A BERRY COLOR DIMORPHISM IN A NEW SPECIES IN THE FAMILY *ERICACEAE*

ZACHARY A. MAYER AND SAMUEL J. HAYNOR

Abstract: The majority of plant color dimorphisms on record are floral dimorphisms, often with each morph having a different reproductive strategy. Fruit polymorphisms are very rare, but potentially important for the seed dispersal strategies of some species. In the páramo of Costa Rica, we found a previously unrecorded species of the family *Ericaceae*, temporarily named *Pernettya saelyeae*. The plant exhibited two berry color morphs, with either red or white mature berries. We recorded the locations of individual plants of both color morphs across seven 30 m x 30 m grids and measured mean mass per berry and mean seeds per berry of each plant. Each color morph was highly aggregated, and percent composition of white morphs was higher in sites with less ground cover. We found that red-berried plants had fewer seeds per berry and invested more berry mass per seed than in white-berried individuals.

Key Words: Pernettya saelyeae, Pernettya prostrata, *berry color dimorphism, Costa Rica, páramo, new species*

INTRODUCTION

Flower color polymorphisms have long been a subject of fascination for botanists. Many plants exhibit environmentally or genetically driven floral color dimorphisms, with fitness tradeoffs between resource allocation and pollinator preference (Jones and Reithel 2001). For example, several plants from the Heliconiaceae family have developed flower color dimorphisms, corresponding to male and female hummingbird nectar reward preferences and energetic requirements (Temeles and Kress 2003). Color polymorphisms can be maintained in plant populations through environmental heterogeneity (Warren and Mackenzie 2001) or by geographic isolation of conspecifics (Schemske and Bierzychudek 2001). While flower color dimorphisms have been extensively documented, dimorphisms of fruit color are rarely reported.

The color of a fruit is essential in its ability to attract effective seed-dispersers while inhibiting visitations from fauna who are poor seed-dispersers (Wheelwright and Janson 1985). Fruit pigmentation also has physiological effects, such as thermal regulation (Willson and Whelan 2001). Color polymorphisms in fruit are hypothesized to be maintained by environmental heterogeneity, with berry pigmentation often varying between stressed and non-stressed plant environments (Mandak and Pysek 1999).

In January of 2007, local conservationists Andrew and Satyia Saelye introduced us to a potential new species of plant in the family Ericaceae on the páramo in the San José province of Costa Rica, which they tentatively named *Pernettya saelyeae* (common name "banapple"). *Pernettya saelyeae* was previously thought to be of the species *P. prostrata*, a Central American shrub with purple berries poisonous to humans in all of its hybrids (Vindas, 2003). However, Andrew and Satyia asserted that the *P. saelyeae* berry was edible and therefore not *P. prostrata*. The authors consumed several hundred berries each and did not get sick, corroborating their assertion.

An interesting, salient characteristic of *Pernettya saelyeae* is that different individual plants exhibit either fully red or fully white berry pigmentations. We noticed immature green berries on both color morphs, and never found a plant that had both fully red and fully white berries on it. This provided evidence against the two colors being developmental stages of the same morphotype. We hypothesized that there was indeed a color dimorphism in *P. saelyeae*, and that the morphs differ in local distribution. Because fruit color polymorphs are often maintained by variation in environmental conditions, we expected individual plants with the same berry color morph to be aggregated in microhabitats. We predicted (1) that the morphs would be aggregated in patches by fruit color and (2) that there would be some difference in mass per seed and seeds per berry between individuals of the two berry morphs as it related to potential seed dispersers. We had previously observed that white berries appeared to be seedier, while red berries appeared to be sweeter, perhaps indicating some dichotomy in seed dispersal strategies.

METHODS

Study system.

Pernettya saelyeae is a dominant shrub in areas above treeline in the Talamanca mountain range of Costa Rica. Hence, the páramo region of Chirripó National Park in Costa Rica on 28-29 January 2007 (during the dry season) proved a good study site to record *P. saelyeae* dimorphic aggregations.

Field Methods.

We haphazardly chose a 30 m x 30 m quadrat in the páramo region of Chirripó National Park where *P. saelyeae* were present. We recorded the location of each *P. saelyeae* individual within this quadrat as a grid coordinate, noting berry pigmentation (red, white, or immature) of the plant, number of branches on the plant, and plant height (cm). For each of the 76 plants recorded within the 30 m x 30 m

grid, we collected ten representative berries and measured mass per berry (g) and counted seeds per berry to determine any differences between color morphs. For each plant, we calculated mean mass per berry and seeds per berry. We used these data to calculate mean berry mass per seed for each plant.

To examine aggregations and abundances of *P. saelyeae* berry color morphotypes across the páramo, we haphazardly chose six additional 30 m x 30 m quadrats where we recorded the location and berry pigmentation of each fruiting *P. saelyeae*. For each quadrat, we observationally recorded vegetative ground cover. Each quadrat was considered to have uniform vegetative cover (dense cover, medium cover, or clear). We defined quadrats with dense cover as those which were overgrown with woody tussock grasses, while medium cover areas were covered only by small herbaceous plants, and clear areas were composed of either human-made trails or large areas of open earth. In one quadrat with a very clear human-made trail (quadrat 7), we also measured the distance from each plant to the trail. We created X-Y plots for each quadrat using the coordinates of each *P. saelyeae* plant sampled, distinguishing plants of each berry morphotype. We sampled 513 total plants in all 7 quadrats, and calculated the relative abundance of each berry morph on the páramo. During this process, we dug holes around what appeared to be several individual plants to be certain that there were no roots connecting multiple individuals.

We also collected 50 mature berries of each color for human taste preference trials conducted at La Estación Biología at Cerro de la Muerte on 30 January 2007. Fifteen volunteer subjects blindly sampled two berries (one red, one white), and then stated their taste preference. We conducted two trials per individual, flipping a coin to determine the color sequence in each trial.

Statistical analysis.

We visually assessed the data from our 7 quadrats to determine whether the berries were obviously spatially aggregated by color. We performed a chi-squared test on the number of red-berried and white-berried individuals in grids with each level of vegetative ground cover to determine if ground cover influenced percent composition of red-berried and white-berried plants. To look at plant morphotype distribution on a smaller scale as it relates to vegetative ground cover, we used a two-tailed t-test to analyze the distance of each individual from a man-made trail in grid number seven. Ground cover decreased as one approached the trail. Analysis of all seven quadrats examined berry morphotype aggregation by ground cover across the páramo, while

analysis of quadrat 7 took a detailed look at the gradient of ground cover in a small section of the páramo.

We used a MANOVA to test whether there was a difference in mean berry mass, mean seeds per berry and mean berry mass per seed between the two berry color morphs. The model was statistically significant, which allowed us to use two-tailed t-tests (assuming unequal variance) to individually compare the mean berry mass, mean seeds per berry and mean berry mass per seed between the two berry color morphs. We used a chi-squared test to analyze taste preference of berry color of the 15 experimental subjects.

RESULTS

We found 407 (79% of total individuals) red-berried and 92 (18%) white-berried *P. saelyeae* individuals in our seven 30 m x 30 m grids on the páramo, with 14 (3%) additional individuals with pre-mature berries.

It is visually apparent that the color morphs of *P. saelyeae* aggregate into distinct clusters (Fig. 1). We found that there was a greater proportion of white-berried individuals in quadrats with less ground cover ($X^2_{4,507}$=46.493, P<.0001, Fig. 2). This lends more support to the idea of microhabitat segregation, suggesting that the white berries are relatively more common in clear areas. In quadrat 7, which had a high density of *P. saelyeae* plants as well as a trail transecting it, we found that white plants were, on average, 35% closer to the trail than the red plants (t_{151}=3.69, P=0.0003). White plants were on average 6.24 meters from the trail, while red plants were on average 9.54 meters from the trail.

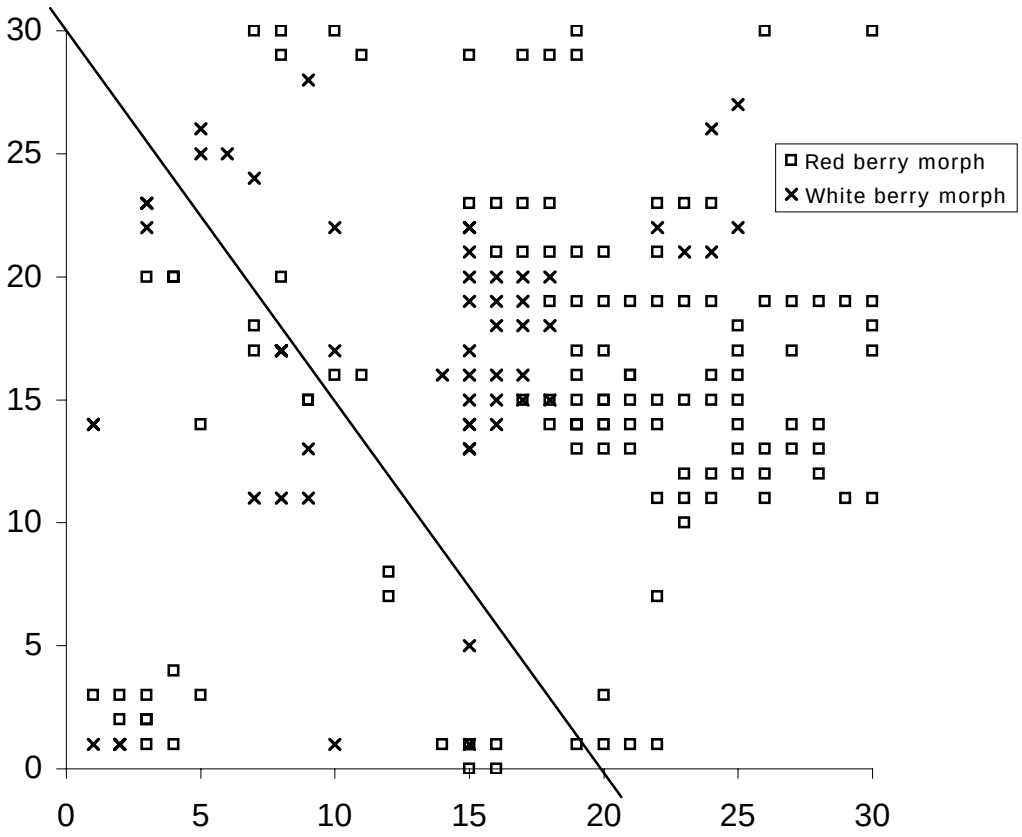

Figure 1. Distribution of *Pernettya saelyeae* berry color morphotypes (red and white) in a 30 by 30 meter grid. The solid line in this figure represents the man made path running through quadrat 7. The number of white morphs and red morphs present are N=57 and N=99, respectively. Bold squares represent two data points.

