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LEAF RESIN VARIATION IN *COPAIFERA LANGSDORFII*: RELATION TO IRRADIANCE AND HERBIVORY

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Key Word Index—*Copaifera langsdorfii*; Leguminosae; seedlings; saplings; leaf resin; sesquiterpenes; irradiance; oecophorid; *Stenoma*; lepidopteran herbivory.

Abstract—The influence of irradiance and herbivory on intraspecific patterns of leaf resin variability was investigated for the leguminous tree *Copaifera langsdorfii* in a Brazilian woodland. An irradiance experiment showed that seedlings in full sun had significantly higher leaf resin yield than in shade (6% full sun), supporting expectations based on plant metabolism. On the other hand, saplings growing under natural conditions had higher resin yields in shade (6% full sun) than in full sun—thus contradicting metabolic predictions. Herbivory by an oecophorid leaf tier was also higher in the shaded than in the full sunlit saplings. Since other factors, such as leaf specific weight, soil nitrogen and soil moisture, were found not to influence leaf resin yield in the two irradiance regimes, it was concluded that the higher herbivory in the shade could have selected for the higher leaf resin yield there.

INTRODUCTION

Insect herbivory is considered to play a prominent role in the evolution of variation patterns in plant secondary chemicals [e.g. 1–5]. Physical environmental factors, such as irradiance, can influence spatial patterns of herbivory within a plant population [e.g. 6–10] as well as influence the levels of secondary chemicals in leaves of plants [11–18]. Thus, spatial patterns of secondary chemical variation within populations may result from both herbivory and irradiance [7].

The objective of this study was to examine the influences of irradiance and herbivory on spatial patterns of sesquiterpene variation in *Copaifera langsdorfii* Desf. This work is part of a long-term investigation on the role tropical environments play in the evolution of copious resin-producing trees [19–21] in which the leguminous genera *Hymenaea* and *Copaifera* (Caesalpinioideae) were selected as model systems. Leaf resin in *Hymenaea* and *Copaifera* is comprised primarily of suites of sesquiterpene hydrocarbons that are essentially the same in both genera [22–24]. Superimposed upon the basic qualitative similarity of leaf sesquiterpenes is quantitative variation in composition (% total) or concentration (mg/g leaf dry wt) and yield (total amount). These quantitative variations have been studied within the plant [3], between parent tree and progeny [25–28], within and among populations [3, 23, 29–32] and between species [23, 33]. Although there are *ca* 15 sesquiterpene hydrocarbons, generally caryophyllene and $\alpha+\beta$ selinene occur in highest concentration, together often constituting 50–70% of the total leaf resin [23, 26, 31–34].

Controlled environment experiments with *Hymenaea* showed little phenotypic plasticity of leaf resin composition in relation to temperature and photoperiod, as well as little phenotypic plasticity of both composition and yield in relation to moisture [35, 36]. Also, both leaf resin yield and composition did not significantly differ in *Copaifera multijuga* Hayne occurring on soils with contrasting physical and chemical properties [37]. However, under field and controlled environment conditions, increases in irradiance have resulted in significant increases in leaf resin yield, but not in composition, in selected species of *Hymenaea* and *Copaifera* [13]. Thus, these experiments generally indicated strong genetic control of resin composition and yield in relation to physical environmental factors, and led to the hypothesis that biotic factors could be influencing the evolution and maintenance of the present patterns of variation [25, 28, 34, 38, 39]. However, these experiments and field observations also point out the necessity for assessing the relative role of irradiance and biotic factors in influencing patterns of leaf resin variation.

Although various kinds of insects have been observed on *Hymenaea* and *Copaifera* leaves throughout their neotropical distribution, lepidopterans (noctuids, geometrids, saturniids but particularly oecophorid leaf tiers) have been the most common herbivores. Laboratory experiments with the generalist noctuid lepidopteran, *Spodoptera exigua* (Hübn), have shown that *Hymenaea* leaf resin yield has dosage-dependent toxic and deterrent properties [38]. Also both caryophyllene and $\alpha+\beta$ selinene concentrations have significant effects on larval growth, but additionally caryophyllene has significant effects on mortality and the selinenes strongly influenced time to pupation [39]. Field studies have further shown the significance of both yield and caryophyllene concentration as defensive properties of both *Hymenaea* and *Copaifera* leaf resins against specialist oecophorids [31, 34, 40]. Moreover, highest terpene yield during leaf development of *Hymenaea* and *Copaifera* occurs just before or when herbivory peaks (34, 40–43).

