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Fitness Consequences of Herbivory: Impacts on Asexual Reproduction of Tropical Rain Forest Understory Plants[1]

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ABSTRACT

Although herbivory can affect plant fitness, few studies have investigated the effects of herbivory on the fitness of plants that reproduce vegetatively via fragmentation. Plants that reproduce in this manner are common in tropical wet forest understories, where they are subject to damage from falling branches and other debris. For these species, the ability to reproduce via fragmentation may be affected by the extent of herbivory. In this study, we determined the effects of natural and artificial herbivory on plant fragments. The leaves of four tropical understory plant species were either damaged artificially or chosen based on the extent of existing damage. Effects of real and artificial herbivory ranged from strong to no effects depending on the plant species and biotic conditions. Insect-damaged fragments showed stronger effects than artificially damaged fragments. Herbivory may be an important predictor of mortality in reproductive fragments of understory plants.

RESUMEN

Aunque la herviboria puede afectar el vigor de las plantas, pocos estudios han investigado sus efectos en plantas que se reproducen vegetativamente por fragmentación. En el sotobosque de la selva tropical, donde las plantas están expuestas a ramas y otras partes de los árboles que les caen encima, es común encontrar especies que se reproducen de esta manera. En estas especies, la herviboria puede afectar la habilidad de reproducirse por fragmentación. En este estudio, se determinaron los efectos de herviboria tanto natural como artificial en fragmentos de plantas. Las hojas de cuatro especies de plantas del sotobosque tropical fueron dañadas artificialmente o escogidas según su nivel de herviboria. Los efectos, tanto en hojas con daños naturales como artificiales, variaron desde grandes efectos a inexistentes, dependiendo de la especie de planta y las condiciones bióticas. Los fragmentos que fueron dañados por insectos presentaron efectos mayores que los segmentos que fueron dañados artificialmente. La herviboria puede ser un factor importante para predecir la mortalidad de fragmentos de plantas características del sotobosque.

Key words: asexual fitness; Costa Rica; Piper cenocladum*;* Piper imperiale*; plant fragmentation; plant–herbivore interactions;* Psychotria elata*;* Solanum enchylozum*; tropical rain forest.*

NUMEROUS STUDIES EXAMINING THE EFFECTS OF FOLIVORY ON PLANT FITNESS indicate that impacts vary widely. These fitness effects can include negative (Marquis 1984, Kerley *et al.* 1993, Gedge & Maun 1994, Sacchi & Conner 1999), undetectable (Sacchi *et al.* 1988, Bergelson & Crawley 1992, Bergelson *et al.* 1996), and positive (Paige & Whitham 1987, Paige 1992, Agrawal 1998) consequences for the plant. These studies typically use a traditional view of fitness focused on sexual reproductive capabilities of plants (Belsky 1986, Schupp 1990), ideally examining total seed production, seed quality, and the probability of establishing seedlings. In contrast, there are few studies that have examined the effects of folivory on asexual reproductive fitness (Capinera & Roltsch 1980, Sagers & Coley 1995).

Tropical rain forest systems contain numerous plant species that reproduce asexually through fragmentation or adventitious shoots (Kinsman 1990) and some tropical plants, such as species in the genus *Piper*, use fragmentation as their primary

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form of reproduction (Gartner 1989, Kinsman 1990). Fragmentation and adventitious shoots are especially useful for understory plants in dynamic tropical rain forests that have heavy epiphyte loads, high winds, or weak soils because these plants are commonly fragmented by falling trees, branches, and other debris from the canopy (Greig 1989, Kinsman 1990). For example, in a Costa Rican forest, 17 of 22 study species of understory shrubs and herbs that were artificially fragmented were successful at establishing and surviving to eight months (Kinsman 1990).

Because it is so common, asexual reproduction by fragmentation can play an important role in the population and community dynamics of understory plant communities in tropical wet forests. Quick establishment by new individuals of successful genotypes (*e.g.,* herbivore resistant/tolerant, successful fragmenters) and rapid colonization of new habitat can allow asexually reproducing plants to establish immediately, thus gaining an edge over slower-colonizing, sexually produced seeds and seedlings (Penalosa 1984). Asexual reproduction, however, will most likely have negative consequences on the genetic heterogeneity of populations.

If fragmentation is the primary mode of reproduction for a plant, then herbivory may exert extraordinary selective pressure on that plant as has been suggested by previous studies (Letourneau & Dyer 1998, Dyer & Letourneau 1999). This potentially large impact of herbivory on plant fitness warrants further investigation. Here, we studied the survivorship of fragments from four common and abundant tropical understory plants representing three families under different regimes of natural and simulated herbivory.