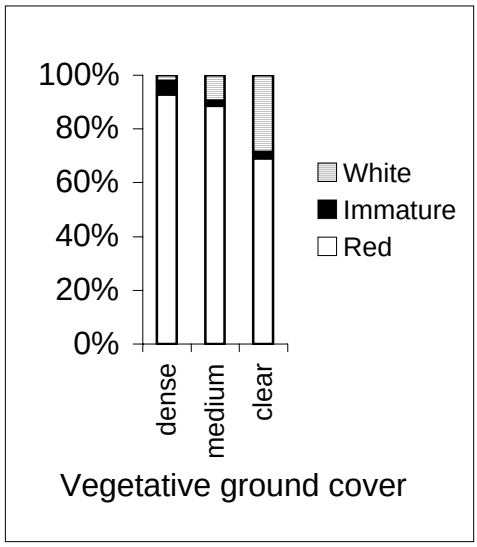

Figure 2. Percentage of *Pernettya saelyeae* berry color morphotypes and individuals with immature berries (white, red, and immature) in seven quadrats at different levels of vegetative ground cover (dense, medium, and clear). As the vegetative ground cover level decreased, the relative percentage of white plants to red plants increased. Total sample size of plant individuals was N=516.

We found significant differences in berry mass, seeds per berry, and berry mass per seed between the two berry morphs (MANOVA: $F_{3, 36}$=1.12, P<.0001). We found no significant difference between the mass of white berries (mean ± 1 SE=0.77 ± 0.078 grams) and red berries (mean ± 1 SE=0.78 grams ± 0.078) ($t_{26.85}$=0.46, P=0.65). However, the standard deviation of the masses of red berries (.43 grams) was nearly twice that of the masses of white berries (.23 grams) ($F_{1,38}$=.935, P=.0041). We found that the white berries contained 23% more seeds than red berries ($t_{33.36}$=4.51, P<0.0001); red berries contained a mean of 29.10 ± 1.57 seeds, while white berries contained a mean of 37.61 ± 1.06 seeds. Furthermore, red berries had 35% more berry mass per seed than white berries ($t_{27.65}$=5.59, P<0.0001). Red berries contained a mean of 0.004276 ± 0.00017 grams of berry per seed, while white berries contained a mean of 0.003191 ± 0.00009 grams of berry per seed.

Finally, in the human berry preference test, red was preferred in 81% of the trials, while white was preferred in 19% of the trials (X^2_1= 9.85, P=.002). In 23 out of 28 trials, the red berry was preferred, while white berries were preferred in only 5 trials.

DISCUSSION

Fruit color polymorphisms are not yet well understood. While dimorphic flowers are commonplace, distinct fruit phenotypic differences in conspecifics are not. Our observation that white and red berries never co-occur on the same individual plant and that immature green fruit are on both plants supported the possibility of a berry color polymorph. Both morphotypes transition though a pinkish pre-mature phase; berries turn their mature color starting from the side opposite their stipule, where their flower dropped from their inferior ovary. These morphotype-specific development observations suggest that there is a color dimorphism in the fruits of *P. saelyeae.* The lack of knowledge about this dimorphic fruit exists in more than just the biological community. Satyia Saelye stated that even local people in the area were unaware that this berry was edible, and confused it with its poisonous relative, *P. prostrata*, also present in the páramo zone.

In our visual analysis of our grid data, we found distinctly segregated clumps of red and white-berried plants, supporting our prediction that the plants would be spatially aggregated by color. This suggests that there are factors such as different microenvironments or

local dispersal patterns that favor the different color morphs. Interestingly, there was a much greater abundance of red-berried morphs than white, even in areas clear of vegetative cover, where white-berries were found in greatest abundance.

The distinctive traits of the two berry morphs may illuminate reasons for this difference in berry color morph abundance. We found a high density of approximately 130 *P. saelyeae* seeds in a single pellet of excrement of a grey junco bird, indicating that birds are an important disperser for this fruit. Although tropical birds vary in their visual preferences for fruit pigmentation, an overwhelming number of studies have found that red is the most common color for bird-dispersed fruits in neotropical regions (e.g., Wheelright and Janson 1985, Janzen 1983). This may mean that the red berries are more likely to be dispersed and possibly that red individuals have higher reproductive success. However, Willson *et al.* (1990) showed that when frugivorous birds are presented with fruits of equivalent nutritional value, they did not show a strong preference for red fruit over other colors. Therefore, color alone may not predict preference and subsequent dispersal rates between the two morphs.

From a disperser perspective, mass of the fruit's mesocarp should be important in foraging preference. Although the mean mass of mature berries between the two berry color morphs were equal, red berries had 35% more berry mass per seed than white berries. This red coloring is therefore probably an honest signal of rewards to a potential seed disperser, and the larger mesocarp in the red morph should create a frugivorous bird preference for red berries over white berries.

Although local dispersal patterns could predict berry color morph aggregations in space, it does not explain why relatively more white-berried individuals are found in areas with less vegetative cover. While some fruiting plant species always produce white fruits, other species produce white fruits only when the plant is under stress (Mandak and Psyek 1999). Stress-induced white pigmentation of fruits has previously been studied in apple cultivars, specifically involving light-stress. This bleaching has been hypothesized to be a result of decreased anthocyanin production. Typically, anthocyanin functions to sequester light energy internally, protecting fruit tissues from high light intensities (Merzlyak *et al.* 2003). At an altitude above 11,000 feet, plants in the páramo have a number of adaptations for the sun. Many plants have rosette formations for indirect deflection of heat. Others have white sheens acting as a "sunscreen," providing protection from intense sunlight. The red berry

morphs of *P. saelyeae* may be a similar adaptation for the high light intensity. Sun-protective anthocyanin production may only be available in areas of high soil nutrient content, which may explain why red berry morphs are found in areas with denser vegetative cover.

It is also possible that genetics plays a role in the berry color morph distribution of *P. saelyeae*. If this is case, anthropogenic factors could contribute to the high aggregation of white-berried individuals in areas with low ground cover such as near the main trail. We found that our volunteers greatly preferred red berries, and thus, human consumption of these red berries along the trail may lead to unsuccessful red berry dispersal in this area. With many tour groups visiting the páramo every week, it seems likely that anthropogenic factors play a role in the distribution of this common fruiting plant.

Our study was not able to determine whether *P. saelyeae* berry color morphotypes are environmentally induced, heritable, or both. To augment our study, future experiments could include growing *P. saelyeae* under different light intensities as well as cross-breeding individuals to look for Mendelian or more complex genetic patterns involved in determining berry morphotype. Knowledge of this newly described plant could contribute to the relatively new field of research on the adaptability and evolution of fruit color morphisms.

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WORKING TOWARDS SUSTAINABILITY: A CASE STUDY OF A TROPICAL MONTANE FIELD STATION

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Abstract: La Estación Biológica de Cuerici in the San Jose province of Costa Rica is a center for ecological conservation and biological education. The private owners have protected 200 hectares of oak and alder forest while conveying the importance of conservation and sustainability to all visiting groups. The station is funded by income from visiting student groups. The food and water networks are highly integrated into the natural environment and the surrounding human community, focusing on reuse, recycling and sustainability. The integrated systems at the station have some vulnerabilities and limitations, including dependence on a single hydropower system and current reliance on outside food sources. Despite these limitations, the station system demonstrates that a practical balance can be attained between conservation and human needs.

Key Words: sustainability, resource cycle

INTRODUCTION

Seventy years ago, the grandparents of Carlos Solano acquired land near Cuerici Mountain in the Talamanca ranges of Costa Rica. Because there was no access road to the property, the family was unable to sell timber. Instead, the family created pastures and agricultural land using slash and burn methods. They hunted animals from the mountain and raised cows. Later, after an access road was built, they began to produce charcoal from dominant tree species, such as oaks (*Quercus copayensis* and *Q. costarricenses*). In the 1970's, the Costa Rican government passed strict laws that made deforestation illegal. A farmer was required to obtain a permit for any tree removal. The family no longer had a livelihood, and his grandparents decided to sell the land as cattle pastures. Although Carlos spent his childhood catching birds and hunting tapirs, he valued the mountain forest and saw it disappearing rapidly. He decided, with the help of eight friends, to buy the land from his grandparents, along with some additional neighboring property.

With his co-owners, Carlos designed a set of laws for the land. He divided up the land for different purposes: a blackberry farm, a small cow pasture, a site for reforestation, a site for forest conservation, and residential space. He preserved riparian buffers by building barbed wire fences to keep cows from disturbing the vegetation near

streams and possibly contaminating the water supply with their feces. He prohibited tree cutting and the use of fire. It was not difficult to convince the small surrounding community to support fire control after a large fire in 1992 spread upslope, destroying farm crops and part of the oak forest. The fire united the citizens with a common goal of conservation. Carlos created La Estación Biológica Albergue Cuerici to save the mountain habitat in which he was raised.

Although Carlos supports nature conservation, he believes a balance between conserving nature and maintaining human needs is more important. According to Carlos, this balance entails using only what is necessary from the land. He has just enough crops to make a small profit to support his family and just enough cows and chickens to supply his family with milk and eggs. He abhors the ideas of silviculture and export agriculture as he believes that greed and exploitation destroy the forest. His life is now devoted to the sustainable use of the land.

Human land use is inevitable. However, sustainable development can allow humans and the environment to coexist healthily. We defined sustainability as a self-propagating system in which resources are provided locally and wastes are contained and recycled. Sustainable living is especially important in the rain forest, where biodiversity is high but is potentially decreasing due to rapid deforestation (Evans 1999). For example, in Costa Rica in 2005, only 7.5% of primary forest remains, and of that, 75% is privately owned (F.A.O. 2005). Carlos and other private landowners like him may become the driving force in protecting new land in Costa Rica. In 1991, Carlos built La Estación Biológica Albergue Cuerici to share his ideas of sustainability with visiting students and teachers (Fig. 1). Students come from around the world to study Cuerici's tropical forest habitat. However, when they leave the station, they take with them not only a biological understanding of the forest, but also an understanding of how people like Carlos survive and successfully conserve.

We explored the central networks of this field station. We conducted personal interviews (Solano *et al. personal communications*) and collected data on the two central resource cycles: water and food. These systems are fundamental to the station's growing resource independence. We interviewed Carlos Solano (trout farmer and station manager), Albert Torres (station administrator and friend of Carlos), Maydou Cordoba Delgado (wife of Carlos and farm manager), Elisa Cordoba Delgado (chef and

relative of Carlos), and Dacia Mena Rojas (chef and neighbor).

WATER SYSTEM

The water system at Cuerici has three main source inputs: a stream to the West, an underground spring to the West, and another underground spring to the East. Incoming water is used in the sewage system, potable water system, trout farm, and the hydroelectric station (Fig. 2). All incoming water is from high in the watershed (>2600 m), and therefore does not contain significant bacterial or toxin contamination.

Drinking Water. The underground spring to the East provides the station and the surrounding homes with all necessary water. This water comes in from the spring and immediately flows through a mesh filtering screen into a sediment tank. Within the tank, the sediment settles to the bottom and water continues through another filtration tube into a holding tank. From this tank, the water is distributed to the station and three surrounding homes that are a part of the station complex.

