9.0	0	2	1	1.00 ± 0.35
9.1–5.0	0	1	2	1.00 ± 0.35
15.1–20.0	1	0	0	0.33 ± 0.20

Fruit development, infestation, and seed-feeding

Flower and fruit visiting. Forty-four per cent ($N=117$) of mature floral buds (with closed petals) had between one and six unidentified Diptera larvae inside the perianth per flower. These larvae complete their development in the flower, feeding on their floral parts and inflicting various levels of damage to them, from minor (a few stamens destroyed) to severe (all floral parts destroyed).

Based on direct observations, four insect categories were observed visiting flowers of *P. towarens*, Diptera, social and other aculeate wasps, honeybees (*Apis mellifera* L.), and various Coleoptera. Of these, Diptera were the most important flower visitors comprising more than 75% of visitors through most parts of the day. Only at noon did the relative importance of flies decrease (down to 40–50% of total visits) with the relative importance of non-braconid wasps (40%) and bees (20%) increasing. About 60% of the floral visits occurred between 11:00 and 15:00 h. No braconid wasps were observed visiting flowers directly, but braconid wasps were filmed visiting inflorescences of *P. towarens*. A visit consisted of a wasp approaching the inflorescence, exploring it in sustained flight, landing on it, and walking over different flowers. They visited inflorescences during daylight hours at an average rate of 6.6 visits per inflorescence per h (± 0.92 SE). The mean duration of braconid wasp visits was 59.5 s (± 5.8 SE) and ranged between 10 and 240 s. A maximum of two wasps were observed visiting the same inflorescence simultaneously. The quality of the film did not allow us to observe any oviposition events. No insect visits to 1-month-old immature fruits were detected in this study either by direct observation or by filming, however, at this stage of fruit development several fruits (5–15%) were already infested with braconid larvae.

Fruit infestation and seed-feeding. Fruit growth was monitored between January and December 2001. By the middle of January, 1-month-old immature fruits were slightly larger ($4.6 \text{ mm} \pm 0.12$ SE) than flowers ($4.25 \text{ mm} \pm 0.09$ SE), and the average fruit volume was 1.09 cm^3 (± 0.05 SE). Fruits developed gradually for approximately 9 months after fertilization (Figure 4a). Mature fruit averaged 2.1 cm^3 (± 1.0 SE), an almost 20-fold increase from the initial size. Between 17 and 46% of fruit was aborted during the last 5 months of development. Fruit infestation by braconid larvae was detected at the first month of development and continued until fruits matured (Figure 4b). As many as 50% of the 1-month-old fruits had braconid larvae inside, and the percentage of infested fruits remained relatively high (50–60%) during the entire period of fruit development. Only one larva was ever observed inside each fruit, always attached to one of the two undeveloped embryos (normally the largest one). During the first 3 months in the fruits, the larvae remained approximately 1 mm long, then at approximately the fourth month of development they started to grow quite rapidly and this continued until pupation between the eighth and ninth month, at which time they were approximately 9 mm long. The start of the rapid phase of larval growth occurred simultaneously with that of fruit and embryo development. This is indicated by highly significant correlations between larva length and embryo volume ($r=0.26$, $P<0.0001$, $N=324$) and larva length and fruit volume ($r=0.48$, $P<0.0001$, $N=324$). The braconid larva feeds on the seed's content, destroying it completely. In multi-seeded fruits, normally only one seed was infested. Once the adult braconid emerges from the pupa, it chews an exit hole through the mesocarp and egresses from the fruit. Emergence of the adult braconids from the fruits occurs when these are still closed and attached to the plant.

Figure 4. Fruit (volume), seed embryo (volume), and larval growth (length) in *Protium tovarense* from 1-month-old fruits until fruit ripening (a) and percentage of fruits infested during the same interval of time (b).

Molecular confirmation of larval identity. A 28S D2 rDNA sequence has been generated from one of the larvae found in the *Protium* fruit and it was identical with that obtained from one of the adult “Bracon” reared from a *Protium* fruit. The sequence obtained has been submitted to the EMBL/GenBank database and has accession number DQ167438.

