Center for Amazon Community Ecology

1637 B North Atherton Street #90, State College, PA 16803
Tel: 814-238-5148; Email: amazonecology@comcast.net

1. Project title:

The insect ecology and harvest of copal (Burseraceae) tree resin in the northeastern Peruvian Amazon

Project description adapted from proposal to INRENA - Peruvian government National Institute for Natural Resources

2. Institution responsible for the project

Center for Amazon Community Ecology, 1637 B. North Atherton St. #90, State College, PA 16803 USA

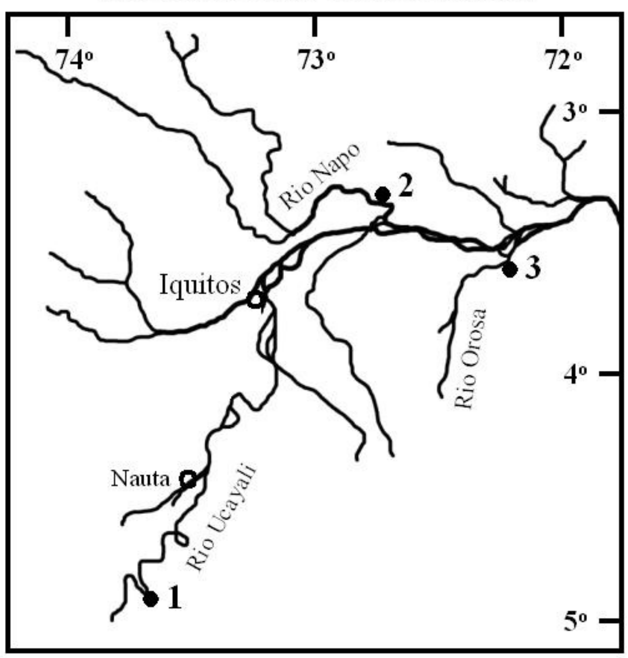
The Center for Amazon Community Ecology is registered as a domestic non-profit corporation in the state of Pennsylvania to promote the understanding, conservation, and sustainable development of human and other biological communities in the Amazon region and other tropical forests. The Center's specific aims are to study the ecology, management, and marketing of tropical forest flora and fauna, assist forest-based communities to safeguard and use local resources to support community development, and educate the public about these activities and related information.

3. Type of study

Ecology, sustainable harvest of non-timber forest products

4. Location of study area

Map of field sites for Copal resin study in northeastern Peruvian Amazon



Site 1 – Jenaro Herrera Investigation Center
Site 2 – Explor Napo Lodge
Site 3 – Madre Selva Biological Station

Site 1. Jenaro Herrera Research Center, Ucayali River, Loreto (Study year 1) The Center is maintained by IIAP (Institute for Investigation of the Peruvian Amazon). The Center is 200 km southeast of Iquitos at 4° 55'S; 73° 45'W.

Site 2. Explor Napo Lodge, Napo River, Loreto (Study year 1). The Lodge is an eco-tourism and research base operated by the Explorama Lodges company in the private Sucusari reserve. The lodge is 160 km northeast of Iquitos at 3° 14' S; 72° 55"W.

Site 3. Madre Selva Biological Station, Oroso River, Loreto (Study year 2). The Station is operated by Project Amazonas, a non-profit organization based in the U.S. and Peru. The station is 150 km east of Iquitos at 3° 37'S; 72° 14'W.

5. Background of the Study

The evolution of resin synthesis in at least ten percent of all plant families, mostly tropical angiosperms, has apparently been driven by plant attempts to deter herbivores and pathogens (Langenheim, 1990; Farrell *et al*., 1991). An important by-product of this defensive strategy is that resins that can be aromatic, anti-microbial, waterproof, sticky and hard have also become essential resources for some insects (Roubik, 1989; Phillips and Croteau, 1999; Plowden *et al*., 2002). Prime examples of resin-using insects are: pine bark beetles (Scolytidae) that incorporate host tree resin into a mating pheromone (Stark, 1982; Phillips and Croteau, 1999), weevil larvae (Curculionidae) that manipulate resin into a protective chamber (Plowden, 2001), syrphid flies (Syrphidae) that feed in resin exudates (Plowden *et al*., 2004), ants (Formidae) that manipulate resin lumps into colonies (Plowden, unpublished observation), stingless bees (Apidae) that gather resin for nest construction and defense (Roubik, 1989; Nogueiro-Neto, 1997), and assassin bugs (Reduviidae) that apply resin to their forelegs to help capture prey (Adis, 1984; Roubik, 1989). People have also found hundreds of ways to use resins in their daily lives and industrial applications (Howes, 1949; Langenheim, 2003).

It is well known which tropical plant families produce resins (Farrell *et al*., 1991; Langenheim, 2003), but relatively little is known about specific interactions between resin-using insects and resin-producing plants (Armbruster, 1984; Roubik, 1989) and how insect resin use and human resin harvest affect each other. Describing these relationships in greater detail would improve our understanding of the evolution and ecology of resinous plants and resin-using insects. Tracking resin flow through tropical forests would highlight a new dimension of community ecology in tropical forests where resin-using bees are key pollinators (Roubik, 1989; Armbruster, 1997; Michener, 2000; Peres, 2000). Such knowledge would help forest-based communities and other managers design systems to sustainably harvest resin and assess how logging and other forms of forest disturbance might affect resin resources used by people, resin-using insects and their associated flora and fauna (Plowden, 2002).