After water is used by the station, there are two output trajectories. Sewage water (from the toilet system) goes into a series of septic tanks. Two are needed to accommodate the volume of sewage coming from the station. Within the tanks, bacteria and other microbes decompose the organic matter. When water leaves the tanks, it travels to a rock and soil filtration system. The system is covered in plastic on the surface to prevent leaching of unprocessed organic matter. He placed the septic tank away from ground water and stream beds, and artificially raised the land at least 2 meters to increase the distance of rock and soil filtration.

All grey water (from showers, kitchen, sinks), goes directly to a separate rock and soil filtration system comparable to the system previously described. It is important that these two water outputs are kept separate because the soap and other chemicals in the grey water would drastically decrease the efficiency of decomposition in the sewage system, leading to a system backup. Each individual house within the station complex also has its own septic system.

Trout. The underground spring and the stream to the West both feed the trout farm, which currently has ca. 50,000 fish. Both the stream and spring waters are from a similar geographic location. The incubation tank directly uses groundwater, while the trout tank water comes from a stream near the groundwater system. These two water sources are kept separate both in the holding tank and all delivery tubes because eggs and hatchlings must be kept in pure spring water.

The source of the spring is covered with plastic to exclude organic matter and sediment. The incubated trout require sediment-free water because they are born with their mouths open, and any small particles in the water could cause suffocation. The spring feeds directly to the incubation tanks at approximately 0.48 L/sec. Water flow was measured by taking five measurements of the surface velocity (using a floating stick). The area of output was then calculated (width of transect and depths at 10 cm intervals were used for these calculations). Incubation pipe inputs were measured using one-liter container fill rates. We decided to quantify as many water inputs and outputs as possible to better understand water cycles of the station complex. The Western stream feeds all other trout tanks, including the four large outside holding tanks and the trout trenches (Fig. 1). Water enters the four holding tanks at a rate of approximately 8.50 L/sec.

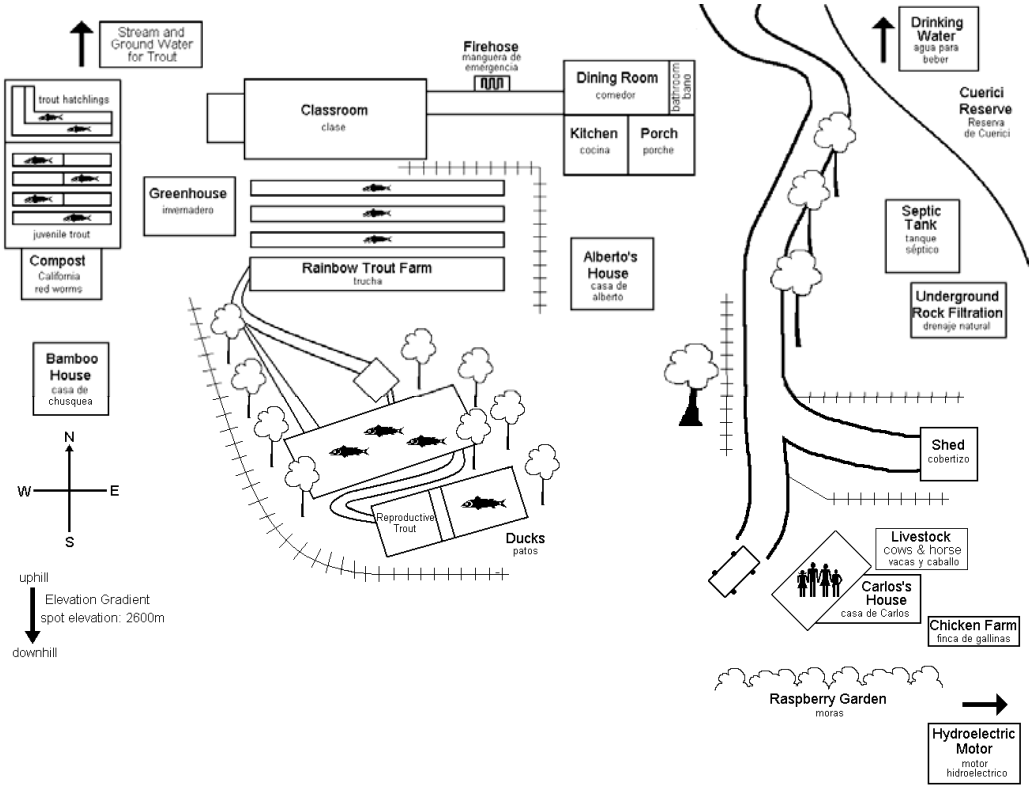

Figure 1. Map of Cuerici Biological Station and all surrounding components. This includes the station and resources used by Carlos, Carlos's daughter, and Alberto.

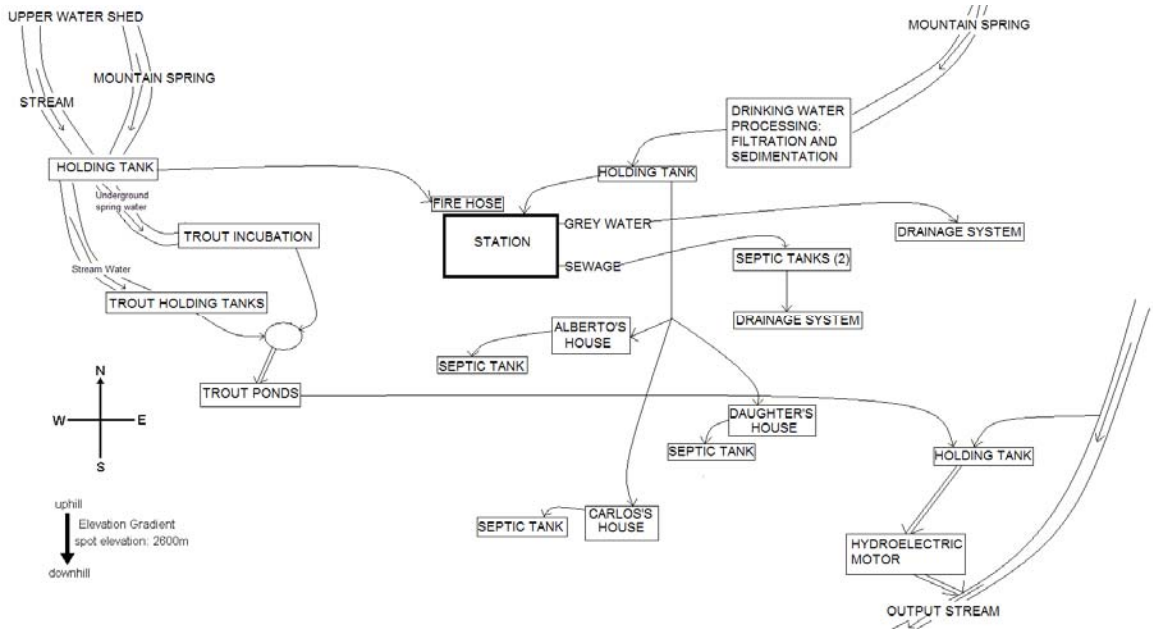

Figure 2. Map of water inputs and outputs throughout the trout farm, water delivery system, and hydroelectric pump.

Incubation begins in the pure, oxygenated spring water. Eggs experience about 50% mortality. The two main causes of mortality are suffocation from the weight of the yolk sac on the embryo and fungal infections of the egg. Although two fungicides are used by the government to control fungal infections (azul de metileno and verde malaquita), Carlos instead picks out dead, infected eggs by hand to prevent water contamination that could harm downstream aquatic cycles and aquatic organism health. This is a very time-intensive process, but is important for maintaining water quality. All incubation occurs in an enclosed room, keeping the eggs protected from outside elements.

After trout incubation, which lasts 25-30 days, the fish are moved to inside tanks, where they grow to three centimeters in length. They are then moved to one of four holding tanks for trout up to approximately four cm in length. Carlos has ca. 7500 fish in each holding tank, and most of these will be sold to other farms. Tanks are arranged by size class to minimize cannibalism of smaller trout. However, within-tank cannibalism still occurs frequently. Small trout (2-4 cm) also die easily during transferring and sorting. Total mortality of small trout is roughly twenty percent. Tanks are cleaned twice a week to remove fish

excrement and to prevent bacterial buildup, which depletes the dissolved oxygen.

Most fish are sold at ca. four cm, but those that are especially large and healthy for their age class are kept for breeding. Trout kept for breeding or consumption are moved to four ground trenches which contain various sizes of fish. Around two years of age, they are moved from these trenches to larger ponds where reproductive trout of all age classes are combined. Trout reproduce annually, usually between September and December, and each female produces 1000 to 3000 eggs. Trout instinctively migrate upstream to lay their eggs. Fish will wait at the gate which leads upstream from the reproductive pond. Here, the fish are collected for egg and sperm extraction. Each fish is removed from the water and eggs or sperm are manually extracted into separate collection jars. The largest eggs and sperm from the healthiest individuals are matched to produce the best possible offspring. Fertilized eggs of many mating pairs are used to maintain genetic variability in each generation. Roughly ten adult trout die every reproductive season from competition and natural causes. This completes the life cycle within Carlos's trout farm.

Hydroelectric System. Water from the stream in the immediate station watershed, water that bypasses the drinking system, water that bypasses the trout system, and trout outputs combine in a holding tank south of the station (Fig. 2). The holding tank is equipped with a large mesh filter in the bottom to prevent leaves and other objects from damaging the hydroelectric turbines. The pipes to the electrical motor are placed at the base of the holding tank to prevent any air from entering the system, which could cause system failure. The water flows through this entrance pipe approximately 25 meters in slope distance downhill to the hydroelectric motor at approximately 5.49 L/sec.

The hydroelectric system provides power to the station, the three surrounding houses in the station complex, and the trout farm. The system is composed of a Baldor 5000 W high efficiency electric motor, a Thomson and Howe Energy System and small hydro controls (which measure water flow, amperes, voltage, and hertz through a Siemans sensor system), and a General Electric converter. There is a small transformer in the hydroelectric pump house which supplies 110 V electricity to a light and an outlet box for use within the pump house. All energy traveling to the houses and station leaves the pump house at 440-460 volts (59-59.5 Hz). Voltage is lost en route to the converter at the station, where it is then transformed to either 110 or 220 V. The water heater and other large

appliances require 220 V. After transformation, the energy passes through a sensor which allocates the energy to different resources throughout the station and to each of the three houses. Because there is no storage device for energy (e.g. battery or generator), the water heater is a buffer for inputs of energy. When there is a change supply, the water heater is able to receive excess power, or shut off, allowing electricity to flow to more critical devices. If the energy input goes outside the range of 50-150 V, the entire system shuts down and must be restarted at the pump house. Carlos was not able to estimate the total power generated, the power used, or the amount of excess energy lost to water heating because the station lacks a battery or generator.