To investigate the influence of irradiance and herbivory on spatial patterns of leaf resin variability, we have examined irradiance with regard to the variation in (i) resin yield, (ii) caryophyllene and $\alpha+\beta$ selinene concentrations and (iii) herbivory in leaves of *Copaifera langsdorfii* saplings. Leaf nitrogen and moisture, as well as leaf specific weight (LSW) were also analysed; these parameters could be complicating factors in assessing leaf resin yield, because they can be influenced by irradiance [44–47] and can also affect herbivory [48–51].

The study was carried out from September 1985 to April 1986 near the city of São Carlos, São Paulo state in southeastern Brazil. The study site (SC) is located in a reserve of the Fazenda Canchim agricultural experiment station (EMBRAPA), where members of our laboratory have worked previously [31, 42, 43]. The site is at an altitude of 650 m; mean annual precipitation is 1476 mm with 80% occurring from October to March. The median temperature during the rainy season (October–March) is 21.5° and 18° during the dry season (April–September) [52]. The vegetation is scrub woodland which occurs as a mosaic of more open patches of shrubs and small trees grading into taller, denser patches of trees with a closed canopy. *Copaifera langsdorfii* is one of the more common tree species with densities of *ca* 1–2 trees/10 m^2 [31], spaced 7 m apart (Macedo, C.A., personal communication). Saplings are abundant in both the open and the shaded, closed canopy patches. On average, irradiance in the shaded patches was 6% of that in the full sunlight of exposed patches.

The oecophorid leaf tier *Stenoma* aff. *assignata* (Meyrick) has been the primary herbivore found on *Copaifera langsdorfii* at the SC site [31, 43] and may be restricted to *Copaifera* (Becker, V.O., personal communication). It was essentially the only herbivore encountered, with two discrete generations appearing during the study period. The larvae live and feed within nests made of tied leaflets, which eventually dry; new leaflets are tied continuously as larvae develop. Late instar larvae nests contain 8–12 leaflets. *Copaifera langsdorfii* leaves flushed from August through September (having been leafless during most of the dry season); therefore, only mature leaves of essentially the same age were available when the leaf tier larvae were first obvious in early October.

Saplings (1.5–2.5 m height) in shaded and in exposed (full sun) patches were assayed for total leaflet loss due to leaf tying, late instar larvae to leaflet ratios and larval reduction in both generations. Larval reduction is a measure of survivorship and represents the percent decrease in the number of larvae on a sapling between mid and late instars. We assume that most of this reduction is due to mortality, primarily as a result of an unfavorable diet; however, adverse microclimatic conditions, predation and parasitism, as well as migration cannot be ruled out. Leaves were collected from the study saplings for analyses of resin yield, total nitrogen, LSW and moisture. Soil samples in shaded and exposed patches were analysed for differences in available soil nitrogen, because it can influence carbon allocation to carbon-based defenses [17, 18, 53–55]. In conjunction with the field study, a seedling experiment was carried out to assess the influence of irradiance on yield under more uniform than natural field conditions. One-month-old seedlings, grown from seed collected from one tree, were submitted to one of two treatments: full sunlight or 6% full sunlight. Mature leaves were collected for analyses of resin yield from all seedlings at the end of the study, and stem heights and number of leaves were recorded.

RESULTS

Seedling experiment

Leaf resin yield was significantly higher for the seedlings in full sun than for those in the shade ($t = -2.78$, $p < 0.01$) (Fig. 1). Irradiance had a significant effect on primary stem heights and number of leaves. Primary stem heights ($t = -1.39$, $p < 0.01$) were significantly lower and number of leaves were higher ($t = 3.39$, $p < 0.01$) in the exposed than in the shaded seedlings.