The Burseraceae family provides a good model plant group to address basic and applied tropical forest resin ecology questions because most of its 200 neotropical species probably have resin ducts (Solereder, 1908; Langenheim, 1990; Gentry, 1993). Rainforest peoples throughout Central America and the Amazon have collected terpene-rich Burseraceae resins for torches, medicines, incense, ceramic glazes and caulking for wooden boats (Pittier, 1926; Howes, 1949; Mors and Rizzini, 1966; Pinedo-Vasquez *et al*., 1990; Schultes and Raffauf. 1990; Balée, 1994; Grimes *et al*., 1994; Boom, 1996; Comerford, 1996; Martinez-Habibe, 1998, Plowden, 2001).

Plowden's (2001) pioneer work with Burseraceae resin ecology in the Brazilian Amazon showed that an undescribed species of *Pappista* weevil (formerly described as *Sternocoelus* in Plowden, 2001) provokes most resin found on *Protium, Tetragastris* and *Trattinnickia* tree trunks. This weevil has a novel strategy for coexisting with resin that deters most herbivores. Unlike bark beetles that form galleries in host tree phloem (Phillips and Croteau, 1999), this weevil's larva carves a chamber in the hardening resin that exudes from holes it makes while feeding (Plowden *et al*., 2002; Figure 1). A complex of other invertebrates use Burseraceae resin stimulated by weevil action. These include *Alipumilio* (Syrphidae) fly larvae that forage inside resin lumps on some host tree species (Plowden *et al*., 2004), stingless bees that harvest fresh resin, and assassin bugs that stalk these bees with resin-dabbed legs. Several types of ants, spiders, millipedes and other arthropods nest or forage in hardened resin lump chambers vacated by weevils (Plowden, 2001).

Resin weevils and other resin-using insects are not restricted to the eastern Brazilian Amazon. In May 2002 and April 2004, resin lumps were observed on Burseraceae trees formed by weevil action at four rainforest sites in the central (Manaus, Brazil) and western Amazon (Iquitos, Peru)(Plowden, unpublished observations). In Brazil where Burseraceae resin is called "breu" and the Peruvian Amazon where similar resins are called "copal," forest-based communities often use and sometimes sell this material. Collectors in northeastern Peru include residents of the Allpahuayo-Mishana Reserve (Plowden, unpublished observation), Maijuna and Yagua Indians (Gilmore and Graham, personal communication). Some resin lumps investigated at forest sites near Iquitos and the Napo River contained both weevil and fly larvae. *Crematogaster* (Myrmicinae) and *Pachycondyla* (Ponerinae) ants from these sites were observed manipulating "copal" resin in different ways to create their own nests (Plowden, unpublished observation; Delabie, personal communication). Both *Trigona* stingless and orchid bees (Euglossinae) were also observed collecting soft "copal" resin throughout this region (Plowden, unpublished observations). There is apparently a continuum of weevil-resin interactions throughout the Neotropics since since weevil larvae have also been documented in *Protium* resin lumps in Colombia

(Martinez-Habibe, 1998) and seem to be responsible for resin lump formation on some *Bursera* trees in Oaxaca, Mexico (Purata, personal communication).

The Cryptorynchinae sub-family of weevils that includes *Pappista* is the most species-rich weevil group in the Neotropics (O'Brien and Wibmer, 1978; Wolda *et al*., 1998). Female weevils' use of a hard beak to place eggs deep in plant tissues may help generate the taxa's great diversity, but the immature weevil developmental phase and habits must be studied more to understand their overall ecological importance and reasons for evolutionary success (Anderson, 1993; 1995; Howden, 1995). Resin weevils, however, do not colonize all Burseraceae species in a given site. While some sites investigated in Brazil have 20 to 40 Burseraceae species (Balée, 1994; Ribeiro *et al*., 1999; Vásquez and Phillips, 2000), resin weevils do not seem to have colonized more than 12 of these species at any one site (Plowden, 2001; Plowden, unpublished observation). While there is a great diversity of Burseraceae species in the Peruvian Amazon, it appears that most resin lumps are found on two *Protium* species and are only found occaisionally on five or six others (Fine, personal communication; Plowden, unpublished observation).

Distinct types of resin lumps also indicate that more than one species of weevil colonizes Burseraceae trees. In the eastern Amazon, some resin lumps were white to light grey, thick, and oval shaped while other lumps developed with irregular shapes and turned from white to black (Plowden *et al*., 2002). Even though all resin lumps in this region were formed by resin exuding from a series of separate bore holes, the resin accumulated in layers over one contiguous chamber that extended to the margin of the resin lump (Plowden, 2001). In the central and western Amazon, some resin lumps were also black, irregular shaped and formed by multiple bore holes. This region also has a type of resin lump that is almost a perfect hemisphere, a type not seen in the eastern Amazon. Some of these resin lumps had a small spout on top in their earlier stages of formation; this structure may serve as a breathing tube for the weevil larva inside. The larva in this round lump chewed contiguous holes under the center of its chamber and manipulated a small volume of resin exudate into thin honeycombed type walls (Plowden, unpublished observations). These observations indicate that resin weevils have developed distinct adaptations to *Protium* resin defenses in the same pattern that different *Blepharida* (Crysomelidae) beetles have evolved distinct behaviors to bypass high exudation pressure and noxious chemicals in leaf resin defenses of *Bursera* species in Mexico (Becerra, 1994; Evans *et al*., 2000).