FOOD CYCLE

When we (19 students and faculty) arrived at Cuerici, we became part of an intricate food web (Fig. 3). To prepare a typical meal, many resources are used. Alberto imports approximately 85% of the food from San Jose and San Isidro, including rice, beans, sugar, meats, and condiments. The other 15% of the food is supplied locally. Carlos's trout, his son's blackberries, and his uncle's carrots and potatoes are also part of the menu. Per day, for 19 people, the chefs use about three kg of dry rice, three kg of dry beans, two kg of meat, and 50 pieces of fruit for juice. The kitchen staff includes Carlos's cousin and close neighbor. They use a propane stove to cook three meals a day.

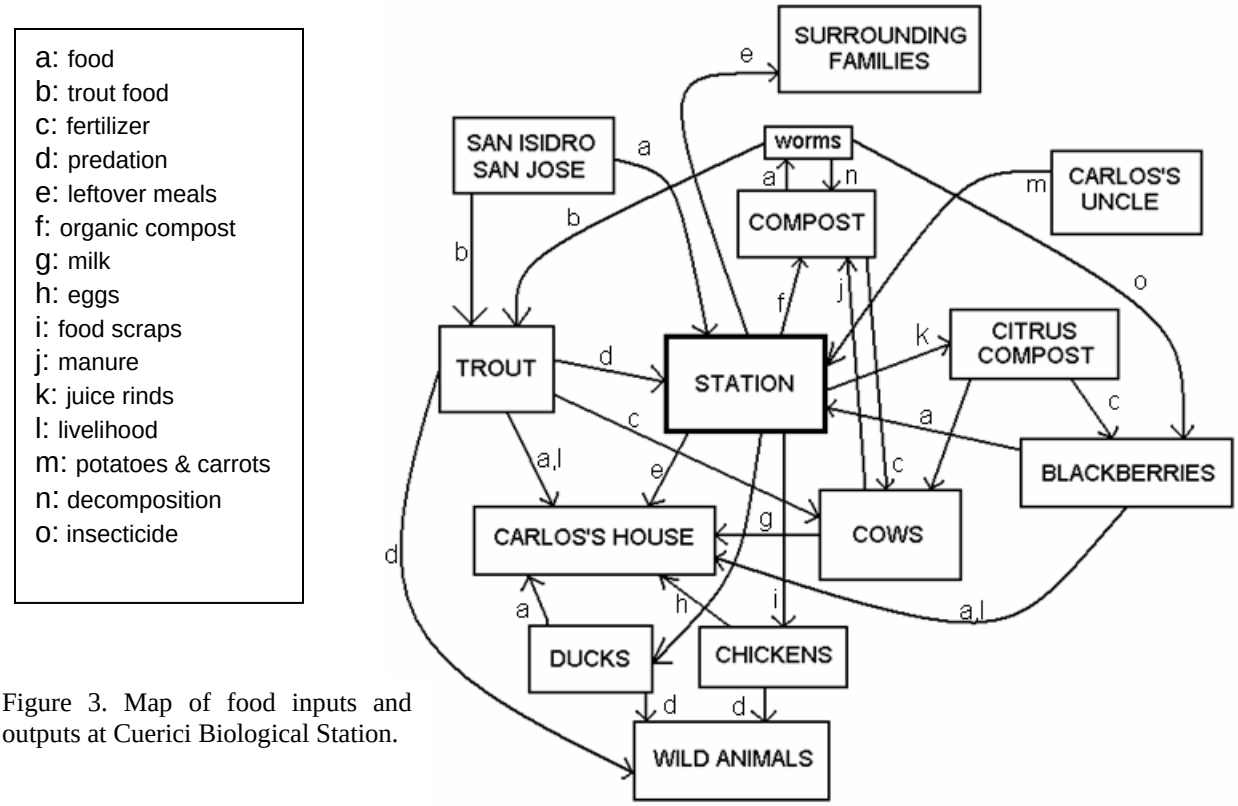

Figure 3. Map of food inputs and outputs at Cuerici Biological Station.

After each meal, students place their remaining food scraps into an organic compost collection bowl. The plates are washed by hand to conserve water. The cooked food that was not served is given to the chefs and surrounding families. It is likely that they eat a portion of it, although some of it is given to the farm animals as feed. Carlos's family has one milk cow, three other cows, thirteen chickens for eggs, six ducks, and one horse. The resources from the animals support only Carlos's family. Occasionally, predators such as pumas, vultures and coyotes eat the ducks and chickens.

To quantify the destination of food at the station, we weighed food before and after each meal for an entire day (29 January 2007), including amount eaten, leftover food, and organic compost. We found that our group of 19 individuals ate 27.63 kg of food, produced 11.66 kg of organic food waste, and left 12.37 kg of food uneaten, totaling 51.66 kg of food per day (Fig. 4).

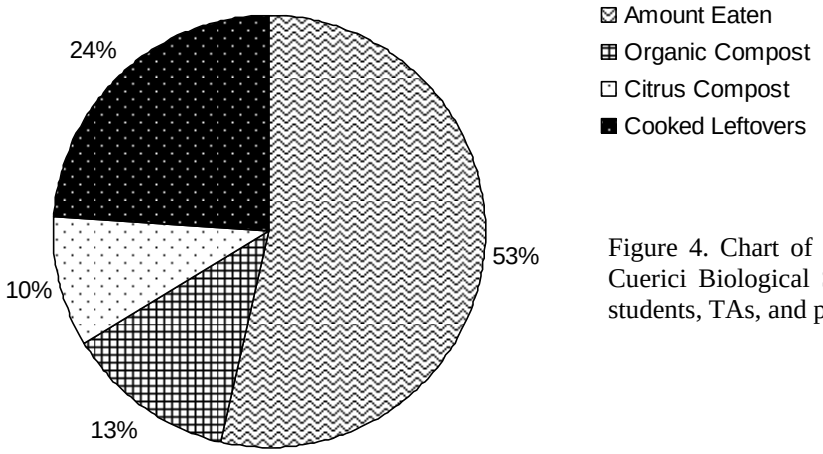

Figure 4. Chart of food consumption over one day at Cuerici Biological Station by group of 19 Dartmouth students, TAs, and professors.

The organic compost is broken down into two categories: citrus (which worms cannot process) and non-citrus (which they can). The non-citrus waste is kept in the juvenile trout building, where California red worms break down the material and also produce a natural insecticide and fungicide used on the blackberry farm. Carlos originally received a small bag of worms from a visiting organic agriculture professor, and now has thousands of them. He uses the worms not only to accelerate the decomposition process, but also as food for the juvenile trout, because the worms have high protein content. The trout give back to the compost through their sediment (i.e.

fish carcasses and excrements), which Carlos cleans from the trout ponds once every three years. Horse and cow manure are also added to the compost, and the final product is then used to fertilize the pasture, and blackberry farm. The small profit from the blackberries is invested in Carlos's trout farm. The trout are sold for profit or used at the station to feed students and expose them to his passion and project.

ECONOMICS

La Estación Biológica Albergue Cuerici is financially distinct from Carlos's rainbow trout farm. The station is under the umbrella organization Explornave. The station currently hosts 8-10 groups of approximately 20 students each per year, and requires the generated income to support its community development goals, including providing jobs for local people and purchasing local fruits and vegetables. Cuerici maintains a strong focus on sustainability; however, its budget is extremely tight. Cuerici charges approximately US$41 per person per night (as of 2007); however, only ca. 10-15% of this is available for investment in new projects because of high overhead costs such as yearly taxes and fees, maintenance costs, and salaries. The 11 business partners are conservationists and any profit feeds directly into the further development of the station. The cost distribution is approximately 30% food products, 30% property maintenance, and 40% salaries, taxes, and fees. To minimize losses from the overhead costs and maximize station development, Cuerici would ideally host 15 student groups per year. With increased visitation, they would be able to invest in another 30-bed bunkhouse and a larger dining room, which would allow the station to host two groups simultaneously.

In hopes of increasing the number of groups visiting the station, Carlos and the reserve administrator, Alberto Torres, have planned an advertising campaign, including a website with articles, pictures, and a video clip of the station. The main marketing areas include the US, Spain, Japan, Germany, and England. Investing in a large advertising campaign is costly; however, the station needs publicity to acquire new student groups. Large, well known institutions such as Dartmouth College and La Organización para Estudios Tropicales could also be important in generating publicity to other institutions that have programs returning to Costa Rica year after year.

Along with the station, one of Carlos's long term investments is his trout farm. He is currently raising the trout to feed both his family and visiting students, and to sell to other farms and fisheries. Although the

trout are not yet profitable, Carlos hopes to profit very soon after his high initial investment. The trout are an integral part of his farm and have the potential to be his main source of income. Carlos has built a reproductively self-sustaining trout hatchery that provides his family with food and future financial support.

DISCUSSION

La Estación Biológica Albergue Cuerici is a center of innovation and responsibility in environmental engineering and conservation. The station uses natural water sources for all processes and effectively prevents downstream contamination. The food cycle efficiently recycles all unused organic matter inputs. However, not all food cycle resources originate within the system, which prevents complete sustainability.

In the future, a planned large-scale (50 M x 18 M) hydroponic greenhouse may provide food to visiting groups. An experimental, smaller scale model is now being used to determine if the greenhouse can tolerate the temperature variations of the montane system. This project is currently the highest priority as it would greatly reduce food costs to the station. Students would be able to pick their own vegetables, connecting them directly to the resources that sustain them while at the station. Although requiring a large initial investment (3-4 million colones; $6000-8000), the greenhouse would both increase resource independence and contribute to the environmental education.

All station electricity is currently self-produced; however, replacement parts for the hydroelectric system are no longer available. Predicting when the system will breakdown is impossible, and the station does not have a generator or battery to provide backup power when the existing system fails. Electricity is essential for the trout farm's water pumps. In addition, reliable electricity is important for student groups who return year after year. The government has recently provided power further up the mountain, but it would be expensive to access the grid (6 million colones; $12000). Because a replacement hydroelectric motor is also expensive (3 million colones; $6000), the government grid may be a good investment as a permanent source of power. The station managers will have to decide between the resource-independence of self-produced alternative energy and the security of government-provided energy.

Sustainable development is very complex on the regional or national scale, and often involves great suffering when projects (e.g.

dam building) for energy sustainability destroy ecosystems and local communities (Isaacman and Sneddon 2000, Murphy 2002). However, La Estación Biológica Albergue Cuerici demonstrates that small-scale sustainable development can produce food, water, and energy while having a low environmental impact.

The station serves as a structural model for other stations and an ideological model for environmentally conscious development. The elevation gradient and clean water sources are central to the system's engineering. Therefore, any developing station in a mountainous environment could base their water and food networks on the described systems. A station in a different habitat may require a different alternative-energy source and more complex water treatment systems; however, Cuerici's engineering still serves as a functional blueprint of food and water networks. For students and conservationists, the station is a model for balancing human needs with conservation, in a community which both employs and protects the land that gives it life.

