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Effects of Temperature and Light Levels on Leaf Yield and Cocaine Content in Two *Erythroxylum* Species

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Published information on the response of *Erythroxylum* crops to temperature and photosynthetic photon flux density (PPFD) is more descriptive than quantitative. The objective of this study was to quantify the effects of temperature and PPFD on leaf growth and cocaine content in the major cocaine-producing species. Plants of *Erythroxylum coca* var. coca (Coca) and *Erythroxylum novogranatense* var. novogranatense (Novo) were grown in artificially-lighted controlled environment chambers with a 12 h photoperiod and at day/night temperatures of 20/16, 25/21, 30/26 or 35/31 °C and at PPFDs of 155, 250 or 400 μmol m^{-2} s^{-1} for 53 d before leaves were harvested for dry weight and cocaine concentration determinations. Subsequently, chamber temperatures were altered to provide constant day/night temperatures of 19, 23 or 27 °C. Plants were grown for 180 d under these conditions and harvested a second time. Leaf yields in response to temperature were best expressed as quadratic functions. The optimum average daily temperature for leaf growth was near 27 °C in both species. Novo was more vegetatively vigorous than Coca. Leaf mass at the first harvest was lowest in plants grown under 155 μmol m^{-2} s^{-1} for both species. At the second harvest the only change was that there was no difference in leaf mass between 155 and 250 μmol m^{-2} s^{-1} in Coca. Leaf cocaine concentration was not affected by PPFDs $\leqslant$ 400 μmol m^{-2} s^{-1} but was affected by temperature. In Coca, leaf cocaine concentration was maximum at a mean daily temperature of 24 °C at the first harvest and at 19 °C at the second harvest. In Novo, leaf cocaine concentration was maximum at a mean daily temperature of 25 °C at the first harvest but there was no effect of temperature at the second harvest. Coca leaves had higher cocaine concentration than Novo leaves at all temperatures at the first harvest but at the second harvest, there was no significant difference in leaf cocaine concentration between species except in the lowest temperature treatment when leaf cocaine concentration was higher for Coca. Cocaine production on a per plant basis was largely a function of leaf mass.

Key words: Coca, cocaine, dry weight, *Erythroxylum*, leaf yield, light, PPFD, species, temperature.

INTRODUCTION

The two species of *Erythroxylum* grown for their cocaine content are *Erythroxylum coca* var. coca (Coca) and *Erythroxylum novogranatense* var. novogranatense (Novo). These perennial, tropical plants are grown as field crops or, in some cases, as understorey crops. Leaves are stripped from the plants as frequently as every 2 months (Rogers, 1963), and are usually air-dried before cocaine extraction.

Most of the literature on these crops provides qualitative knowledge about their growth. Quantitative data are sketchy and based solely on field observations. For example, Coca has been reported to grow well at mean temperatures of 19 °C (Mackay, 1886), 20–24 °C (Deneumostier and Jacob, 1912), and 18–25 °C (Martin, 1952). Gade (1975) suggested the same temperature range as Martin for optimal growth of Coca, adding that growth was retarded by temperatures averaging below 10 or above 35 °C.

As little as we know about how these species respond to temperature, we know even less about how they respond to light. Coca and Novo are acknowledged by those familiar with their cultivation, as understorey and full sun species, respectively (Plowman, 1984), but no growth, biochemical, or physiological data exist to confirm this conclusion.

Neither do we know whether environmental conditions that favour plant growth also favour cocaine production. Plowman (1981) concluded that leaf cocaine concentration was under some genetic control as well as being influenced by growing conditions. He found different cocaine levels in two varieties of *Erythroxylum coca* grown under similar environments. He did not report whether the varieties required similar environmental conditions for optimal leaf growth. Nor did he indicate whether conditions that optimized leaf growth also optimized cocaine accumulation in the leaf. Variations in leaf cocaine concentrations have been reported for field- and greenhouse-grown plants ranging from 0·13 to 1·05% for Coca and from 0·17 to 0·93% for Novo on a leaf dry weight basis (Holmstedt *et al*., 1977; Plowman and Rivier, 1983; Lydon *et al*., 1993).

The purpose of this work was to quantify temperature and light effects on leaf growth and cocaine concentration in Coca and Novo. Results of leaf yield will be incorporated into a crop simulation model that will be used to predict growth of Coca and Novo under a variety of soil, cultural, and weather conditions.