MATERIALS AND METHODS

Study site and system.—The research was conducted at La Selva Biological Station, Costa Rica, from 1996 through 1999 during both the wet and dry seasons. The station is located at 10°25′N and 84°05′W, on the Caribbean slope of Costa Rica. It is classified as Tropical Wet Forest, receiving 4000 mm of rain per year, with all months receiving a minimum of 150 mm (McDade *et al.* 1994). The plants studied were *Piper cenocladum*, *P. imperiale* (Piperaceae), *Solanum enchylozum* (Solanaceae), and *Psychotria elata* (Rubiaceae). All are common understory plants and are representative of asexually fragmenting species in the shrub and herb layer of tropical forests (Greig 1989, Kinsman 1990).

Natural-damage experiments.—This experiment was performed exclusively with *P. cenocladum.* We collected 88 *P. cenocladum* fragments from sites throughout La Selva in January 1996. Each fragment was clipped from a unique plant and had three leaves. Natural herbivory was visually estimated and categorized as either "low herbivory" (visual mean 8% leaf tissue removed; $N = 42$) or "high herbivory" (visual mean 24% leaf tissue removed; $N = 46$). Natural levels of herbivory in this species have a bimodal distribution with no overlap of means (Letourneau & Dyer 1998, Dyer & Letourneau 1999). To simulate asexual reproduction through fragmentation, *P. cenocladum* fragments were randomly placed on the soil every meter on alternate sides of a transect located in primary forest. Plant survivorship was noted after 18 months (all living fragments were fully rooted and established by 3 mo). A X^2 analysis was used to examine the association between surviving plants and level of herbivory.

A second experiment was conducted at a wetter site (a marshy area with standing water throughout the year) where *P. cenocladum* is more common. Forty paired clones were created by clipping from one shrub the terminal three leaves of two different branches; all clones were matched for equal levels of herbivory and were placed no less than 5 m from each other in the field. We measured total leaf area and herbivory by counting the number of 0.23 cm^2 grid squares on a transparent thermoplastic overlay (herbivory was measured as leaf area eaten). Herbivory was categorized as described above and we used a X^2 analysis to examine the association between surviving plants and level of herbivory. The effect of genotype on survivorship was studied by examining the difference in fragment survivorship of clones. We used a X^2 analysis to test for departure from a 1:1 ratio.

Artificial-damage experiments.—For all four study species, we created 80 individual plant fragments all originating from different individuals and each containing at least two leaves. These fragments consisted of leaf clusters with at least the second leaf pair below the meristem still attached. The leaf areas and existing herbivory of all the leaves were measured as described in the natural-damage experiment. Each leaf was then subjected to one of four levels of damage using scissors: 100, 50, and 25 percent leaf area removed, plus a control. The 100-percent damage category was created by clipping each leaf at the distal end of the petiole; the 50-percent damage category was set up by cutting longitudinally along the midvein; the 25-percent damage category was made by cutting longitudinally along the midvein to the midpoint of the leaf and then randomly picking one side of the leaf and cutting laterally into the midpoint. For *S. enchylozum* and *Ps. elata*, the fragments were measured and damaged *in situ* and then left on the plant, allowing the plant to physiologically adjust to the damage before the fragments were created.

The fragments were mixed in a bag and laid out randomly along line transects in primary forest habitat (except for *Ps. elata*, which occurs in secondary forest) where other individuals of the same species could be encountered. Fragments were placed at least 10 m apart and were pushed into the ground slightly, with the leaf lying flat, as if the stem were driven somewhat into the ground. No water or other supplements were given to the fragments. They were checked after 18 months when survivorship was noted. For *P. cenocladum* and *P. imperiale*, fragments were planted in December 1998 and examined for survivorship in May 1999; for *S. enchylozum* and *Ps. elata*, the fragments were planted in March 1999 and examined for survivorship in August 2000.

Logit models were used to examine artificial damage treatments on survivorship, with each level of artificial herbivory as a dichotomous (yes/no) predictor variable and result (dead or alive) as the response variable. To examine the effects of initial low levels of naturally occurring damage on *P. imperiale*, *S. enchylozum*, and *Ps. elata*, survivorship of the control plants was a response variable in logistic regression, with naturally occurring percent herbivory on these plants as predictor.