Sapling study

The mean leaf resin yield for the shaded saplings was significantly higher than for the full sun ones according to a Wilcoxon 2-sample test ($z = -3.47$, $p < 0.01$) (Fig. 2A). There was no difference in the means for caryophyllene concentration between irradiance regimes, but the shaded saplings had significantly higher mean concentrations of $\alpha+\beta$ selinene (Wilcoxon 2-sample test, $z = -4.0$, $p < 0.01$).

In both insect generations the mean leaflet loss and tier (larvae) to leaflet ratio were significantly higher for the shaded saplings than for the full sun ones, resulting in significantly higher mean total leaflet loss (total during study, October–March) for the shaded saplings (Wilcoxon, $z = -2.64$, $p < 0.01$) (Fig. 2B). Larval reduction was significantly higher for the full sun saplings than for the shaded ones in both the first (Wilcoxon, $z = 2.6$, $p < 0.01$) and second (Wilcoxon, $z = 3.14$, $p < 0.01$) insect generations.

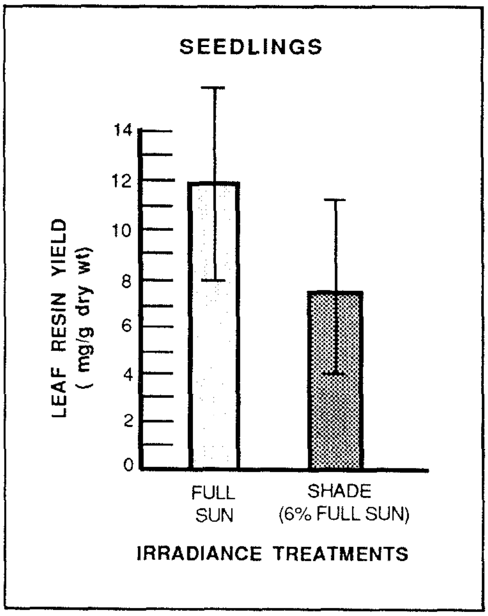

Fig. 1. Comparison of means for leaf resin yield in *C. langsdorfii* seedlings submitted to two irradiance treatments ($n = 12$, $n = 14$ for the full sun and shaded seedlings, respectively).

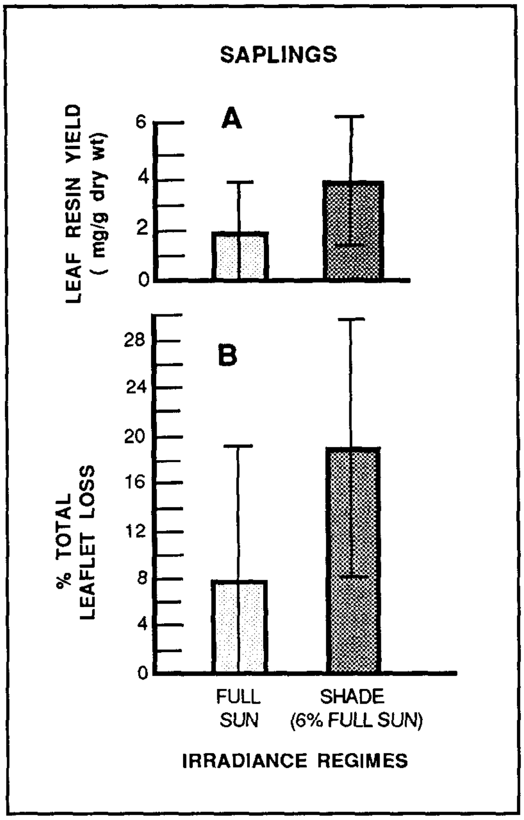

Fig. 2. Comparison of means for leaf resin yield and leaflet loss due to herbivory in *C. langsdorfii* saplings between two field irradiance regimes. (A). Leaf resin yield. (B). Total leaflet loss due to herbivory ($n = 36$, $n = 39$ for the full sun and shaded saplings, respectively).

Coefficients of variation for resin yield, and for caryophyllene and $\alpha+\beta$ selinene concentrations were substantially higher than for the other leaf parameters for both shaded and full sun saplings (Table 1). The shaded saplings had significantly lower variability in all of these resin parameters than did the full sun ones (Table 1).