Systematics

Bracon phytophagus Quicke sp. n. (Figures 5–8)

Adult female

Length. Length of body 10.5 mm, of fore wing 10.5 mm and ovipositor (part exerted beyond apex of metasoma) 9.5 mm.

Head. Antennae with 58 flagellomeres. Terminal flagellomere strongly acuminate. Median flagellomeres strongly oblique, almost diamond-shaped in dorsal aspect, slightly longer laterally than wide. First flagellomere 1.0 and 0.95 times length of 2nd and 3rd flagellomeres, respectively, the latter being 1.45 times longer (maximum length) than wide. Mandibles twisted so only a single tooth visible in anterior aspect. Inter-tentorial distance: tentorio-ocular distance=1.6:1.0. Inter-tentorial distance: height of clypeus=2.85:1.0. Face largely smooth and shiny, with three distinct areas of setosity on either side, one

Figure 5. Habitus and details of claw and ovipositor of *Bracon phytophagus* sp. n., holotype female. (a) Habitus; (b) oblique ventral view of mid-telotarsus showing large basal lobe; (c) apex of ovipositor and ovipositor sheaths; (d) detail of apex of ovipositor showing extra serrations.

bordering the lower half of the eye and extending to the malar region, a band running dorsally from the region of the anterior tentorial pits to approximately the mid-height of the face and a single line of setae submedially. Height of eye: shortest distance between eyes: width of head=1.0:1.05:2.0. Oculo-antennal groove well developed. Frons shiny, weakly impressed and weakly minutely coriaceous. Stemmaticum rather rectangular. Shortest distance between posterior ocelli: transverse diameter of posterior ocellus: shortest distance between posterior ocellus and eye=1.0:1.2:3.8.

Mesosoma. Mesosoma 1.68 longer than maximally high, largely smooth and shiny. Pronotum smooth. Notauli obsolescent, weakly indicated on anterior of mesoscutum. Scutellar sulcus narrow and minutely punctate. Scutellum smooth. Median area of metanotum large, flat, without carina anteriorly. Propodeum smooth.

Figure 6. Habitus and features of the metasoma of males of *Bracon phytophagus* sp. n. illustrated using Automontage®. (a, c) Dark morph; (b, d) yellow form.

Wings. Fore wing: lengths of veins SR1:3-SR:r=6.5:4.8:1.0. Lengths of veins 2-SR:3-SR:r-m=1.4:2.9:1.0. Vein 2-M 1.35 times 3-SR. Vein 1-M nearly straight. Vein 1-SR+M moderately curved posteriorly. Vein r-m with two bullae. Vein 1-SR forming an angle approximately 80° with vein C+SC+R. Vein m-cu 0.35 times 1-M. Vein cu-a interstitial. Hind wing: vein 1r-m:R1=1.0:2.0. Apex of vein C+SC+R with one especially thickened seta (basal hamule). Base of hind wing with medium-sized glabrous area distal to vein cu-a on posterior half of cell.

Legs. Claws with pointed basal lobe (Figure 5b). Lengths of fore femur (excluding trochantellus):tibia:tarsus=1.0:1.05:1.35. Fore tibia with transverse apical row of markedly thickened bristles which curve slightly dorsally. Lengths of hind femur:tibia:basitarsus (based on male paratype)=1.58:2.5:1.0

Metasoma. Smooth and shiny with seven exposed, moderately densely setose tergites. First metasomal tergite 1.2 times longer than maximally wide; median area largely smooth and shiny; dorsolateral carinae very strong and sub-lamelliform. Second tergite 1.9 times wider than medially long, with medium-sized, nearly pentagonal, smooth mid-basal triangular area formed posteriorly into a mid-longitudinal carina that extends approximately 0.6 times length of tergite; with a pair of posteriorly diverging sublateral grooves. Second suture bisinuate, strongly crenulated; abruptly terminated sublaterally. Third tergite 2.25 times wider than medially long, without transverse, subposterior groove; with very weakly indicated antero-lateral areas. Tergites 4-7 largely smooth, moderately setose.