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SIZE-SPECIFIC AND PARTIALLY INDUCIBLE DELAYED GREENING IN *ANTHURIUM HOFFMANNII* (ARACEAE)

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Abstract. Herbivory rates are much higher in tropical forest plant communities than in comparable temperate systems, even with the higher anti-herbivory defenses employed by tropical species. For example, some plant species exhibit delayed greening (i.e., delaying chloroplast production in the youngest leaves until they have built up quantitative defenses). In a tropical oak forest, we explored how the patterns of leaf greening in *Anthurium hoffmanni* (Araceae) varied with stem size and along an herbivory gradient. For each focal plant, we measured the relative size of the first leaf, the stem diameter (the square of which we used as a surrogate for carbon storage), the hue of the newest expanding leaf, and the amount of herbivory on the plant. We predicted that smaller plants would not invest in delayed greening because they would not have sufficient carbon stores to subsidize non-photosynthetic leaves. We found that the smallest plants had no delayed greening. We also hypothesized, and found, that on plants with more herbivore damage, young leaves remained red longer, suggesting that the timing of greening may be phenotypically plastic, and a partly inducible anti-herbivore defense. To our knowledge, this is the first demonstration of apparently adaptive intraspecific variability in delayed greening.

Keywords: herbivory, plant defense, Araceae, *induction, plant development*

INTRODUCTION

Rates of herbivory in tropical forests are higher than in comparable temperate systems, even though tropical species are better defended (Coley and Barone 1996). While most damage in temperate species occurs on mature leaves, tropical species accrue the most damage on young leaves (Coley and Barone 1996; Numata et al. 2003). In addition, tropical plants in sun-lit canopies suffer less damage from herbivory than those in shaded subcanopies (Coley and Barone 1996). The leaf lifespan of tropical shade-tolerant plant species averages 2-4 years, with 70% of lifetime damage occurring during the first two weeks of expansion (Coley and Kursar 1996). Herbivores prefer young leaves because of their lower toughness and high water and nitrogen content, which give them a higher nutritional value than mature leaves (Coley and Kursar 1996). Early damage to leaves from herbivory alters plant growth and reproduction and consequently, the abundance and distribution of plants (Clark & Clark 1985). Therefore, in tropical regions, studies aiming to better understand herbivory defense

strategies need to focus on the defense mechanisms of the youngest leaves (Coley and Kursar 1996).

The young leaves of many shade-tolerant tropical plant species have high concentrations of red anthocyanins, and little or no chlorophyll. In delayed greening, anthocyanin content decreases more gradually than in plants with normal greening, as the young leaves mature and slowly begin to produce chlorophyll (Cai et al. 2005). Previous studies have suggested that delayed greening is an adaptation to reduce the amount and costs of herbivory (Coley 1983, Coley and Barone 1996, Coley and Kursar 1996, Cai et al. 2005). In the developing leaves of plants with delayed greening, chloroplast development occurs only after the leaf has reached a certain size, and is better protected from herbivores by quantitative defenses that take some time to develop (Coley and Barone 1996). Quantitative defenses include compounds such as phenols, tannins and lignins that are present in high concentrations and increase the toughness of leaves, or reduce digestibility (Coley et al. 1985). Young leaves with delayed greening can have *c.* 10-20% less light harvesting proteins, photosynthetic enzymes, chlorophyll, and lipid-rich membranes compared to normally greening leaves. Thus, the resources lost from a young leaf for a given amount of herbivory are reduced compared to older leaves (Coley and Barone 1996). It may be especially advantageous for tropical shade-tolerant species to have delayed greening under conditions of low light and high herbivory (Coley and Barone 1996). Although delayed greening reduces photosynthesis during leaf expansion, the benefits of herbivory reduction can outweigh the costs of reduced photosynthesis (Coley and Kursar 1996).

We studied the relationship between herbivore damage and delayed greening in the shade-tolerant understory plant, *Anthurium hoffmannii* (Araceae) in a tropical oak forest. Previous studies have observed delayed greening, and explored its value as an anti-herbivory defense, and its effects on herbivory among species (Coley 1983, Coley & Barone 1996, Coley & Kursar 1996, Numata et al. 2004, Cai et al. 2005, Coley et al. 2005). However none, to our knowledge, has explored variation in delayed greening within a species or the effects of herbivory on delayed greening within the lifetime of a plant.

We reasoned that two factors, plant size and herbivory intensity, might alter the amount of delayed greening in plants within a species. First, smaller plants have limited carbon stores and may simply be unable to support a new leaf that does not fix carbon: delayed greening in small plants could result

in high mortality. Therefore, we hypothesized that the smallest plants would not delay greening in their young leaves. Second, the size of the expanding leaf at which the plant begins to invest in chloroplast production may vary depending on the level of herbivory experienced by an individual plant. Specifically, we hypothesized that plants experiencing less herbivory would invest in chloroplasts at an earlier stage in the expanding leaf development than plants with higher levels of herbivory. Plants with more herbivory are more likely to lose valuable resources in their expanding leaves, so the benefits of delaying investment in chloroplasts in the developing leaf should outweigh the costs due to forfeited photosynthetic gain.

METHODS

We sampled 82 *A. hoffmanni* plants along the loop trail of the Cuerici Biological Reserve, Costa Rica from 28 to 29 January 2007. We randomly sampled plants of all sizes along a 50 m stretch of trail. We measured stem diameter on each plant directly under the node of the newest leaf. The square of the stem diameter is proportional to stem cross-sectional area; we used this as a surrogate for the carbon storage capacity of each plant.

Sampled plants had 3.36 ± 1.20 leaves per plant (mean ± 1 SE). For each plant, we measured the dimensions of each of its leaves as the length of the central vein and the leaf width at the base of the central vein. The average leaf width and length per plant were highly correlated ($r = 0.9682$, $P < 0.0001$, $n = 80$ leaves). We calculated a leaf size index as the product of the width and length. We used the proportional size of the first leaf, relative to older leaves, to describe the relative size of the newest leaf on each plant:

$$\text{Relative Size of the First Leaf} = \frac{\sum_{i=1}^{N-1}\left(s_x / s_i\right)}{N},$$

where N = total number of leaves on the plant, s_x = size of the newest leaf on the plant, and s_i = size of the ith older leaf. Using this formula, we were able to compare the relative sizes of new leaves among individual plants, in spite of the differences in average leaf size per plant.

We processed leaf images using Adobe Photoshop CS (v. 8.0). From each photo, we took a 50 pixel x 50 pixel sample close to the main vein, which we considered to be

representative of the entire leaf. Then, we applied an Average Blur filter to each sample to create a single, composite mean color from the 2500-pixel sample (Figure 1). From this sample, we extracted numeric values for red and green content as well as hue, using values provided by the software. Hue represents the frequency of light on a color scale, where red has the lowest hue and violet the highest. Red and green values represent the saturation of each color in the image (i.e. the "density" of each color), independent of other color saturations. While individual color values are independent of one another, hue values represent the contribution of all color values (i.e. green, red, and blue all contribute to hue). Hue values represent overall changes in leaf color while green values are probably more specific in describing chlorophyll concentration. We used hue to quantify delayed greening on the newest leaf. We considered a plant with low hue (i.e., red color), on leaves with larger sizes relative to the size of older leaves, to exhibit a high degree of delayed greening.

Figure 1. The gradient of composite sample colors from digital photographs of the youngest leaf of *Anthurium hoffmannii* (Araceae) in Cuericí Biological Reserve, Costa Rica. The lowest hue values are on the left (red) and the highest hues are on the right (green). Composite sample colors were calculated by using an Average Blur filter in PhotoShop 8.0; the colors in the gradient represent the average hue of the entire leaf (n = 120 leaves). The differences in saturation and brightness (i.e. darker greens and reds) do not influence hue.

We quantified herbivory as the mean percent tissue lost on extant leaves for each plant. We visually estimated tissue loss by dividing each leaf into quarters and estimating the percent of leaf area lost to herbivory in each section. We averaged the percent tissue damage over the four quarters for each leaf. Finally, we visually noted the color of the first (newest) leaf (red, transition, or green) and photographed it with a Nikon Coolpix L3 digital camera (5.1 Megapixels).

Statistical Analyses.

To determine whether plants with smaller carbon stores would be less likely to invest in delayed greening, we used two separate regression analyses to test the relationship between stem size and hue and green saturation in the youngest leaves of different plants. To determine how the color in the newest leaf varied with the relative size of the first leaf, we used three separate regression analyses to test the relationships between the

relative size of the first leaf, and hue, red, and green saturation.

To determine the effects of herbivory on delayed greening, we used a multiple regression to test the effects the relative size of the first leaf, mean percent leaf damage, and their interaction on leaf hue. We used an arcsin square root transformation to achieve normality in our mean percent leaf damage data.

RESULTS

In smaller-stemmed plants (presumably with lower carbon stores) there was a greater tendency for new leaves to be green than in larger plants. As stem size increased, hue and green saturation of the youngest expanding leaves decreased (Figure 2a, b). In addition, plants with larger stem diameters had proportionally smaller youngest expanding leaves; the relative size of the first leaf decreased with the square of the stem size ($F_{1,78} = 21.9647$, $r^2 = 0.2197$, $P < 0.0001$). As the relative size of the first leaf increased, leaves had higher hues and were greener (Figure 2c, d). Overall, the relative size of the first leaf accounted for only a small amount of the variation in red saturation ($F_{1,78} = 8.5049$, $r^2 = 0.0983$, $P < 0.005$). In plants with a stem size below 0.5 cm, we did not observe red coloration in any of the youngest expanding leaves.

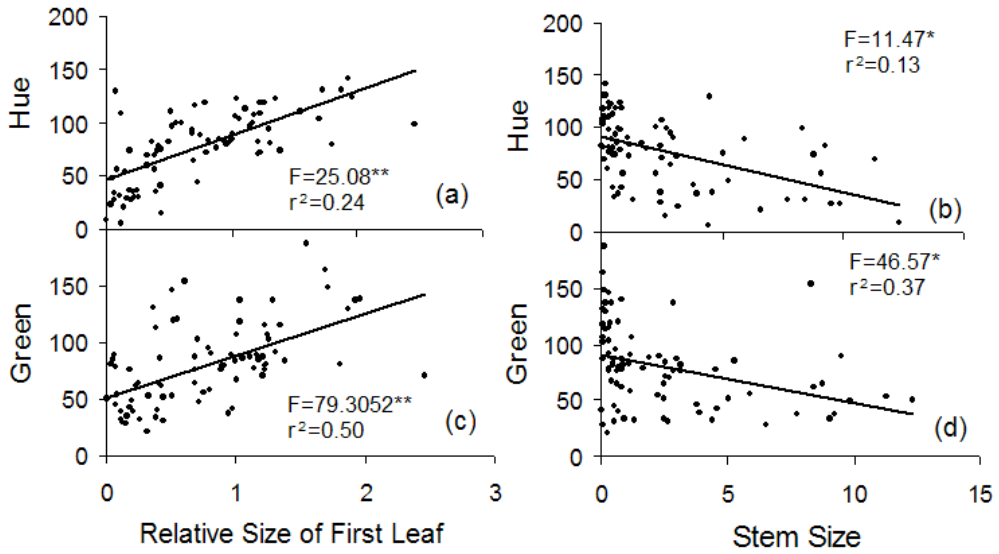

Figure 2. Linear regressions demonstrating negative relationships between stem size (surrogate for carbon stores) and hue and green saturation (a and b) and positive relationships between the relative size of the first leaf and hue and green saturation (c and d) in *Anthurium hoffmannii* at Cuericí Biological Reserve, Costa Rica. A low hue value indicates red coloration and a high hue value indicates green coloration. * indicates $P < 0.005$ and ** indicates $P < 0.0005$. Each point represents one plant (n = 82 plants).