There was no difference in leaf nitrogen of the saplings between irradiance regimes (Table 2). This result might be expected in that nitrogen in the soil (upper 40 cm of a sandy Oxisol), where the saplings were studied, did not differ between the shaded and exposed patches. The mean LSW was significantly lower and leaf moisture significantly higher for the shaded saplings than for the full sun ones (Table 2).

DISCUSSION

Leaf resin yield varied in relation to irradiance in both the saplings and seedlings, but in opposing ways. Even though sunflecks among the naturally shaded saplings, but not occurring in the experimentally shaded seedlings, could increase the photosynthetically active irradiance on these saplings, we nonetheless anticipated that exposed saplings would have a higher leaf resin yield than shaded ones. This prediction is not only suggested from our seedling experiment, but also from previous growth chamber studies showing an increase of resin yield with an increase in irradiance in seedlings of *Copaifera pubiflora* Benth. and *C. officinalis* L. [13]. These *Copaifera* species occur in similar dry forest habitats to those of *C. langsdorfii*. Also, resin yield increased from shade to full sun in planted *C. multijuga* saplings in Amazonia [13]. Studies of other carbon-based compounds, such as leaf phenolics, have shown increases of these compounds with increases in irradiance [11, 15, 16, 18, 27]. In fact, Bazzaz *et al.* [17] have suggested that, generally within a species, resource allocation to carbon-based defenses increases with increases in irradiance.

LSW, soil moisture and soil nitrogen were also considered under the different irradiance regimes, because they may influence leaf resin yield. Lower LSW in shaded leaves compared to exposed ones could result in higher resin yields, which were measured on a dry weight basis. However, this is not likely, because the shaded experimental seedlings (which had a lower mean LSW (45 g/m^2) than those in the sun (80 g/m^2)), had significantly lower leaf resin yield than did the full sun seedlings. Furthermore, resin yield in *Copaifera langsdorfii* increases throughout leaf development, and thus is highest at the time of greatest leaf sclerification [42]. We would not expect soil moisture to influence the comparison of resin yields for several reasons. First, moisture conditions did not affect leaf resin yield in growth chamber experiments of *Hymenaea courbaril* seedlings [36]; also *C. multijuga* trees growing on soils with significantly

Table 1. Coefficients of variation and comparison of variance of resin yield, major sesquiterpenes and other leaf parameters of *C. langsdorfii* saplings between field irradiance regimes by F tests (one tailed)

Leaf parameter	Coefficients of variation Full sun $n = 36$	Shaded (6% full sun) $n = 39$	F
Resin yield (mg/g)	100	68	1.84*
Caryophyllene (mg/g)	169	83	4.78†
$\alpha+\beta$ Selinene (mg/g)	139	78	2.92†
Total N (%)	9	8	1.39
LSW (g/m^2)	13	15	1.39
Moisture (%)	15	10	1.78

*$p < 0.05$.
†$p < 0.01$.

Table 2. Comparison of means for leaf parameters of *C. langsdorfii* saplings between field irradiance regimes by t tests

Leaf parameter	Full sun $n=36$	Shaded (6% full sun) $n=39$	t
% Total N	2.36 ± 0.22	2.34 ± 0.18	0.43
LSW (g/m^2)	87.50 ± 11.20	64.21 ± 9.50	9.7*
% Moisture	38.80 ± 5.72	44.74 ± 4.36	−5.0*

$P < 0.01$.

different equivalent humidity had similar leaf resin yield [37]. Secondly, the differences in soil moisture between the exposed and shaded patches of saplings probably were not great, as it was the rainy season during the study period, and the leaves were formed and resin synthesized under these conditions. Nitrogen is probably not an influencing factor on leaf resin yield in the saplings, because there were no differences in either soil or leaf nitrogen between irradiance regimes.

Therefore, differences in LSW, soil moisture and nitrogen are not factors to explain higher leaf resin yield in the shaded saplings, which contradicts results from the seedling experiment and theoretical expectations based on plant metabolism. If irradiance alone were determining the spatial pattern of leaf resin variability, then the shaded saplings would have lower yields than the exposed ones. However, the higher mean resin yield in saplings under shaded conditions could be the result of selection by the higher levels of herbivory in the shade. A similar conclusion was reached in a field study with *Satureja douglasii* (Benth.) Briq., in which higher monoterpene-yielding genotypes occurred under shaded conditions [7]. It was suggested that this spatial pattern probably resulted from selection by an herbivorous slug that is both restricted to shaded conditions and is inhibited by the leaf monoterpenes [56].