Figure 7. Features of final instar larva of *Bracon phytophagus* sp. n. taken from chill-killed specimens. (a) Frontal view of head capsule showing large, multi-toothed, heavily sclerotized mandibles; (b) oblique view of head showing (arrowed) the small papilliform antenna; (c) lateral view of mature larva showing large humps on abdominal segments 2–7.

Hypopygium sharply pointed, reaching end of apex of metasomal tergites. Ovipositor sheaths 2.9 times longer than hind tibia. Ovipositor very slender, apically markedly darkened (Figure 5c); dorsal valve with a distinct nodus; ventral valves apically with three normal teeth and with nine more basal weaker serrations (Figure 5d).

Colour. Head black, with black setae but palps (except 2nd segment of maxillary palp which is largely black) and narrow marks near orbit of eye and a mark behind each antennal socket orange-yellow; mesosoma largely bright yellow, the mesoscutum more reddish yellow; metasoma largely yellow but with 4th and more posterior tergites becoming increasingly extensively black marked and hypopygium largely black; legs yellowish with telotarsi largely black, apices of mid- and hind tibiae narrowly dark brown to black; antenna and ovipositor sheaths black. Wings boldly patterned: basal 0.4 of fore wing yellowish, broad transverse band between apex of pterostigma and apex of 2nd submarginal cell pale yellow hyaline, the remainder brown to dark brown, pterostigma black with apical 0.1

Figure 8. Prepupa, pupa, and traces of silk in pupation chamber of *Bracon phytophagus* sp. n. (a, b) Prepupa, in lateral and ventral views, showing “S”-shaped curvature and beginning of development of ovipositor structures posteriorly (lower left); (c) posterior of pupa in situ showing ovipositor with remains of larval skin (head capsule: arrowed) adhering to ovipositor; (d) frass from inside pupation hole with traces of thin silk-like material.

yellowish; hind wing yellow with transverse dark band at level of vein R1, and narrowly dark apically.

Male

Smaller than female (7–9 mm), probably polymorphic for colour (see Figure 6). The yellow form has slightly more extensive dark markings with a dark patch also on the mid-ventral part of the hind tibia, and with submedial pairs of nearly black marks on the 3rd metasomal tergite (Figure 6b, d).

Etymology

Named because it is the first known phytophagous braconine.

Type material examined

Holotype: female; North Venezuela: Cordillera de la Costa, Altos de Pipe (10°20'N, 66°55'W); reared from fruit of *Protium towarens*. The type specimen is deposited in the Natural History Museum, London. Paratypes: four males, with same data as holotype, two in the Natural History Museum, London, two in the Instituto Venezolano de Investigaciones Científicas, Venezuela.

Notes

The new species is closely related to *B. flavipalpis* replacement name (Szépligeti) (= *Iphiaulax flavipalpis* Szépligeti, 1901, n. comb., not *B. flavipalpis* Thomson, 1892) and *B. glabrescens* (Szépligeti) (= *Iphiaulax glabrescens* Szépligeti, 1901, n. comb.) because it is large (more than 8 mm long), has a pointed basal lobe to the claws, and has a very slender ovipositor. It can be distinguished from them in having the mesosoma red yellow rather than largely black, in having the fourth and/or fifth metasomal tergites black (at least posteriorly), and in having the hind legs largely yellow.

Two males reared from the fruits of *Protium towarens* were far darker than the other males or the female (compare Figure 6a, c and Figure 6b, d). These are excluded from the paratype series in case they are not conspecific, though they have identical ITS-2 sequences to the predominantly yellow typical forms. The ITS-2 sequences of the five sequenced individuals have GenBank/EMBL accession numbers DQ167439–DQ167443; the specimens are also in the collection of the Natural History Museum, London. These darker specimens also differ from the two Szépligeti species named above in that they have the hind legs the same colour as the middle legs.