We found that, controlling for relative leaf size, herbivory damage had a marginally significant negative effect on hue (Table 1). Plants with higher herbivory delayed greening in their youngest expanding leaf longer than plants with lower herbivory. For example, a 10% increase in herbivory would result in a 16% decrease in hue.

Table 1. Multiple regression statistics for testing the effects of mean percent herbivory damage (arcsin square-root transformed), relative size of the first leaf (log transformed) and the herbivory*leaf size interaction on hue for *Anthurium hoffmannii* (Araceae) in Cuericí Biological Reserve, Costa Rica (n= 82 plants). A low hue value indicates red coloration and a high hue value indicates green coloration.

Source	DF	SS	F	P
Relative leaf size	1	30168.041	51.6291	<.0001
Leaf damage	1	2096.049	3.5871	0.0620
Relative leaf size*Leaf damage	1	302.911	0.5184	0.4737

DISCUSSION

We found significant variation in patterns of delayed greening across size classes within the herbaceous plant, *Anthurium hoffmannii*. Our results confirmed our first prediction: investment in delayed greening increased with stem size (larger plants had lower hues and green saturation, Figure 2a, b). We suggest that the smallest plants (which had no delayed greening) simply do not have sufficient carbon reserves to subsidize leaves that cannot photosynthesize fully. Thus, delayed greening in small plants may increase their risk of mortality. By exerting strong selection for alternative developmental pathways in the young leaves, this increased risk may inhibit the delays on chlorophyll production that characterize the young leaves of larger plants, in delayed greening species.

While previous studies have quantified the patterns of delayed greening among species, none to our knowledge has documented how delayed greening varies across size classes within a species. Our results suggest that, within a species, the delayed greening anti-herbivory defense may not always be the optimal strategy. Plants may be able to regulate how much delayed greening they exhibit. Further studies could quantify chlorophyll production as individuals of delayed greening species mature, to ensure that the pattern from our static observations also occurs over developmental time.

As the relative size of the youngest leaves increased, we found that greening increased (plants with relatively larger leaves had higher hues and green saturation, Figure 2c, d). We found that both hue and

green saturation increased linearly with the relative size of the first leaf (and correspondingly, red saturation decreased slightly with leaf size). The change in hue as relative leaf size increases is likely due to increasing concentrations of chlorophyll, which masks the red anthocyanins (Cai et al. 2005). While some studies have found that chlorophyll increases linearly with leaf expansion (Kursar and Coley 1992), consistent with our results, other studies have hypothesized that complete chlorophyll production is delayed until the new leaf has amassed sufficient levels of secondary compounds (Coley and Kursar 1996). For example, in *Desmopsis panamensis* (Annonaceae) and *Annona spragueii* (Annonaceae), the newest leaves are white and then abruptly accumulate chlorophyll while species with red leaves gradually accumulate chlorophyll (Kursar and Coley 1992). Future studies could include chemical analyses on the secondary compounds in the youngest expanding leaves of white and red delayed greening species to quantify how photosynthetic and non photosynthetic pigments change with leaf development.

Finally, we found support for our prediction that young leaves would prolong delayed greening when herbivore damage on the plant was high. We showed that there is variability in the phenotypic expression of delayed chlorophyll production that may, at least in part, be influenced by the amount of herbivory an individual plant experiences. Our findings also imply that the importance of herbivory to delayed greening may vary with plant size. The trend for larger plants with high herbivory to have more delayed greening suggests that delayed greening may be an inducible defense. Inducible herbivory defenses may be especially important in the tropics where herbivory on young leaves is high (Coley and Barone 1996). Future studies could test the degree to which delayed greening is inducible, by quantifying the levels of chlorophyll in the new leaves of plants experiencing differing levels of herbivory over time.

While other studies have shown that delayed greening may be beneficial for some species and not for others, our study suggests that optimal delayed greening strategies may also vary within a species and even within an individual. The plastic expression of delayed greening within a species demonstrates the capacity of plants to alter programs of leaf development, in response to variability in damage by natural enemies.

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EFFECTS OF DEFORESTATION AND SUCCESSION ON SOIL AT CERRO CUERICÍ, COSTA RICA

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Abstract: The consequences of changes in land use are often apparent in the plant community, but effects on soil characteristics are frequently overlooked. We attempted to quantify differences in pH, conductivity, and the depths of the O and A horizons brought about by changes in land use by analyzing soils of the Cerro Cuericí region of Costa Rica. Three common community types of this region are pasture, secondary-growth alder forest, and primary-growth oak forest. We predicted that pH would be lowest in the pasture and highest in the alder forest. We expected to find the thinnest O and A horizons in the pasture, and deepest horizons in the oak forest. By sampling soil pits in the three sites, we found that oak forest and alder forest pH were similarly low, while pasture pH was significantly higher. The O horizons of the oak forest and alder forest were significantly deeper than those of the pasture, and the A horizons of the alder forest and pasture were significantly deeper than those of the oak forest. Our results suggest that the creation of pasture through deforestation and burning can significantly alter the chemical and physical properties of soil.

Key Words: Quercus copeyensis, Q. costaricensis, Alnus alcucuminata, *oak, alder, pasture, land use, pH, soil horizon depth, organic matter*

INTRODUCTION

The characteristics of a given soil are determined by parent material, climate, biota, topography, and time (USDA 2000). When forested land is cleared, the subsequent alteration of organic inputs and sunlight and the loss of chemical and physical processing of rainfall by the vegetation can have a significant impact on the chemical and physical properties of the soil (Schulz 1960, Parker 1985, CONF 2003). That impact is further magnified when cleared land is burned for agriculture. Burning volatilizes much of the nutrient content of the organic material in the soil, and subsequent agriculture or grazing removes some of what remains (NRCS 1996).

Until the mid-20th century, the slopes of Cerro Cuericí, in the San José province of Costa Rica, supported primary-growth oak forest dominated by *Quercus copeyensis* and *Q. costaricensis* (Fagaceae). Beginning in the 1940's, settlers cut sections of the forest and burned them to create pasture and farmland. Since that time, parts of the deforested areas have regenerated, producing secondary stands dominated by nitrogen-fixing alders, *Alnus alcucuminata*

(Betulaceae). Other areas remain as grazed land (*Carlos Solano, personal communication*).

To investigate how the historical patterns of deforestation, burning, grazing, and secondary growth in this system may have altered the chemical and physical properties of the soil, we made three assumptions. First, each of the study areas originally consisted of old-growth oak forest with similar soil characteristics, including low pH. Second, the land which is currently pasture or secondary alder forest was burned after the original oak forest was cleared. Third, the parent material and rainfall regime were constant across all sites.

We hypothesized that the transition from oak forest to pasture, and succession to alder forest, would cause significant changes in pH, depth of O horizon (the organic layer), and depth of A horizon (the topsoil). To more fully document differences among sites, we also measured soil conductivity; however, we had no basis for predicting a particular trend in conductivity.

Compared to oak and alder soils, we expected pasture soils to have a lower pH due to anticipated higher leaching rates in the pasture, which would cause mineral cations to be replaced with hydrogen ions (Kricher 1997). Because oak frequently tends to dominate in highly acidic soils (Finzi 1998), we predicted that oak soils would have a lower pH than alder soils.

Nutrient turnover rates should be highest in the pasture due to the rapid consumption of biomass by cattle, accessibility of nutrients in manure, and high decomposition rates under intense sunlight. Low input and rapid uptake result in a low net deposition of organic matter and nutrients, leading us to expect the O and A horizons to be thinnest in the pasture. We predicted the O horizon to be thickest in the oak forest because of lower litter temperatures under the dense canopy and high tannin concentrations, resulting in slow decomposition rates (Coley 1983). Additionally, we predicted that leaching rates would be low in the oak forest due to rapid uptake of precipitation by the standing biomass, thereby allowing dissolved organic material to accumulate in the A horizon. Finally, we predicted that the O and A horizons would be of intermediate thickness in the alder forest. Decomposition rates should be faster than in the oak forest because of high concentrations of nitrogen and lower concentrations of tannins in the leaf litter, encouraging microbial activity (Coley 1983).

METHODS

Study system: Our study sites were located on the property of the Estación Biológica Cuericí (elevation

2600 m), in San José province, Costa Rica. We selected three sites for our study, one for each of three successional stages: grass pasture, alder forest, and oak forest. The pasture site was immediately to the east of the biological station, the alder site was 60 m to the southwest, and the oak site was 200 m to the northeast. To ensure that the soil column was not artificially homogenized, we chose a pasture site that had never been tilled. To minimize the effects of erosion we selected sites with similarly shallow slopes.

Field methods: We sampled on 28-29 January, 2007. At each site, we established a haphazardly-oriented 35 m transect. We randomly selected five distances along this transect, and for each distance we randomly selected points between zero and three meters from to the left or right of the transect. At the first, third, and fifth points, we dug a soil pit deep enough to determine depth of the O and A horizons. We qualitatively observed the density of the roots in both the O and A horizons within each soil pit. For each of the five points on each transect, we collected 20 mL of A horizon soil from each of three holes within a 1.5 m radius and combined them into a homogenized three-part composite sample for each point. Using this composite soil sample, we measured pH and conductivity using 50 : 50 soil : water slurries.

Statistical analysis: A MANOVA was used to determine the differences in pH, conductivity, O horizon depth, and A horizon depth among the three sites. We then performed four one-way ANOVAs comparing each of the four variables among the three sites. Finally, we conducted Tukey-Kramer Highly Significant Differences tests (α = 0.05) for pH, O horizon depth, and A horizon depth, and we conducted a power analysis for conductivity.

RESULTS

We found significant differences in the depth of the O horizon, the depth of the A horizon, pH, and conductivity among the three sites (MANOVA: $F_{8,6} = 53.11$, $P < 0.0001$). There were significant differences among sites for pH (ANOVA: $F_{14} = 74.97$, $P < 0.0001$), O horizon depth (ANOVA: $F_8 = 27.62$, $P = 0.0009$), and A horizon depth (ANOVA: $F_8 = 40.53$, $P = 0.0003$), but not for conductivity (ANOVA: $F_{14} = 3.50$, $P = 0.063$). Conductivity was marginally significantly higher in the pasture than in either the alder or the oak. A power analysis of conductivity showed that only 3 more samples were needed to demonstrate significant differences. pH was significantly higher in the pasture than in either forest type (Fig. 1). There was no significant difference in pH between alder and oak forests (Fig. 1). There was no

measurable O horizon in the pasture. Although the mean depth of the O horizon was 35% greater in the oak forest than in the alder forest, this difference was not statistically significant (Fig. 1). The A horizons in the pasture and the alder forest were 73% and 77% deeper, respectively, than that of the oak forest (Fig. 1). The A horizon was 19% deeper in the pasture than in the alder forest; this difference was not statistically significant (Fig. 1). In the alder and pasture sites, we noted that the majority of roots were located in the A horizon. However, in the oak forest, fine roots dominated the organic layer.