In a previous study of *Copaifera langsdorfii* at the SC site and a nearby site, Langenheim *et al.* [31] reported a highly significant negative regression between resin yield and caryophyllene concentration with leaf loss by oecophorids, along with a threshold concentration of selinenes above which damage levels dropped. These results indicate that the herbivores can be inhibited by different leaf resin yields or concentrations of individual sesquiterpenes.

In the present study, however, correlation analysis showed no relation between leaf resin yield, caryophyllene or $\alpha+\beta$ selinene concentration, and leaflet loss for the shaded saplings. In all cases where *Hymenaea* and *Copaifera* leaf resin has been shown to be inhibitory to lepidopterans, it has been a dosage-dependent response. Thus, the lack of a negative correlation between herbivory and leaf resins among the particular plants in this study may be related to the low levels of leaf resin yield in the saplings (0.2–0.4% dry wt) when compared to the levels in the seedlings (0.8–1.2% dry wt), and to levels reported in previous studies (0.9–1.6% dry wt) in which oecophorid inhibition by *Hymenaea* and *Copaifera* leaf resins was demonstrated [31, 34]. Adult *C. langsdorfii*, from the same area and analysed during the same period as the sapling study, have a mean leaf resin yield of 0.4% dry wt and, similarly, showed no correlation between leaf damage and leaf resin yield [40]. Previous studies have also reported *Copaifera* seedlings to have higher leaf resin yield than adults [27, 31, 40, 42].

In this study, the reduction in leaf resin yield between seedlings and adults also occurs between seedlings and saplings. Leaflet loss in seedlings may have a greater effect on these plants than on saplings and adults, thus resulting in selection for higher resin yields. The relative impact of herbivory increases with a lower growth rate [57]. The seedlings are slower growing and have longer-lived leaves than the saplings and adults (Langenheim, J. H. and Macedo, C. A., personal communication) and thus would be expected to allocate more carbon to secondary chemicals for defense [17, 57–61]. Moreover, physiological maintenance costs per area of foliage would be less for seedlings than for saplings and adults, leaving more carbon available for allocation to secondary compounds in seedlings [55]. Preferential herbivory on shaded seedlings with lower resin yields could result in only those with higher yields surviving. Exposed plants have less herbivory and, therefore, both low and higher yielding seedlings survive. Although leaf resin yield changes during ontogenetic development of the plant, the differences in resin yield between the shaded and exposed plants could be maintained in the sapling stage. This reduction in leaf resin yield in saplings and adults suggests a balance between the allocation of carbon to defense, and to growth and reproduction, which is in accordance with the optimal defense theory [17, 62, 63]. Although an examination of the relationship between growth and resin yield in the seedling experiment would be of interest, the short duration of this experiment did not allow conclusions to be drawn from the growth data.

We would further predict that stronger selection by the herbivores would result in lower variability of resin yield and concentrations of major sesquiterpenes in the shaded than in the exposed saplings. High interplant resin variability available for selection is demonstrated from both the seedling experiment, in which there were high standard deviations for resin yield for seedlings originating from a single parent tree, and from the saplings which had high coefficients of variation for all resin parameters. The significantly lower variability of resin yield, and of caryophyllene and $\alpha+\beta$ selinene concentrations in the shaded than in the exposed saplings may be another indication of the stronger selection by herbivores in the shaded patches.

Higher levels of herbivory in the shade than in the full sun are expected for several reasons. Lower larval reduction and higher number of larvae in the shaded saplings can be related to the lower LSW and higher leaf moisture which favour growth and survivorship of immature lepidopterans [51, 64]. Avoidance of dessication is a basic problem facing small lepidopteran larvae [65]; thus, temperature and humidity in the shade are also likely to be more favorable for them. Likewise, damage to *Copaifera multijuga* in an Amazonian rainforest by oecophorids was higher on shade leaves than on sun leaves [27]. Furthermore, the higher density of adult *Copaifera* trees in the shaded patches may enhance larval recruitment of *Stenoma* aff. *assignata* onto the shaded saplings.