Larval description

Final instar larva (Figure 7a–c). The mature larvae are white, rather “C”-shaped, with smooth cuticle. First thoracic spiracle located near posterior margin of 1st segment. Abdominal segments 1–7 with distinct dorsal humps, these being particularly prominent on segments 2–6. The head capsule is strongly sclerotized: with a pigmented band running more or less concentrically with the epistoma from near the antennae to connect with the hypostoma, antennae papilliform but without distinct encircling ring, epistoma complete and wide, clypeus dark and sclerotized, inner pleurostomal margin and mandibular processes darkened, hypostoma with lower edge dark, hypostomal spur slender, stipital sclerite strongly “U”-shaped, cardo large, irregular and pigmented, prelabial sclerite virtually absent, mandibles massive, virtually black, strongly hooked with four strong tooth-like processes along ventral margin. Prepupa (Figure 8a, b) distinctly “S”-shaped in female, with posterior end curved dorsally as a result of development of ovipositor sheaths.

Discussion

Reproductive phenology of Protium towarens

The reproductive phenology of *Protium towarens* seems relatively variable over time, with periods in which the species is a supra-annual reproducer, and other periods in which it reproduces regularly every year, at the end of the rainy season. Many studies on the reproductive phenology of neotropical and paleotropical forests have identified an important proportion of species with supra-annual phenological patterns (Hart 1995; Williams-Linera 1997; Camacho and Orozco 1998; Berlin et al. 2000; Kang and Bawa 2003; Numata et al. 2003; Hamann 2004). Normally, these species display synchronized and massive production of flowers, fruits, and seeds during their main reproductive intervals (Kang and Bawa 2003). Contrary to this profile, individuals of *P. towarens* did not display a synchronized reproductive pattern in the subpopulations examined. Besides this, no mass flowering was observed during the 21 years monitored. Our estimates of flowering

and fruiting intensity were relatively low. In reproductively active trees, only a few branches produced flowers and fruits per reproductive season. These differences with respect to recognized supra-annual species lead us to question the supra-annual condition of *P. towarensis*. Our observations were restricted to just two subpopulations separated by less than 2 km and located at similar altitudes. Given the limited spatial scale covered in this study, we cannot discard the possibility that other subpopulations of the species were reproductive at the time in which no reproduction was observed in the trees examined. The fact that the two subpopulations monitored presented such contrasting reproductive profiles (F1 reproductive versus F2 non-reproductive) indicates a high level of spatial heterogeneity. Only by conducting a phenological study over a larger geographic scale, considering several subpopulations under differential physical conditions, will we be able to determine if the supra-annual pattern suggested from our results is the true reproductive pattern of *P. towarensis*.

The relationship between climatic factors and reproductive patterns in tropical forests around the world is not well understood. In several tropical rain and cloud forests, minimal night temperatures, drought, and El Niño/La Niña events have been considered to be good predictors of reproductive activity in these plant communities (Ashton et al. 1988; Camacho and Orozco 1998; Sakai et al. 1999; Yasuda et al. 1999). However, in other cases, climatic factors including El Niño and La Niña phenomena, do not appear to be directly responsible for triggering and synchronizing phenological events (Hamann 2004), and climatic conditions were not good predictors of the reproductive status of *P. towarensis*. Over the 21 years of this study, flowering has occurred during both dry and rainy seasons, and only during the last 4 years, the main peak of flowering concentrated at the end of the rainy season and during the dry period, as occurs for other montane forest trees in the neotropics (Camacho and Orozco 1998). No temperature or precipitation estimates exist for the study site since 1976 and therefore we cannot determine if these factors play a relevant role in the reproductive phenology of this species. The reproductive activity of *P. towarensis* seems to be independent of the occurrence of El Niño and La Niña events, flowering and fruiting occurred during years with El Niño, La Niña, or none of these phenomena. It is important to remark, however, that La Niña events occurred in two out of three extended time intervals (2–3 years) without reproductive activity in the study site. Biotic factors such as pollination service and pre-dispersal seed-feeding can also be partially responsible for the reproductive timing of tropical forest trees. For example, high pre-dispersal seed-feeding in a given year can increase flower production during the following year (Wright and Meagher 2003). In *P. towarensis*, with fruit infestation reaching as high as 70%, year-to-year fluctuations in the intensity of seed-feeding could modulate the production of flowers in the population.