Some resin lump types are used more than others by flies, ants, bees and people (Plowden, 2001; Plowden *et al*. 2004). So far syrphid fly larvae have only been observed in black type resin lumps in Brazil and Peru (Plowden, 2001; unpublished observations). It seems possible that this resin may be a richer source of the bacterial and fungal microbes these larvae likely feed on (Rotheray, personal communication) than white resin lumps in the eastern Amazon and round lumps on Burseraceae trees farther west. The round resin lumps also seem less attractive as a substrate for ant colonies and source of fresh resin for stingless bees (Plowden, unpublished observation). What remains unknown is whether any of these insects have become so specialized in their use of Burseraceae resin lumps that they indirectly depend on certain types of resin weevils in order to survive. Both *Pachycondyla* and *Crematogaster* are considered opportunistic arboreal ants (Longino, personal communication), but since a *Crematogaster* was found to finely manipulate Burseraceae resin to fashion a chamber for a developing gyne (Plowden, unpublished observation), it seems possible that at least one species in this genus has become a highly specialized if not obligate user of this resource (Delabie, personal communication). A small-scale experiment in Brazil revealed that *Trigona* stingless bees readily harvested fresh "black breu" resin while chunks of "white breu" resin went largely untouched (Plowden, 2001). The ecological significance of these relationships may extend considerably beyond the immediate resin system since some types of these insects are known to be key pollinators of many plants (Roubik, 1989; Vockeroth and Thompson, 1987).

6. Summary of study objectives

1. Document basic ecological aspects of the relationship between Burseraceae trees in the northeastern Peruvian Amazon and weevils that stimulate resin lump formation.

2. Identify which other insects utilize Burseraceae resin and begin to describe the nature and importance of these resins in the life history of these insects. These other insects include at least several taxa of flies, ants and bees.

3. Formulate recommendations for ways that Burseraceae resin may be sustainably harvested for use and sale by local communities.

7. Project Participants

Principal Investigator:
Dr. James Campbell Plowden, President, Center for Amazon Community Ecology (CECAMA), State College, PA, USA.

Principal collaborator:
Cesar Delgado Vásquez, M.S.c.: Entomology investigator, PBIO-IIAP, Iquitos, Peru.

Field assistants:
Victor Alberto Raygada Guerra: Independent forestry technician, Iquitos, Peru.
Angel Eduardo Raygada: Forestry Student, National University of the Amazon (UNAM), Iquitos, Peru.
Nestor Jaramillo Jarama: Independent field botanist, Iquitos, Peru.
Marissa Kusuda Plowden: Independent field assistant, State College, PA, USA.

8. Materials and Equipment

<u>Tree inventory and resin sample collection</u>
compass, measuring tapes, diameter tape, hand held GPS unit with antenna, aluminum tags, nails, flagging tape, duct tape, binoculars, plastic ziplock bags

<u>Plant specimen collection and preservation</u>
Pole tree pruner, tree climber, alcohol, newspaper, plant press, drying oven

<u>Insect capture and storage equipment</u>
Net, wire screen mesh, rearing boxes, pins, trap adhesive, aspirator, marking kit, boxes, alcohol and preservative, glass vials, scizzors, knife

<u>Documentation and measuring equipment</u>
Marking pens, waterproof notebooks, digital camera, digital camcorder, laptop computer, portable precision balance, portable digital balance, calipers, field scope with camera attachment, dissecting microscope

9. Detailed study goals and methods

9.1 Investigation Goals and Objectives:

1. <u>Document basic ecological aspects of the relationship between Burseraceae trees in the northeastern Peruvian Amazon and weevils that stimulate resin lump formation.</u>

This will include identifying which species of Burseraceae trees have resin lumps on them that are formed by weevil colonization and describing the relevant stages of the natural history of these resin weevils. Specific objectives will be to determine: 1) the relationships between host tree species and size (diameter), weevil species and resin lump type, 2) the amount of resin that accumulates with different resin lump types during resin weevil development, 3) the time required for resin weevil development with different types of resin lumps and resin weevil species, 4) the rate of resin accumulation with different resin lump types in undisturbed and harvested trees, and 5) the relative importance of Burseraceae species, host tree diameter, weevil species, resin lump type, number of resin lumps counted or removed at an earlier time, and time since previous monitoring or resin harvest on the amount of total resin accumulation per tree.

2. <u>Identify which other insects utilize Burseraceae resin and begin to describe the nature and importance of these resins in the life history of these insects.</u>

Specific objectives will be to: 1) determine which species of host trees and resin lump types are used by each of these insects, 2) identify the taxa of flies, ants, bees, or other insects that inhabit Burseraceae resin lumps or use this resin, and 3) describe the relative importance of Burseraceae resin as a nest-building material for resin-using ants and bees.