This study shows how both irradiance and herbivory can influence the level of a leaf resin. If irradiance alone were influencing the spatial pattern of resin variability, we would expect the saplings in full sun to have higher leaf resin yields than the shaded ones, as occurred in the seedling experiment. However, in the saplings studied, herbivory seems to override irradiance in determining the spatial pattern of leaf resin yield variability. These results, although preliminary, support the hypothesis that biotic factors have probably played an important role in the evolution and maintenance of patterns of resin variation in the genera *Hymenaea* and *Copaifera* [19–21]. Moreover, they emphasize the need for further investigation of changes in secondary chemicals during plant ontogeny, as implied by Swain in 1977 [66], as well as changes in the role of selection pressures exerted by herbivores during the ontogeny of trees.

EXPERIMENTAL

40 saplings in shaded and 40 in exposed patches, all between 1.5 and 2.5 m height (DBH < 2.7 cm) were tagged. On average, irradiance in the shade was 125 μmol/sec/m^2 and in the full sun was 2000 μmol/sec/m^2 (measured at noon in February using a Licor LI-185B light meter with a quantum sensor). The total number of leaf tier nests and larvae on each sapling was counted twice (mid and later instars) for both insect generations. Larval reduction was calculated as the % reduction in the numbers of larvae between mid instar and late instar census. The total number of leaves and the number of leaflets per leaf were counted for each sapling in early October before the first insect generation. For calculations in the second insect generation, the total number of leaves was subtracted by the number of leaves lost to the larvae in the first generation. The tier to leaflet ratios were calculated by dividing the total number of late instar larvae by the total number of leaflets. The average number of leaflets tied in late instar nests was found to be 10. Thus, no. late instar nests × 10 = no. leaflets lost in each insect generation. Total leaflet loss represents % of the initial number of leaflets that were lost during the study (October–March).

Leaves for terpene analysis were air-dried and shipped to the University of California, Santa Cruz (UCSC). Leaves were ground with a mortar and pestle and extracted with high purity pentane, filtered and concentrated with a mild stream of N_2. A known amount of *n*-tetradecane was added as an int. standard. The solution was analysed directly by GC (3 μm DB-WAX 0.53 mm × 30 m capillary column, 130°, 5 psi He carrier gas, FID, electronic integrator).

Leaves for total N were oven-dried for 3 days at 80°, digested according to a micro-Kjeldahl method [67] and nitrogen assayed colorimetrically using a Technicon 2 Autoanalyzer. % leaf moisture was determined by the difference between fresh and oven-dried weight (3 days at 80°). LSW was determined by weighing oven-dried leaf disks (0.6 cm diam) made by a hole puncher from fresh leaves.

For the seedling experiment, one-month-old seedlings (20–30 cm tall only with cotyledons present), grown from seeds from a single adult *Copaifera langsdorfii* (EBF801), were potted in plastic bags with sieved soil from the upper 20 cm collected at the field site from which the litter layer had been removed. The experiment was conducted on a plot at the Universidade Federal de São Carlos. 15 seedlings were placed in full sun (2000 μmol/sec/m^2) and 15 in shade (125 μmol/sec/m^2) from the beginning of October to mid March. Shading was provided by 3 layers of plastic shade cloth, together providing a 94% irradiance reduction. Seedlings were watered to field capacity every 3 days. No apparent insect damage occurred to the seedlings. Two sets of leaves were formed under experimental treatments; the second of which was used for chemical analyses.

For the soil analyses, 7 samples from exposed patches and 7 samples from shaded patches were collected in February, mixed and homogenized to yield 2 samples from which 2 subsamples were taken and sent for analysis at the soils laboratory of the Universidade de São Paulo, Piracicaba, Brazil.

Data were analysed with SAS on a Magnuson M80 computer at UCSC. Voucher specimens have been deposited in herbaria at UCSC and in Brazil at the Universidade Estadual de Campinas (UNICAMP) and Universidade de Brasilia, D.F.

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