For most of the study period flowering occurred at low intensity, for relatively short periods, and only in a fraction of the population, and these features greatly limit the fruit set capability of the population in any given year. In addition to these limitations, high levels of fruit infestation by *B. phytophagus* sp. n. diminish the proportion of healthy seeds available even further. Finally, the substantial mortality at the seedling stage (54–57%) and during the subsequent years of development, contributes even more to reduce levels of recruitment. The age structure of the populations studied, with virtually no seedlings and a dominance of 15-year-old or older saplings, suggests that recruitment is relatively uncommon and probably occurs under particular and infrequent environmental conditions.

Pre-dispersal seed-feeding in Protium towarens

Even though our observations on flower visitation are relatively limited, they suggest that Diptera are the most likely pollinators of *P. towarens*. Besides their superiority in terms of frequency of visits, they also contacted the reproductive parts of the flowers. *B. phytophagus* were only filmed visiting inflorescences during the time dedicated to observing flowers and fruits of *P. towarens*. These observations suggest that braconids must oviposit at a very early stage of fruit development, at the flower/fruit transition, and braconid larvae were detected in several fruits at the initial stage of development (<1 month old). The more heavily sclerotized ovipositor tip of *B. phytophagus* compared with those of nearly all other known braconines (cf. doryctines; see Quicke et al. 1992) suggests that they may be able to penetrate older and harder fruit. Even though many seed predator larvae develop from eggs laid on floral parts (e.g. anthomyids, Zimmerman 1980; beetles, Cummings et al. 1999; moths, Wright and Meagher 2003), there are also examples of insects that oviposit directly inside developing fruits, including parasitic wasps in *Palicourea salicifolia* Standl. (Rubiaceae) (Wesselingh et al. 1999) and weevils in dipterocarp (Toy et al. 1992) and *Vaccinium* (Mahoro 2003) species.

Pre-dispersal seed-feeding by insects is a relatively common plant–animal interaction described for temperate and tropical species of plants and covering a wide range of plant families (Crawley 1992). In most cases, larvae belong to three orders, Diptera, Coleoptera, and Lepidoptera. Hymenoptera, and in particular, members of the Braconidae, are less common examples of pre-dispersal seed feeders. *B. phytophagus* sp. n. larvae appear to develop slowly inside the fruits with adult egress starting after about 9 months. During the time inside the fruit, two different larval phases were identified. During the first 3 months the larva does not grow, then starting from the fourth month, this “diapause” phase is terminated and the larva grows simultaneously with the seed’s embryo. At the final stages of development, the seed embryo is fully grown and the larva feeds on it until pupation. Losses of seeds due to insect larval feeding can be quite variable among species of host plants, but in many cases the percentage of seeds eaten is relatively high (>90%) (Janzen 1971; Mattson 1980; Randall 1986; Crawley and Gillman 1989). The impact that *B. phytophagus* larvae have on the population recruitment of *P. towarens* must be high, because the percentage fruit infestation ranged between 30 and 70% over the period of fruit development. Fruit set is not massive in this species, therefore satiation (Janzen 1971) has to be discarded as a viable strategy to escape from seed feeders. On the other hand, the impact of seed-feeding is probably reduced as a result of the typically large interplant distances. The limited reproductive success of *P. towarens* make it seem a rather unreliable host plant for a specialized seed-feeding insect such as *B. phytophagus*.

Evolution of phytophagy in a braconid wasp

Most phytophagous insect species are specialists (Jaenike 1990). Specialization for a particular host plant implies, in the broadest sense, that this plant should be able to support a population of seed-eating insects and to permit their propagation (Dethier 1954). Based on these criteria, *P. towarens* does not appear to make a good host plant for a monophagous pre-dispersal seed feeder, because it has a temporally and spatially variable reproductive pattern (mixture between supra-annual and annual patterns), low flowering and fruiting intensity, low population densities and low survival of young plants.

The above prompts us to ask how *B. phytophagus* evolved into a phytophagous species in association with *P. towarens*. We can imagine two possible scenarios in which this

interaction could be maintained over time. In the first, the braconid wasp behaves as an oligophagous or polyphagous species, not depending exclusively on *P. tovarense* to complete its life cycle and so can use fruits of different species with different reproductive phenologies that complement or even overlap each other. The second possibility is that the wasp is monophagous but very well adapted to finding suitable host trees, and is perhaps quite long-lived as an adult.