3. <u>Formulate recommendations for ways that Burseraceae resin may be sustainably harvested for use and sale by local communities.</u>

This will utilize information gathered while investigating the first two sets of study goals. Specific questions to be addressed will be: 1) what is the maximum amount of resin that could be harvested from a given area during an initial harvest, 2) how much resin could be harvested from a given area if harvests are conducted 6, 12, 18, or 24 months after an initial harvest, 3) what harvesting protocols might help maintain resin yield and conserve resin availability for resin-using insects.

Subsequent studies will build on this work to investigate how the physical and chemical properties of resins and resin delivery systems in different species of host trees may explain differences in insect resin use in Burseraceae trees. If Burseraceae resin seems to be a significant resource for some bees and ants, future work will examine its relative importance to other types of resin used for nest construction. While limited markets exist for these resins in an unprocessed condition, the additional line of research will explore if there are ways that forest-based communities could sustainably harvest and transform these resins into a value-added product that could be sold to help generate more local income.

9.2 Hypotheses

Goal 1.
1a. Null hypothesis: There is no difference between the relative density of all Burseraceae tree species and the relative density of Burseraceae tree species with resin lumps (Objective 1.1).
1b. Null hypothesis: There is no difference between the relative abundance of host tree species with resin lumps and the relative abundance of host tree species associated with each type of resin lump (Objective 1.1).
1c. Null hypothesis: There is no difference in the species of resin weevil associated with different types of resin lumps (Objective 1.1).
1d. Null hypothesis: There is no difference in the amount of resin that accumulates on one resin lump with different types of resin lumps and different species of resin weevils (Objective 1.2).
1e. Null hypothesis: There is no difference in resin weevil development times with different types of resin lumps and resin weevils (Objective 1.3).
1f. Null hypothesis: There is no difference in the total amount of resin that accumulates on host trees with different types of resin lumps (Objective 1.4).
1g. Null hypothesis: There is no difference between the amount of resin found on undisturbed trees and trees whose resin was harvested up to 24 months earlier (Objectives 1.4).

Goal 2.
2a. Null hypothesis: There is no difference between the relative proportion of Burseraceae tree species whose resin lumps contain fly larvae with the relative proportion of all Burseraceae tree species with any resin lump (Objective 2.1).
2b. Null hypothesis: There is no difference between the relative proportion of types of resin lumps containing fly larvae with the relative proportion of all types of resin lumps (Objective 2.1).
2c. Null hypothesis: There is no difference between the relative proportion of Burseraceae tree species whose resin lumps contain ants with the relative proportion of all Burseraceae tree species with any resin lump (Objective 2.1).
2d. Null hypothesis: There is no difference between the relative proportion of types of resin lumps containing ants with the relative proportion of all types of resin lumps (Objective 2.1).
2e. Null hypothesis: There is no difference between the relative proportion of Burseraceae tree species whose resin is harvested by bees with the relative proportion of all Burseraceae tree species with any resin lump (Objective 2.1).
2f. Null hypothesis: There is no difference between the relative proportion of types of resin lumps whose resin is harvested by bees with the relative proportion of all types of resin lumps (Objective 2.1).
2g. Null hypothesis: Only one species of fly develops in resin lumps of all species of Burseraceae trees (Objective 2.2).
2h. Null hypothesis: Only one species of fly develops in all types of resin lumps (Objective 2.2).
2i. Null hypothesis: No ant species makes its nest exclusively in Burseraceae resin lumps (Objective 2.3).
2j. Null hypothesis: There is no difference in the growth rate of resin lumps that are harvested and not harvested by bees.

Objectives 2.3: More basic information about ant and bee Burseraceae resin use is first needed to formulate and test relevant hypotheses.

Goal 3.
No hypotheses are relevant to Objectives 3.1, 3.2, and 3.3.

9.3 Variables in the study

Burseraceae trees species: There are various species in four genera of Burseraceae trees in the study areas. Samples of leaves, flowers and fruits will be collected when possible from study trees. These materials will be examined in the field by the investigators and later if necessary by a Burseraceae taxonomic specialist to determine the scientific name of each tree as closely as possible.

Tree diameter: Tree diameter at breast height (DBH) will usually be measured at 1.3 meters above the ground. When necessary, the diameter will be measured higher up on the trunk above a buttress root.

Insect species: Immature and adult insects will be collected from resin lumps and host tree trunks during the study. Attempts will be made to rear immature insects in laboratory conditions to maturity. Specimens will be sorted in the field into distinct morpho-species and examined later by relevant taxonomic specialists to determine the scientific name of each insect as closely as possible.

Resin lump type: Resin lump types will be assigned based on the following characteristics: color of resin, size and shape of resin lump, presence of unusual structures such as a spout, number and pattern of bore-holes in the host tree, structure of larval chamber inside resin lump.

Resin lump number: The number of resin lumps on a host tree will generally mean the number of separate resin lumps on a tree. Where resin lumps are merged, this number will be based on the best estimate of the number of resin lumps formed by the action of individual weevils.