RESULTS

In the two natural-herbivory experiments with *P. cenocladum*, fragments with low levels of herbivory were significantly more successful at establishment than fragments with high levels of herbivory. In the first experiment on drier soils, mortality was high (89%); only 2 fragments with high levels of herbivory survived while 8 fragments with low levels of herbivory survived ($X^2 = 4.7$, df = 1, $P = 0.03$; Fig. 1). The second experiment on wetter soils yielded similar results but with lower overall mortality (40%); 17 fragments with high levels of herbivory survived and 31 fragments with low levels of herbivory survived ($X^2 = 4.5$, df = 1, $P = 0.03$; Fig. 1). Half of the plants in this experiment were paired clones and the effect of genotype on survivorship was studied by examining the difference in fragment survivorship of clones. The four possible outcomes for each pair of clones were: one clone dies and one clone lives (there are two ways for this to occur), both clones live, or both clones die. The latter two possibilities are expected to occur more frequently if ability to fragment successfully has a strong genetic component. If there is no genetic component to fragmentation success, the expected outcome would be a 1:1 ratio. The outcome was not significantly different from the expected ratio of 1:1 ($X^2 = 0.6$, df = 1, $P = 0.5$).

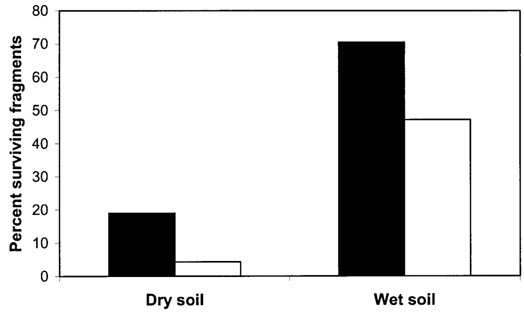

FIGURE 1. Effects of natural herbivory on survivorship of *Piper cenocladum* fragments. Black bars represent fragments with low herbivory (<10%) and open bars represent fragments with high herbivory (>15%). In both dry and wet soils, low herbivory had significantly greater survivorship ($P > 0.05$). There was no genetic component for the ability to successfully fragment (i.e. clones responded similarly to fragmenting; there were equal numbers of dying–surviving clone pairs versus dying–dying or surviving–surviving clone pairs).

In the artificial-herbivory experiment, all four species responded in a similar manner to the artificial leaf removal treatments with a general trend of lower survivorship for all artificially damaged plants versus the controls (Table 1); however, significant predictors of survivorship differed among species. For *P. imperiale*, the artificial herbivory treatments did not significantly affect survivorship of fragments at any level of leaf removal. The 100-percent leaf removal treatment significantly predicted survivorship for *P. cenocladum* and *Ps. elata* but not for *S. enchylozum*. The 50-percent leaf removal treatment predicted survivorship for *P. cenocladum* and *S. enchylozum*, but not for *Ps. elata*. The 25-percent leaf removal treatment significantly predicted survivorship only for *S. enchylozum*. The low levels of naturally occurring herbivory on the control plants were not significant predictors of survivorship for *P. imperiale* ($X^2 = 1.9$, df = 1, $P = 0.2$), *Ps. elata* ($X^2 = 0.01$, df = 1, $P = 0.9$), or *S. enchylozum* ($X^2 = 0.01$, df = 1, $P = 0.9$).

TABLE 1. *Effects of artificial herbivory on survivorship of plant fragments. Eighty individuals of the species noted in the plant species column were haphazardly selected. The average natural herbivory at time of collection is in parentheses. Treatment columns contain percent survivorship for fragments with statistics in parentheses* (X^2, *df,* P). *The control column contains percent survivorship for control fragments, with statistics in parentheses for the most parsimonious nonhierarchical model that fits the data* (X^2, *df,* P *[a nonsignificant* P*-value indicates a good fit]). Values in bold indicate that the variable was a statistically significant predictor of survivorship for that species.*

Plant species (initial herbivory %)	Artificial Herbivory Treatments			
	Control (%)	25% (%)	50% (%)	100% (%)
Piper cenocladum (10)	65 (0.6, 3, 0.9)	55 (0.04, 1, 0.9)	**40 (5.9, 1, 0.02)**	**40 (5.9, 1, 0.02)**
Piper imperiale (24)	42 (3.3, 4, 0.5)	33.3 (0.5, 1, 0.5)	37.5 (1.3, 1, 0.3)	38.9 (1.3, 1, 0.3)
Psychotria elata (8)	75 (0.1, 2, 0.9)	60 (0.9, 1, 0.3)	55 (1.7, 1, 0.2)	**35 (6.3, 1, 0.01)**
Solanum enchylozum (6)	80 (0.4, 2, 0.8)	**45 (4.8, 1, 0.03)**	**50 (3.7, 1, 0.05)**	65 (0.9, 1, 0.4)