Through the first of these strategies, the braconid wasp would be able to complete its life cycle using the available host plants for a particular year. Different factors, including unpredictable habitats (Lacy 1984), plant rarity or unpredictability (Jaenike 1990), nutritional deficiencies derived from use of a single food type (Ballenbeni and Rahier 2000), and great resource availability, can favour generalization (Thompson 1982). Tropical cloud forests do not fit into the category of rare or unpredictable habitats, however, plant phenology can be highly variable in the tropics (Newstrom et al. 1994), and this species is a good example. Based on its population density, *P. tovarense* can be considered an uncommon species in this forest, though the cloud forest examined has a moderate plant species richness with a total of 144 species having been collected in an area of approximately 1.6 ha (Sobrevila and Kalin-Arroyo 1982). We cannot discount the possibility that some of these species could be alternative host plants of *B. phytophagus*.

If the wasp is a specialist on *P. tovarense* then it must presumably be well adapted to locating the apparently rare and unpredictable host resource, and in this context it may be significant that members of the genus *Protium* are well known for producing essential oils (Ramos et al. 2000; Machado et al. 2003), with intensive and characteristic odours, that could be used as signals to identify these plants. In either scenario it seems likely that these will play an important role in host plant location by the wasps.

The discovery of a completely phytophagous braconine is of interest for several reasons. It is the first record of phytophagy in the subfamily, the species involved is one of a poorly known group of the largest “*Bracon*” species, so far known only from the Neotropical region; the larval features show a high degree of specialization towards phytophagy with highly modified mandibles; the association with the host plant does not seem to involve gall formation; and apparently the males at least display marked colour dimorphism.

Routes to phytophagy

The close relatives of at least the new species of *Bracon* are parasitoids of bruchid beetles which are obligate seed feeders as is *Stenocorse* Marsh which is closely related to the phytophagous doryctine braconid *Allorhogas* (Belokobylskij et al. 2004). The sister group of the gall-forming Australian genus *Mesostoa*, on the basis of DNA sequence data (Belshaw and Quicke 2002), is a clade associated with galls, including the enigmatic braconid tribe Hydrangeocolini which have been reared from cecidomyiid galls (see Oda et al. 2001), and the closest relative of the recently described fig-inhabiting braconine genus *Ficobracon* van Achterberg and Weiblen appears to be *Syntomernus* Enderlein, which has been reared from galls (D.L.J.Q., unpublished observations; Professor M. Yang, Taichung, personal communication). The genus *Simplicibracon* Quicke also appears to be close to *Syntomernus*, and has been reared from cecidomyiid galls (Maetô 1991). Members of the ichneumonid genus *Poecilocryptus*, which are almost certainly partially phytophagous, have been reared from galls made by a wide variety of hosts (including by the cecidogenic braconid *Mesostoa*) (Gauld and Holloway 1986; Austin and Dangerfield 1998; Gauld and Wahl 2000). Thus it seems likely that phytophagy among braconids may have evolved from

parasitism of either seed-feeding hosts such as bruchid beetles or of gall-inducing hosts such as fig wasps, and that both gall and seed tissue, being relatively nutritious and palatable, became incorporated into the wasp larva's diet.

Larval adaptations

The larval head capsule of the new species shares a number of features in common with that of the labenine ichneumonid, *Poecilocryptus*, in having extensive sclerotization including a large zone running from near the antennae round to the hypostoma, massive heavily sclerotized mandibles (though these are apically, broadly bidentate in *Poecilocryptus* rather than having subapical teeth) and a dark sclerotized clypeus (Short 1978). The larval head capsule of the cecidogenic braconid, *Mesostoa*, also shares large heavily sclerotized mandibles (though lacking teeth), a broad epistoma and sclerotized pigmented clypeus (Quicke and Huddleston 1989). The hypostomal spurs of both *Poecilocryptus* and *Bracon phytophagus* are weak and somewhat reduced, and in *Mesostoa* they are wholly absent, and the ventral cephalic structures show little sign of sclerotization. Thus, it appears that phytophagy leads to increased strengthening of the dorsal cephalic structures, especially a wide epistoma and sclerotized clypeus, and large heavily sclerotized mandibles, but does not necessarily involve any particular development of the labrum and other ventral structures.