Amount of resin: When resin lumps are harvested, their basal area and weight will be recorded directly. When tracking resin lump growth rate, the basal area of resin lump will be measured on the tree. The relationship between the basal area and weight of harvested resin lumps will be used to estimate the weight of resin lumps at different stages of development. Resin lump area will be measured to the nearest 0.1 cm^2; weight will be measured to the nearest 0.1 g.

Resin weevil development time: The end point of resin weevil development will be defined as the point when resin lump growth ceases prior to the emergence of an adult weevil. Resin lump growth rates recorded for small resin lumps will be used to estimate the actual beginning point of larval development. The combined pupal and teneral development phases will be defined as the time between the estimated end point of larval development and the time of adult emergence.

9.4 Methodology

Goal 1
Objective 1.1/Hypothesis 1a:
Initial survey. This survey and all subsequent activities will first be conducted at study Site 1 at the research center Jenaro Herrera on the Ucayali River. In order to gather a breadth of information from different locations and forest types in the region, similar surveys will be conducted later at the Explor Napo and Madre Selva sites. Burseraceae trees with resin lumps will be located in ten (10) 20x500 m (1 ha) parallel plots of intact forest. All Burseraceae trees with DBH (diameter at 1.3 m) $\geq$ 5 cm will be located in five (5) 20x25 m sub-sections of each 1 ha plot chosen through stratified random sampling. The exact configuration of these plots may vary at different sites if suitable plots or transects have already been established. The diameter (DBH), GPS position, and number and general description (color, shape, unusual features) of resin lumps will be recorded for each tree. Digital photographs with a scale will be taken of each resin lump to estimate basal area. All resin lumps will be harvested from 50% of the trees to obtain additional information on the pattern of bore holes, resin lump weight and structure and descriptions of insects inside. Harvested trees will become study trees for testing Hypothesis 1i. Each tree will be marked with a numbered aluminum tree tag. Leaves, flowers, and fruits when possible will be collected from each sampled tree unless the trees without resin lumps can be positively identified to species without collecting such materials. Voucher specimens will be collected to allow identification and deposition in Peruvian herbaria.

Results will first be analyzed to calculate the density (trees/ha) of each Burseraceae tree species with resin lumps and the density of all Burseraceae trees (with and without resin lumps) located in the sub-plots. These densities will be ranked from lowest to highest values and compared with the Spearman Rank Correlation to test to test the hypothesis. These results will demonstrate if resin weevils preferentially colonize some Burseraceae tree species more than others.

Objective 1.1/Hypothesis 1b:
Based on the measurements and descriptions of resin lumps in the initial survey, each lump will be assigned to a distinct resin lump type based on the features that most clearly separate different types of resin lumps (i.e. small grey hemisphere shaped lumps with one bore-hole vs. large black irregular shaped lumps with multiple bore-holes). Data will be analyzed to calculate and rank the proportion of host tree species associated with each type of resin lump. These rankings will be compared with the proportional abundance of each species in the total sample with the Spearman Rank Correlation to test the hypothesis.

Objective 1.1/Hypothesis 1c:
Several complementary methods will be used to determine how many resin weevil species may be present in this system and describe their relationship to different types of resin lumps. The first method will utilize larvae removed from the resin lumps harvested in the Initial survey. These larvae will be examined under a dissecting microscope; to the extent possible, they will be classified using features described in Marvaldi (2003) to determine if any individual or combinations of morphological characteristics reliably permit distinction of weevil taxa found in different types of resin lumps.

Second, wire mesh traps will be placed around at least thirty intact resin lumps of each resin lump type (on at least six different host trees). Traps will be fastened to the tree so they can be easily removed to allow inspection of the resin lump. Traps will be checked once every two weeks to recover any adult weevils (or other insects) that have emerged from the resin lumps. This method has the advantage of positively linking adult weevils to a particular resin lump type and host species, but since pilot studies indicate weevil development may take 18 months or more (Plowden, unpublished observations), this method will not generate large numbers of specimens. Any specimens recovered will be preserved in alcohol for examination and identification to the extent possible by a weevil specialist. Voucher specimens will be deposited at institutional collections in Peru. Results will be analyzed to determine if there are distinct taxa of resin weevils associated with different types of resin lumps.

As noted above, resin weevil emergence from traps is unpredictable due to their slow development, and it is generally easier to note species level distinctions in very young than older larvae (O'Brien, personal communication). A third method will, therefore, be used to collect resin weevils specimens. Since this group of weevils appears to be nocturnal like other relatives in its group (Plowden, unpublished observation; O'Brien, personal communication), adult weevils foraging at night on host trees with resin lumps will be captured with a net and circle traps attached to the trunk (as described Bloem *et al*. 2002). At least five pairs will be caged on the host tree. Another five pairs will be placed on host tree bark in cages in an indoor setting at the study site. Females will be closely observed to watch for oviposition behavior. If this is observed, neonatal larvae may be recovered from the bark within several days or weeks. Weevil larvae and adults will be examined by weevil specialists. Voucher specimens will be deposited at institutional collections in Peru.

In each method of obtaining weevil larvae and adult specimens, the frequency of distinct weevil species or morpho-species associated with different types of resin lumps will be compared with a chi-square analysis to test the hypothesis.