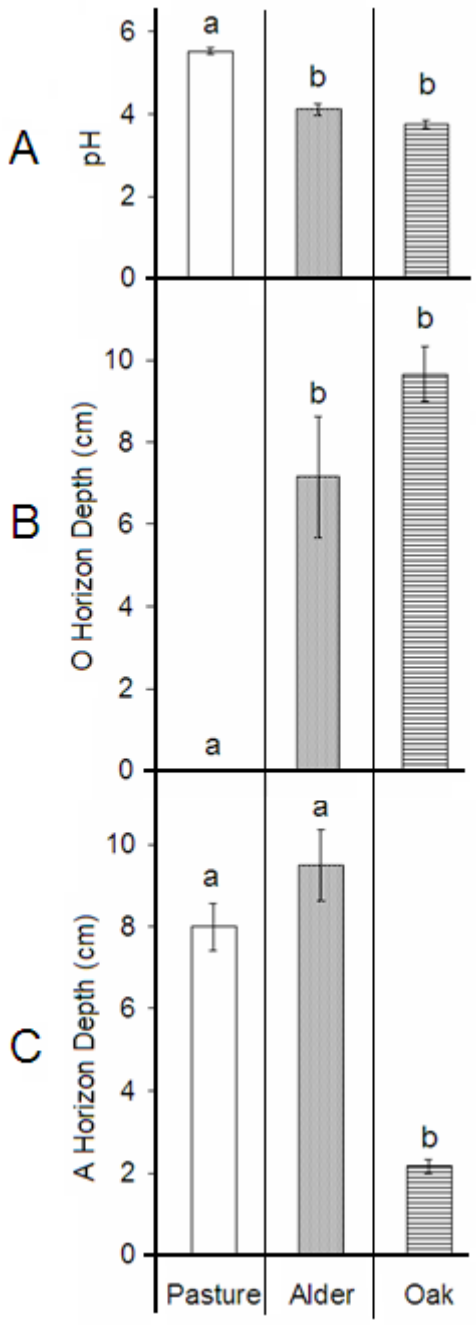

Figure 1. Each panel shows comparisons of soil characteristics: (A) pH, (B) O horizon depth, and (C) A horizon depth across sites. Different letters (a,b) indicate significantly different values among sites.

DISCUSSION

Human alteration of forest and subsequent succession can have substantial impacts on the physical and chemical properties of soils. We found significant differences in pH and soil horizon depth between primary oak forest, open pasture, and secondary alder forest.

The trend observed in conductivity, with higher conductivity in the pasture compared to the oak and alder forests, may be biologically significant. Although direct correlations between conductivity, pH, and nutrient availability have not been established, it was shown in Heinger et al. 2003 that conductivity is one of several variables that influence changes in nutrient concentrations. This trend in

conductivity is consistent with differences found in the other soil characters measured here, and the potential for direct relationships between conductivity and factors such as soil nutrients may be further investigated in future studies.

Contrary to our predictions, pH was highest in the pasture and not significantly different between the two forested sites. A potential explanation for the high pH of the pasture lies in the fact that ash is alkaline. Therefore, the slash-and-burn agriculture practiced here could have increased soil pH (Kricher 1997). It is possible that the alder soils had lower pH than expected because the relatively rapid decomposition of alder leaf litter may produce humic acids (Anon. 1975).

As predicted, the pasture site had the thinnest O horizon. However, the alder and oak O horizon depths were not significantly different from one another. The slow decomposition rate of oak leaves may be offset by relatively low input rates of oak litter, while the rapid decomposition of alder leaves may be offset by relatively high input rates of alder litter. Oak litter input rates may be lower than alder input rates because later successional trees, such as oaks, tend to have leaves with longer lifespans than those of secondary growth trees, such as alders. The net result is an O horizon of comparable depth in both forest types.

The unexpectedly thin A horizon in the oak forest could be related to our observation that fine roots and mycorrhizal hyphae were abundant throughout the O horizon. This finding indicates that a large portion of nutrients mineralized from organic matter is taken up directly by vegetation via mycorrhizae and is rapidly incorporated into standing biomass. Therefore, it is likely that organic material has no opportunity to accumulate in the A horizon of the oak forest. In contrast, the alder forest lacked the dense mat of fine roots and mycorrhizal hyphae present in the oak forest. We assumed that the young volcanic soils of the region are relatively phosphorous-rich; thus, local plant communities are nitrogen-limited. Since the alders fix nitrogen, they are less constrained by nutrient availability in the litter layer. Therefore, alder leaf litter is free to move as fine organic material into the A horizon.

Despite our expectation that the alder forest A horizon would be thicker than that of the pasture, the two were not significantly different from each other and both were deeper than that of the oak forest. Since the alder forest was originally pasture, we suggest that its deep A horizon formed at that time, probably because decomposition

rates in pasture are high and nutrient uptake rates are low, contrary to our original hypothesis. Transition to forest may have simultaneously increased both litter input and nutrient uptake, while maintaining the alder A horizon depth at levels characteristic of pasture.

Our results show that changes in land use around the Cuericí Biological Station have significantly altered the chemical and physical properties of the soil. Specifically, the creation of pasture through deforestation and burning increased soil pH, and subsequent biological activity deepened topsoil at the expense of the organic layer. By determining the input rates of litter in alder and oak forests and the nutrient limitations in these communities, further studies could substantiate the mechanisms we propose to explain the observed differences between successional stages. Although the patterns we observed may be specific to this oak-pasture-alder system, they demonstrate that the impacts of succession extend beyond the above-ground biotic community to the soil beneath.

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INSECT COMMUNITY STRUCTURE IN A SPECIES OF YELLOW ASTER

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Abstract: Insects use flowers for a variety of reasons, including food, shelter, and oviposition sites. While many studies have tested how insect communities within a flower affect the flower's fitness, we aimed to measure the effect that the flower's life cycle has on insect community structure. We predicted that insect community abundance, richness, and evenness would vary across four flower age classes, being highest in the mature flowers because these would have the most floral resources available to insect inhabitants. We found the greatest number of individuals and the greatest richness and evenness of insect morphotypes in mature flowers. However, once we rarefied our data to equalize the numbers of individuals in each flower age class, we found no difference among age classes in richness or evenness.

Key Words: Asteraceae, florivory, succession, rarefaction, diversity

INTRODUCTION

Many insect species are closely associated with flowers as pollinators, florivores, or as a combination of both (Armbruster 1997). From the insects' perspective, a flower is an environment that provides not only food, but also habitat for entire communities of invertebrates. While many studies that explore the relationship between plants and their insect inhabitants have looked at the effect of insect florivory on the plant (Irwin *et al.* 2003, McCall and Irwin 2006), few have examined the complementary effects that the flower has on resident insect communities.

In the family Asteraceae, a flower develops from a closed bud to an open disk. Disk florets open and produce pollen and nectar, and ovules may be fertilized. The flower then withers and the plant begins to allocate resources to the developing seeds (Heywood 1993). For invertebrate inhabitants, these changes over time in flower morphology, size, and chemical secretions might represent important changes in the quality and quantity of available resources and habitat.

Florivorous insect species commonly consume particular flower parts or a combination of parts (e.g., sepals, petals, stamens, pistils, pollen, and ovules) (McCall and Irwin 2006). In addition, other insects associated with flowers are carnivorous and prey on different kinds of florivores. Because many insects are specialized with regard to the resources they obtain from the flower, different amounts of these resources over the life of the flower could drive corresponding changes in the resident invertebrate community composition. Furthermore, because we expect total resource availability in the flower habitat to be positively correlated with invertebrate abundance, and

because higher numbers of individuals are more likely to contain more species (Fisher *et al.* 1943), changes in total resource abundance in the flower could drive corresponding changes in invertebrate community richness.

We measured invertebrate abundance, richness, and evenness in Asteraceae flowers of four age classes within one species to understand how flower development affects the invertebrate communities living in flowers. Because mature flowers are at the height of their resource production (of nectar, dehisced pollen, and developing ovules), we predicted that mature flowers would have higher abundance, richness, and evenness of insects than other age classes.

METHODS

Study system.

We collected our data on 28-30 January, 2007 at the Estacion Biologica Cuerici, San Jose Province, Costa Rica (elevation 2600 m). We chose to use an Asteraceae species because we found many invertebrates in Asteraceae flowers and because Asteraceae flowers can be easily categorized into age classes by examining the numbers of closed, open and spent disk florets. The species we studied, referred to as "yellow aster" in this paper, is a scandent shrub which produces abundant clusters of yellow composite flowers about 4 cm in diameter. It is common around the field station, occurring in open sunny gaps along either side of the road between the stream and the farmhouse belonging to Abel, the uncle of the director of the station, Carlos Solano.

Field methods.

We studied ten clusters of the yellow aster that were all at least three meters apart from each other. The clusters were located on either side of the road between the stream and Abel's farmhouse. In each cluster, we chose four individual flower heads representing young, mature, old, and very old age classes (Table 1). The proportions of florets at each stage represent both flower age and available resources, and petal number may represent flower attractiveness. We cut each flower and placed it in a separate bag. In the laboratory, for each flower, we measured the diameter of the disk, the numbers of closed, open, and spent disk florets, the numbers of closed, open, and withered ray florets, and the number and kinds of invertebrates. We identified the invertebrates to the lowest taxonomic level possible with the resources available (Table 2).

Table 1. Yellow aster flowers were grouped by age class according to the criteria defined below. Values represent mean ± 1 SE (n=10 flowers per age class). Different letters represent significantly different age classes (=0.05).

Age	Description	Open Florets		Petal Number	
Young	Contains mostly closed florets; petals open or closed	B	2 ± 1.0	A	9 ± 0.4
Mature	Contains mostly open florets; petals open	A	32 ± 3.2	A	9.4 ± 0.4
Old	Contains mostly spent florets; petals open or withered	B	5.3 ± 3.4	B	6.7 ± 0.9
Very Old	Contains mostly spent florets; petals mostly withered or fallen off	B	0.6 ± 0.43	C	2.2 ± 0.6

Table 2. Insects found in the yellow aster flowers, identified to the lowest possible taxonomic level.

Common Name	Taxon Level	Latin Name
weevil	family	Curculionidae
thrip	family	Thripidae
fly	family	Drosophilidae
chigger	family	Trombiculidae
crab spider	family	Thomisidae
leafhopper	family	Cicadellidae
ant	family	Formicidae
tent worm	order	Lepidoptera
inchworm	order	Lepidoptera
gnat	order	Diptera
unidentified spider	order	Araneae
unidentified grub	class	Insecta
unidentified nymph	class	Insecta
unidentified gall larva	class	Insecta

Statistical analysis.