Apparent concentration of phytophagous braconids in the Neotropics

To date, all known cases of phytophagy in the Braconidae, with the exception of the new species described here, are through gall formation, and with one exception, all are from the Neotropics. Whether this is indicative that there is something special about the Neotropics is unclear and although the biologies of quite a few Holarctic taxa have been studied, and all to date are parasitoids, very little work has been carried out on species in South-East Asia or the Afrotropical Region. In a recent survey (Quicke 1988b) it was shown that only 21% and 25% of braconine genera in these two regions, respectively, had associated host records compared with 43% for the neotropics and 85% for the West Palaearctic. Therefore it is quite possible that other phytophagous species occur there. Recently, van Achterberg and Weiblen (2000) described a genus of braconine, *Ficobracon*, also belonging to the Braconini, reared from fig syconia in Papua New Guinea and commented that it was either a parasitoid of Agaonidae or phytophagous, and one of us (D.L.J.Q.) has seen a probably related wasp ovipositing in figs in Uganda, and its size would suggest that it was too large to be a simple primary parasitoid of the fig wasp, again suggesting the possibility of partial if not complete phytophagy. Thus, it may be that incidences of phytophagy among Old World braconids have simply gone virtually unnoticed to date.

Another aspect of larval phytophagy in the apocritan (wasp-waisted) Hymenoptera is that the mid- and hind guts are not connected and therefore bulky non-digestible plant material cannot be evacuated. Gall formation in which the host plant is caused to produce highly nutritious, largely digestible tissues is one solution. The very long lag after the *Bracon phytophagus* sp. n. hatches before it starts to grow rapidly might be important here, because the seed tissue found surrounding larvae and pupae (see Figure 8) appeared moist and somewhat decomposed, and therefore microbial activity and/or autolysis could be important in reducing the indigestible bulk of the seed tissue.

Generic placement and relationships of new species

“*Bracon*” has been shown to be paraphyletic with many genera of the tribe Braconini being derived from within it (Belshaw et al. 2001). This is not surprising given that most species are small (3–6 mm) and consequently tend to have reduced features. In identification keys, therefore, “*Bracon*” species tend to come out near the end, after taxa with more obvious apomorphies have been excluded. Morphologically, the new species is essentially a large “*Bracon*” with slightly more heavily sculptured metasoma, but it still keys out to the genus easily in the keys by Quicke (1987, 1997). Within the Neotropical Region, several large “*Bracon*” species are known, and because of their size and often bright colour, those that have been described were usually originally placed in the genus *Iphiaulax* Foerster in which the great majority of such species were placed in the early 20th century. Actually, the New World species placed in *Iphiaulax* all belong to other genera, mostly in *Digonogastra* Viereck, which is convergently similar but not closely related (Quicke 1988a). “*Bracon*” species differ from members of *Digonogastra* in that they have a small, simple scapus that is shorter ventrally than dorsally in lateral aspect, and (nearly all) have the basal lobe of the claw large and pointed or truncate (Figure 5b).

The new species belongs to a subset of relatively large Neotropical “*Bracon*” species which we designate here as the *B. flavipalpis* (Szépligeti) group and which also includes *B. glabrescens* (Szépligeti) (Szépligeti 1901, p 382, 390). This group is characterized by having a relatively strongly sculptured metasoma and having the ovipositor very slender with a somewhat darker tip (Figure 5c, d). Some other, undescribed, species in this group are smaller and more sombrely coloured, but all have a well-developed elongate mid-longitudinal area and strong, sublateral, posteriorly diverging grooves on the 2nd metasomal tergite, though the degree of sculpturing varies. A series of individuals of one such species seen by one of us (D.L.J.Q.) is labelled as having been reared from bruchid beetles, and bruchids are specialist seed feeders.

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