Objective 1.2/Hypothesis 1d.
Resin lumps that are enclosed in the wire mesh traps for the second method of testing Hypothesis 1c will have their basal areas measured at least once per month using a wire and grid pattern as described in Plowden (2001). The weight and growth rate of these resin lumps will be estimated using a best fit regression formula derived from a comparison of basal area to weight measurements from resin lumps harvested for testing Hypothesis 1c. Mean weights and growth rates for trees with different types of resin lumps (and different resin weevil species when possible) will be compared with an Analysis of Variance procedure to test the hypothesis.

Objective 1.3/Hypothesis 1e.

The amount of developmental time for resin weevil larvae and pupae (as described in the Variable section 2.4) will be measured for each resin lump that is monitored in the second method of testing Hypothesis 1c. Mean larval and pupal developmental times for different types of resin lumps (and resin weevil species when possible) will be compared with an Analysis of Variance procedure to test the hypothesis.

Objective 1.4/Hypothesis 1f.
Data from resin lumps harvested in the Initial Survey will be analyzed to calculate the total weight of resin lumps on each tree. The hypothesis will be tested by comparing the mean total weight of resin on trees with different types of resin lumps through an Analysis of Variance.

Objective 1.4/Hypothesis 1g.
The regrowth study will include an equal number of trees whose resin was and was not harvested in the Initial Survey. The sample in each group will include at least ten trees with each type of identified resin lump. Trees will be monitored on a monthly basis for 24 months. The number of resin lumps per tree and basal area of each resin lump will be recorded at each visit. This data will be used to estimate the total resin weight present on the harvested and unharvested trees. The hypothesis will be tested by comparing the mean weight of resin per tree for these two groups at each monitoring period through Analysis of Variance. The procedure will be repeated comparing specific resin lump types in both groups. The regrowth data of the two groups will be further analyzed to observe or predict when the amount of resin present on harvested trees equals or will equal the amount on the unharvested trees.

Objective 1.5
Six different factors and time since initial observation or resin harvest will be examined alone and in combination in a best-fit regression model to test the model's power to predict the actual or estimated total weight of resin on a tree. These factors will be: host tree species, host tree diameter, resin lump type, resin weevil species (if possible), resin lump number during Initial Survey, resin lump actual or estimated weight at Initial Survey. The procedure will allow development of a model to predict total resin amounts with and without harvest at different points in the future.

Goal 2.
Objective 2.1/Hypothesis 2a/b.
Resin lumps harvested in the Initial Survey will be examined for the presence of syrphid and other types of fly larvae. Results will be analyzed to show what percentage of resin lumps containing fly larvae are associated with each species of Burseraceae host trees and each type of resin lump. These percentages will be put in rank order. First, the proportion of each host tree species in the total sample will be compared to the order of those with fly larvae using the Spearman Rank Correlation to test Hypothesis 2a. Next, the proportion of each resin lump type in the total sample will be compared to the order of those with fly larvae using the Spearman Rank Correlation to test Hypothesis 2b. If sample sizes permit, these tests will be repeated to examine the correspondence between particular taxa of flies with different species of host trees and resin lump types.

Objective 2.2/Hypothesis 2c/d.
Resin lumps harvested in the Initial Survey will also be examined for the presence of ants. When ants are found in resin lumps, the following information will be recorded: 1) the species of host tree, 2) the type of resin lump, 3) the general description of the ant (size, color, and if possible genus), 4) the presence of gynes, queens and workers, and 5) ant use of resin and other materials to make their nest.

Results will be analyzed to show what percentage of resin lumps containing ants are associated with each species of Burseraceae host trees and each type of resin lump. These percentages will be put in rank order. First, the proportion of each host tree species in the total sample will be compared to the order of those with ants using the Spearman Rank Correlation to test Hypothesis 2c. Next, the proportion of each resin lump type in the total sample will be compared to the order of those with ants using the Spearman Rank Correlation to test Hypothesis 2d. If sample sizes permit, these tests will be fine-tuned to examine the correspondence between particular taxa of ants with different species of Burseraceae host trees and resin lump types.

Objective 2.1/Hypothesis 2e/f.
Data will be gathered to test these hypotheses regarding bee visits to resin lumps in two ways. The systematic method will be to randomly select five (5) trees with fresh resin from each Burseraceae species found in the Initial Survey. This sample should include at least five (5) trees with each type of resin lump. Since stingless bees do not forage for resin at night (Roubik, personal communication), each tree will be observed in

successive thirty minute periods during daylight hours for one week every three months so that observations are conducted during parts of the rainy and dry seasons. Specimens of bees visiting these resin lumps will be captured with a net or suction tube trap and preserved for resin studies (Objective 2.3/Bee resin harvest – Part I) and identification. One visit will be counted as an observation of one bee harvesting resin from a particular tree and then leaving. Data will be analyzed to calculate the percentage of total bee visits associated with each species of host tree and each type of resin lump. The hypotheses will be tested by using the Spearman Rank Correlation to compare these ranked percentages with the ranking of the total time spent observing host trees of particular species and particular types of resin lumps. If sample sizes permit, these comparisons will also be done with individual species of bees observed or captured in these activities.

Additional valuable data on the association of bees with Burseraceae resin lumps will be collected in the course of working with these trees for other objectives of this study. This information will be presented qualitatively.