We verified that our flower age classes differed by comparing the number of open florets and the number of petals among age classes, with two separate ANOVAs. Next, to control for possible effects of disk size on abundance, we examined the relationship between disk area and invertebrate abundance with a correlation analysis. We calculated evenness, J, for each flower as the Shannon-Weiner diversity divided by the natural log of richness:

$$J = -\sum_{i=1}^{S} (P_i \times \ln P_i)/\log(S),$$

where P_i equals the proportion of total individuals in the ith species and S equals species richness in the sample.

We log-transformed total insect abundance and arcsine square-root transformed evenness to approach normality, because the raw data were left-skewed. We compared the abundance, richness, and evenness of invertebrate flower inhabitants across the four age classes using three separate ANOVAs. To account for variation among clusters, we used a randomized block design, using flower cluster as the blocking factor for each ANOVA. After finding significant differences in invertebrate abundance, richness, and evenness among age classes, we tested which classes were different from each other with Tukey Highly Significant Difference multiple comparisons tests with α=0.05. Statistical analyses were performed using JMP 5.1.2.

We then rarefied the richness and evenness data to determine how these response variables were affected by unequal abundances among age classes. We calculated evenness J as the rarefied Shannon-Weiner diversity H, divided by the natural log of rarefied richness.

Because many flowers had very low abundances of invertebrates (25% of the flowers contained less than three individuals), we could not rarefy our data at the level of the individual flower sample. Instead, we summed the number of individuals of each species for each age class (pooling all flowers in an age class), and from those data we rarefied richness and evenness down to 60 individuals (the lowest number of insects found in any age class, after pooling replicates) using EcoSim (Gotelli and Entsminger 2001). Because we grouped our replicate flowers for the rarefaction, we could not perform significance tests on our rarefied richness or evenness.

RESULTS

The number of open florets varied with flower age ($F_{3,36}$=38.5, P<0.0001); on average, mature flowers had more open florets than all other stages (Fig. 1, Table 1). Petal number also varied with flower age ($F_{3,36}$= 31.7, P<0.0001, Table 1). Mature and young flowers had the most petals, followed by old, and very old flowers had the least (Table 1).

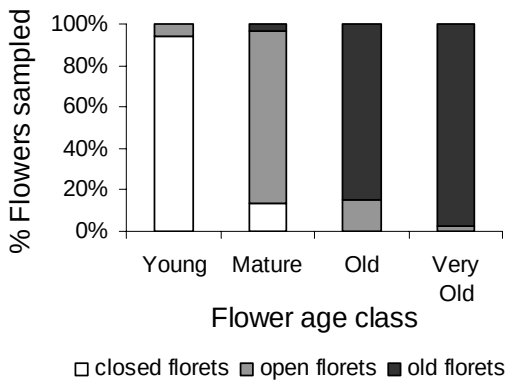

Figure 1. The proportions of closed (white), open (gray), and old (black) disk florets found in yellow aster flowers, in each of four age classes (Table 1).

There was a weak positive correlation between the log total abundance of invertebrates and disk area (r_{34}=0.38, P=0.02). Additionally, the mean difference between young and mature flowers in average disk diameter was 1 mm, which represents a 14.0% increase in disk diameter between young and mature flowers.

Age class, but not flower cluster, affected invertebrate abundance (Age: $F_{3,27}$=7.92, P<0.002, Fig. 2; Cluster: $F_{9,27}$=1.81, P>0.2). Mature flowers had more invertebrate individuals than very old and young flowers, while mean invertebrate abundance for old flowers did not differ significantly from the other age categories (Fig. 2). Eighty-three percent of the total abundance across all age classes was thrips (Thripidae), 13% was weevils (Curculionidae), and 4% was other invertebrates.

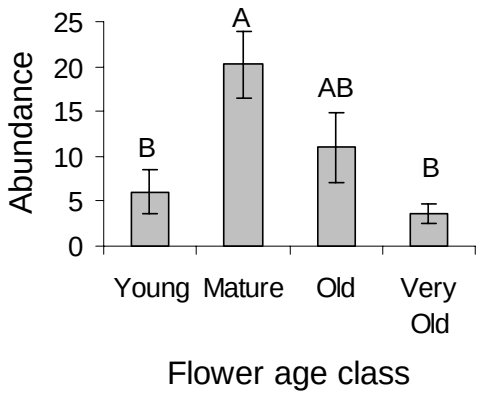

Figure 2. Average abundance of insects across four yellow aster flower age classes (means ± 1 SE; n=10 flowers per age class). Different letters represent groups with significantly different mean abundances.

As with abundance, invertebrate morphotype richness varied with age class, but not cluster (Age: $F_{3,27}$=4.76, P<0.02, Fig. 3; Cluster: $F_{9,27}$=1.07, P>0.5). Mature flowers had significantly higher richness than very old and young age classes, and richness for old flowers did not differ significantly among the three other age categories (Fig. 3). Invertebrate evenness also varied among age classes, but not flower clusters (Age: $F_{3,27}$=4.43, P<0.05, Fig. 4; Cluster: $F_{9,27}$=1.29, P>0.50). Mature flowers had higher evenness than the young flowers, but mature flower invertebrate evenness was not significantly different from the old or very old.

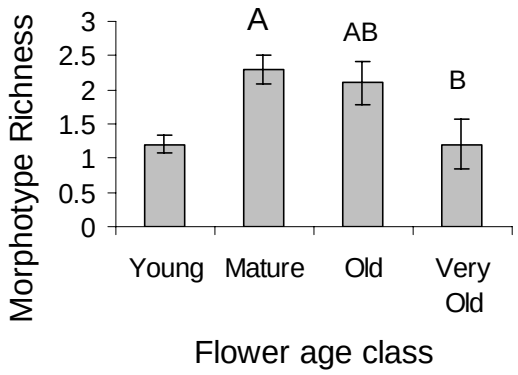

Figure 3. Average unrarefied morphotype richness across the four flower age classes of yellow asters with error bars showing ± 1 SE (n=10 flowers per age class). Different letters represent groups with significantly different mean richness.

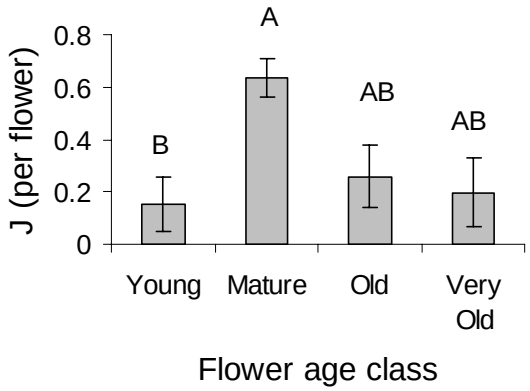

Figure 4. Average unrarefied invertebrate evenness in yellow aster flowers, with error bars showing ± 1 SE (n=10 flowers per age class). Different letters represent groups with significantly different mean evenness.

The rarefied richness for the four age classes (pooled data across replicate flowers) had large 95% confidence intervals, and we were unable to conclude that richness differed across age classes (Fig. 5). Since the 95% confidence intervals for rarefied Shannon-Weiner diversity were also very large, there were no significant

differences in evenness among age classes (Fig. 6).

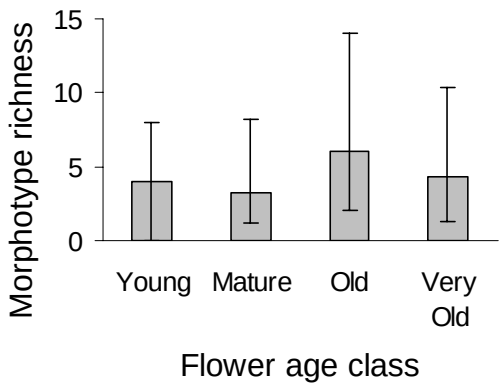

Figure 5. Rarefied invertebrate richness in yellow aster flowers and 95% confidence intervals, combining all flower samples within each age class.

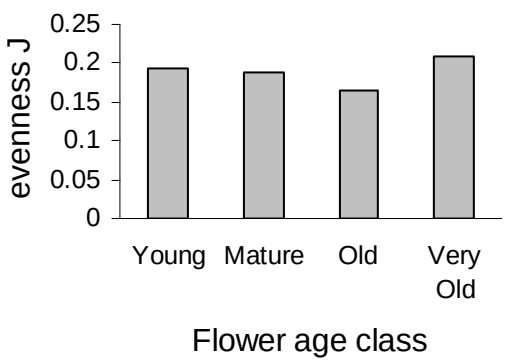

Figure 6. Yellow aster invertebrate evenness calculated from rarefied Shannon-Weiner diversity and richness among age classes.

DISCUSSION

Our data supported our prediction that mature flowers would have the highest insect abundance. However, abundance did not differ significantly between mature and old flowers, which may indicate a time lag in the response of invertebrate populations to decreases in flower resources.

Disk diameter was weakly but significantly correlated with invertebrate abundance disk diameter increased by 14% from young to mature flower stages. Since larger habitat area is correlated with higher abundance and species diversity (Denslow 1995), this difference in disk size may be responsible for some of the increases in invertebrate abundance and richness observed between young and mature flowers.

The higher invertebrate abundance in mature flowers may have also influenced the observed richness patterns (Fisher *et al.* 1943). Because our study used entire flower habitats as replicate units, and because we took absolute censuses of those habitats, the variability in abundance among age classes represents an intrinsic property of the study system, rather than an artifact of sampling effort. However, the natural variability in invertebrate abundance among flower age classes meant that flowers of different ages contained different numbers of total individuals from which to calculate richness. Rarefaction is a useful tool for comparing species richness while controlling for unequal numbers of individuals sampled. However, not all data sets are equally suitable for rarefaction. For example, the low invertebrate abundance found in some flowers forced us to pool our replicates for each age class, and thus prevented us from performing significance tests on

the rarefied richness and evenness. Furthermore, because many of the morphospecies we found were represented by a very few individuals, the rarefied values for richness in any age class were highly variable, producing large confidence intervals around our richness estimates (Fig. 5) and preventing us from drawing a satisfying conclusion about the results. These complications in our analysis of richness and evenness demonstrate some of the difficulties of comparing diversity between habitats, especially when the realities of the natural study system produce a data set whose characteristics are not ideal for analysis by rarefaction models.

Although our study's main conclusive results were limited to invertebrate abundance, further studies in this system might reveal interesting patterns in species diversity, functional group diversity, food web structure, and other community characteristics of flower-dwelling invertebrates. Further studies could address broader ecological questions about the dynamics of community succession occurring in biotic habitats. Community succession in newly available abiotic habitats, such as recently cleared ground, is generally viewed as being driven entirely by endogenous community dynamics, such as differential dispersal and competitive abilities of colonizing organisms. In contrast, in newly opened biotic habitats, such as a flower bud, colonization and community succession are further affected by the biological growth and development of the living habitat.

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