DISCUSSION

Herbivory can have profound and often overlooked fitness consequences for plants that reproduce asexually. In two experiments, we have shown that under naturally high levels of herbivory, fragments of *P. cenocladum* suffered significantly greater mortality, but there was no effect of genotype, when holding herbivory levels constant, on fragment survival. Although herbivory has been shown to have little effect on fitness of sexually reproducing plants, herbivory may play a stronger ecological role in determining survival of asexually produced fragments via decreased vigor of offspring. Thus, in understory plants that reproduce primarily, or even often via fragmentation, natural selection for anti-herbivore defense can act through asexual selection, resulting in strongly defended plants. *Piper cenocladum* has a suite of highly effective defensive compounds that act as herbivore deterrents (Dodson *et al.* 2000; Dyer *et al.* 2001; L. Dyer, pers. obs.) and most individuals house ant mutualists that defend the plant from potential enemies (Letourneau 1983), thereby decreasing herbivory and increasing fragment survival.

The high mortality experienced by *P. cenocladum* plants in the first natural-herbivory experiment in drier soils was not surprising. Other studies have demonstrated that abiotic factors can greatly alter the effects of herbivory (Maschinski & Whitham 1989) and influence plant resistance to herbivore attacks (Waring & Cobb 1992). Our results showing the increased survivorship of *P. cenocladum* fragments in wet soils versus dry soils indicates the possibility that not only is herbivory a determinant of fragment survival, but soil moisture and possibly other abiotic factors (*e.g.,* light, nutrients) are important for asexual reproduction. While this mode of asexual reproduction is common in cloud forests (Kinsman 1990) that have wet soils, our results suggest that it is unlikely to be a viable adaptation in drier forests; however, we know of no studies examining fragmentation in moist or dry forest life zones.

In the second series of experiments, all species had lower asexual fragment survival when leaf tissue was artificially removed when compared with the controls. *Piper cenocladum* and *Ps. elata* were the only species that had significantly higher fragment mortality at the 100-percent leaf removal level. *Piper imperiale* was not significantly affected by any of the leaf removal treatments, which conforms with field observations indicating that *P. imperiale* individuals experience relatively high levels of herbivory (24% initial herbivory for experimental plants; Table 1). For this species, other factors such as microhabitat may be stronger or more important than herbivory in determining survival of asexual fragments. Furthermore, the relatively low overall fragment survivorship (38%) of *P. imperiale* at our study sites suggests that it may be reproducing primarily by seeds.

Solanum enchylozum had significant responses only at the 25-percent and 50-percent leaf removal levels. It is possible that remaining leaf material posed physiological drains (*e.g.,* induced chemical defenses, increased water loss), which caused greater mortality when any leaf material remained but lower mortality in the absence of damaged leaf material.

As in our experiment, other studies have used mechanical means to simulate herbivory, but the correspondence between mechanical and biotic damage has come into question (Baldwin 1990). Several empirical studies have compared plant biochemical and physiological responses to mechanical, invertebrate, and vertebrate herbivory (Capinera & Roltsch 1980, Dyer 1980, Detling & Dyer

1981, Baldwin 1990). Most of these have indicated that plants respond differently to mechanical and biotic herbivory, primarily because of toxins and hormones secreted by insects (Detling & Dyer 1981). Results from our experiment on artificial herbivory and asexual fragment success generally demonstrated that the simple act of leaf removal is a significant predictor of survivorship; however, comparison of the *P. cenocladum* artificial and natural herbivory experiments revealed that there are other aspects of herbivory that also reduce fitness—artificial herbivory required at least 50-percent removal of leaves to affect mortality, but much lower levels of natural herbivory had significant effects on mortality.

Our study has shown that herbivory can be a predictor of asexual reproduction in plants of an understory tropical wet forest. In tropical rain forest understories, ignoring asexual reproduction may discount a significant percentage of overall fitness. These major effects of herbivory on this aspect of plant fitness could affect community dynamics in all ecosystems and should be considered in debates about the role of consumers in agricultural or natural ecosystems.

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