Objective 2.2/Hypothesis 2g/h.

Fly larvae obtained from *Protium* resin lumps in Brazil have been identified as belonging to the genus *Alipumilio*, but adult specimens will need to be examined in order to identify these or similar resin flies to the species level (Rotheray, personal communication). This will be attempted by using the same type of wire mesh enclosures constructed to capture adult weevils described in methods for Hypothesis 1c. In addition to monitoring the traps established for monitoring the emergence of all types of insects (weevils, flies and ants) on a bi-weekly basis, traps will be placed around an additional ten (10) resin lumps that are known to or probably contain fly larvae. This will be determined by partially opening and carefully examining the inside portions of resin lump types shown through investigations of Hypotheses 2a and 2b to likely contain resin fly larvae. Interior resin sections containing fly larvae are readily detected since fly larvae foraging transforms white resin into a dark brown stretchy material (Plowden *et al*. 2004). Such short-term disturbance seems unlikely to disturb the larvae or disrupt the viability of the resin lump since its base will not be detached from the host tree. These resin lumps will be monitored on a weekly basis during four one-month periods to capture emerging fly or other types of insect larvae. If more than one species of resin fly is identified in these traps, chi-square analysis will be used to test Hypotheses 2g and 2h of correspondence between resin fly species and particular types of resin lumps and host tree species.

Objective 2.3/Hypothesis 2i.

One of the goals of Objective 2.3 will be to determine if the ants found in Burseraceae resin lumps use this resource opportunistically or if any of them have become highly specialized users. The following methods will be used to test the hypothesis. First, when ant specimens are collected from resin lumps and identified, the entomological literature will be searched to see if there are documented observations about the nesting habits of these species in other settings. Several techniques will be followed in the field to explore this issue. First, ants using the resin lumps will be observed at twenty different trees to see if they are collecting and using materials other than resin to build their nests. Small pieces of the nests will be removed to observe if the ants use resin from the host tree or other materials to repair them. Second, these observations will include watching the ants to see if they travel to other nests in similar or different types of trees. Third, the trunks of 20 randomly selected trees in the vicinity of these ant resin study trees will be examined to see if there are other arboreal ant nests visible. These trees will be selected in sequence by first choosing a compass bearing at random, following that compass bearing for a randomly selected distance of one to fifteen meters and making observations at the nearest tree ($\geq$ 5 cm DBH) to that point. Specimens of these ants will be collected if possible for comparison to the ants found in the resin lumps. The percentages of all trees with resin lumps found to contain ants of a certain species will be compared with the percentages of the randomly chosen trees containing nests of the same species with a chi-square procedure to test the hypothesis.

Objective 2.3/Bee resin harvest – Part I

The first step of assessing the importance of Burseraceae resin to bees will be to estimate the frequency and amounts of this resin that are used by particular types of bees. The same five trees used to make observations on bee visits to resin trees for Objective 2.1/Hypothesis 2 e/f will be used to estimate bee resin harvest. These will be observed in successive thirty-minute periods for five days every three months for the duration of the study. Stingless bees do not forage for resin at night (Roubik, personal communication) so observations will be restricted to daylight hours. The number of bees that remove resin during each observation period will be recorded. A sample of bees representing each species gathering resin will be collected for identification. Resin will be removed from their hind legs and weighed on a portable digital scale to estimate the amount of resin removed per bee visit. The resin collected per visit will be multiplied by the number of visits per tree per day to estimate the amount of resin removed by bees per tree per day. Results from all trees will be analyzed to calculate the mean value for this value of resin use. This mean value will be combined with the density of trees with fresh resin to estimate the amount of Burseraceae resin use by bees per hectare of forest during different stages of the rainy and dry seasons.

In the course of observing bees in this study and observing trees for ant nests, researchers will also try to note the location of nests of any resin using bees. One good indicator of this is a tubular structure protruding from a hollow tree that is guarded by bees (Roubik, 1989). Locating such nests will be an important first step to assessing the overall amount of resin use and the relative importance of Burseraceae resin to resin using bees in this area. If accessible stingless bee nests are found in the course of the project, a sample of the nest entrance tube and resin being brought to the nest will be collected for later analysis and comparison with the chemical profiles of known Burseraceae resins. This method was used to match floral resins with resin compounds found in a bee nest in Venezuela (Tomas-Berberan *et al*., 1993).

Objective 2.3/Hypothesis 2j.
The second method that will be used to estimate the amount of Burseraceae resin used by bees will be to use the trees being monitored to capture emerging weevils and flies (Hypotheses 1c, 2g) and resin lump growth rate (Hypothesis 1g). The screen traps that are designed to prevent the escape of emerging insects will also exclude bees from harvesting fresh resin. Changes in basal areas over time will be converted to an estimate of resin lump growth in weight. The difference in resin lump growth rate between the caged and uncaged resin lumps will be a rough estimate of the amount of resin removed from the outside of the resin lumps by bees. The mean resin growth rates in these two treatments will be compared through an Analysis of Variance to test the hypothesis. One factor that may reduce the reliable interpretation of this method's results is that covering resin lumps with screens may reduce the rate of resin lump weight loss due to volatization and drying of the resin. This effect, however, is likely minimal in the shady understory.

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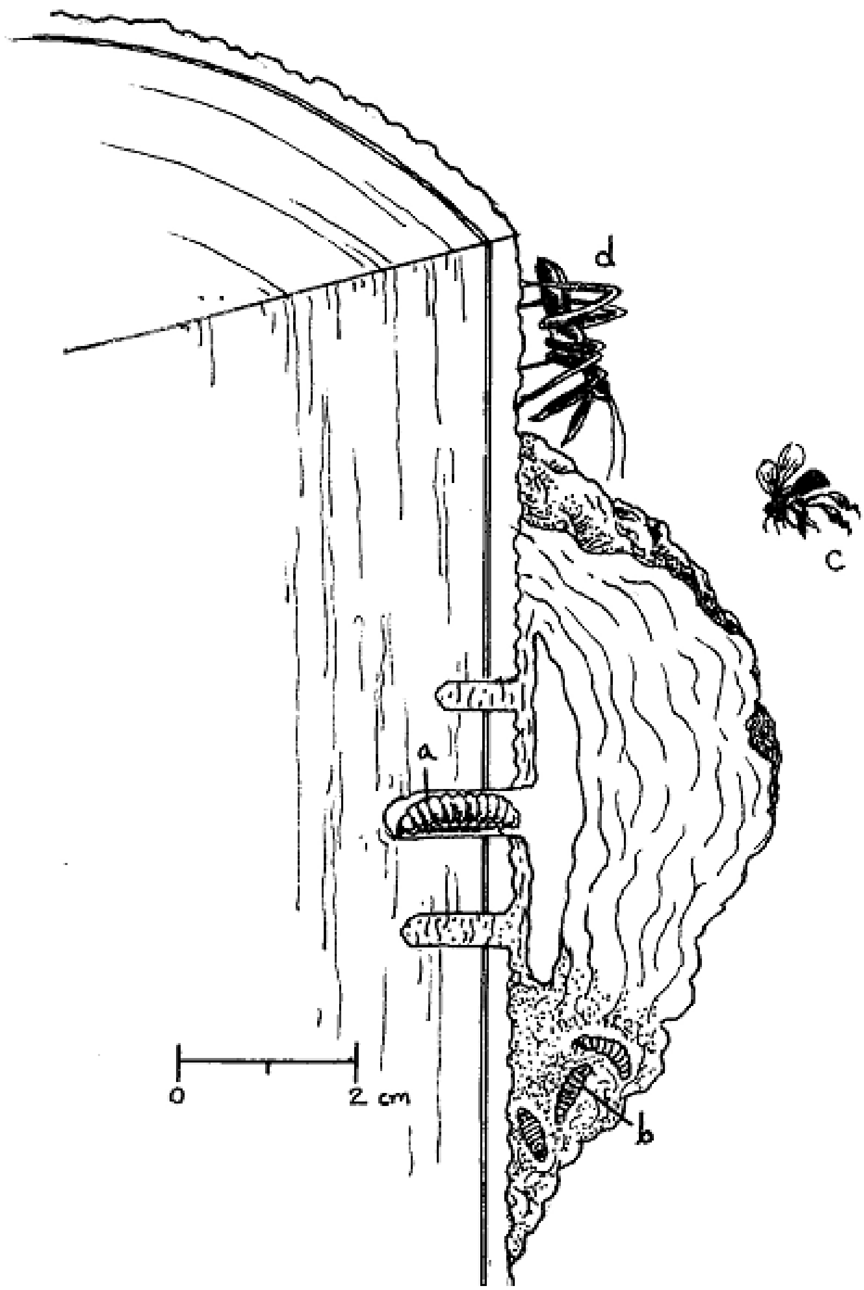
People Cited in Proposal (Personal Communications)

Dr. Jacques Delabie: Staff entomologist, Ant Laboratory, Center for Cacao Research, Itabuna, Bahia, Brazil
Dr. Paul Fine: Post-doctoral student, Field Museum of Chicago, Chicago, IL.
Dr. Michael Gilmore: Department of Botany, Miami University, Oxford, OH.
Dr. Devon Graham: President/Science Director, Project Amazonas, Ft. Lauderdale, FL.
Dr. John Longino: Professor of Entomology, Evergreen State University, Olympia, WA.
Dr. Charles O'Brien: Retired professor of Entomology, Florida A&M University. Green Valley, AZ.
Dr. Silvia Purata Velarde: Staff Ecologist, Institute of Ecology, Xalapa, Veracruz, Mexico.
Dr. Graham Rotheray: Curator Entomology, Royal Museums of Scotland, Edinborough, Scotland.
Dr. David Roubik: Staff Scientist, Smithsonian Tropical Research Institute, Balboa, Panama.

Figure 1.
Illustration of insects associated with Burseraceae tree resin lump in eastern Amazon
Adapted from Forest Chemical News



a. *Pappista* weevil larva feeding on phloem in borehole adjacent to chamber in resin lump
b. *Alipumilio* fly larvae in resin lump found on some host trees
c. *Trigona* stingless bee collecting fresh resin for nest construction
d. Reduvid assassin bug stalking bees and other insects attracted to fresh resin