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MATERIALS AND METHODS

Seeds of Coca and Novo were sown in early May 1989 in 0·32 l pots containing media composed of greenhouse potting media (sandy loam) and Promix BX [Use of tradename does not imply endorsement of product by USDA]. (Premier Brands, Inc., New York, NY, USA) (7:3 by volume, pH 6·1, 4·7% organic matter). Seedlings were grown for 12 months under greenhouse conditions at latitude 39 °N. After 10 months, plants were transplanted to 1·5 l pots and shoots were pruned to a height of 12·5 cm. When the plants were 12 months old, they were pruned to remove all leaves and transferred to growth chambers. The first experiment included four day/night temperature regimes (20/16, 25/21, 30/26, and 35/31 °C) at three PPFDs (155, 250, and 400 μmol m^{-2} s^{-1} at the top of the plant canopy) for a 12 h photoperiod. There were nine plants of each species for each temperature and PPFD combination. During the first and last 90 min of the photoperiod, lights were ramped between zero and full PPFD to eliminate abrupt changes in PPFD and to create a changing light environment that had some resemblance to natural daylight. PPFDs were varied by using neutral density filters and maintained by adjusting the distance between the top of the plants and the light source. The light source was three banks of cool white fluorescent tubes (one tube per 900 cm^2 of chamber area). PPFD at the top of the plant canopy was measured each week with a quantum sensor. The humidity was controlled so that each of the four chambers was held at the same vapour pressure deficit of 1·13 kPa. Nine plants occupied a bench space of 0·9 × 0·75 m. Plants were watered as needed and fertilized with 125 ml of Peters' soluble fertilizer (20·0N:8·7P:16·6K) at a nitrogen concentration of 500 mg l^{-1} every fourth watering.

All leaves were harvested when the plants were 13·5 months old. Subsamples from the harvested leaves from each treatment were freeze-dried and stored over silica gel at −20 °C. The remaining leaves were oven-dried at 80 °C and the weight recorded.

Cocaine analysis was performed as described by Lydon *et al.* (1989, 1993). Freeze-dried leaf tissue was powdered in an A-10 analytical mill (Tekmar, Cincinnati, OH, USA) and extracted with 20 ml HPLC grade methanol (MeOH) per 100 mg d. wt tissue for 1 h at room temperature with shaking. The extract was filtered, evaporated to dryness under vacuum at room temperature, and resuspended in 1 ml MeOH containing 1 μg μl^{-1} 4-androstene-3,17-dione (Sigma Chemical Co., St. Louis, MO, USA) as the internal standard. Quantitative analysis of extracts was performed on a Hewlett-Packard 5890A gas chromatograph equipped with a hydrogen flame-ionization detector, a 30 m × 0·25 mm (i.d.) dimethylsilicone capillary column with a film thickness of 0·25 μm, and helium as the carrier gas. Inlet and detector temperatures were 285 °C, column temperature was 70 °C initially, increased 25 °C min^{-1} for 8·4 min, and held at 280 °C for the final 8 min of the run. The system was calibrated using varying concentrations of pure cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) dissolved in MeOH containing 1 μg μl^{-1} 4-androstene-3,17-dione. Samples from the harvested leaves were taken from each species for cocaine analysis. Leaf weight was recorded on the remaining leaves after they were oven-dried at 80 °C.

After the first harvest, temperature treatments were changed from 20/16, 25/21, 30/26 or 35/31 °C to a constant day/night temperature of 19, 23, 27, or 31 °C, respectively. This change eliminated the temperature extremes and made it easier to justify using temperature as a quantitative (rather than a categorical) variable. Temperature and PPFD treatments were given to five plants of each species. Temperature effects were observed on plants grown at 400 μmol m^{-2} s^{-1}. Effects of PPFDs were observed on plants grown at the near optimum temperature of 27 °C. During the course of the experiment, the growth chamber set at 31 °C failed and the treatment was dropped from the study. Leaves were harvested from the remaining chambers when the plants were 19·5 months old. Leaf dry weight and leaf cocaine concentration were determined as described above.

For the first harvest, regression equations were fitted for each species with mean daily temperature being treated as an independent quantitative variable and leaf dry weight, leaf cocaine concentration, and plant cocaine content (leaf dry weight × leaf cocaine concentration) as the dependent variables. The optimum temperature was determined by taking the first derivative of fitted quadratic equations and solving for temperature when the derivative = 0. Means for light intensity and for temperature treatments at the second harvest were separated by l.s.d. at $P < 0{\cdot}05$. Pairs of species means were compared using Student's *t*-test at $P < 0{\cdot}05$.

RESULTS AND DISCUSSION

Phase I. First harvest

Temperature. The response of leaf dry matter to temperature was quadratic for both Coca and Novo plants. The optimum temperature for leaf production as determined by the regression models was 26·2 °C for Coca and 27·0 °C for Novo (Fig. 1 A).

Temperature affected leaf cocaine concentration. The highest leaf cocaine concentration occurred at ≈ 24 °C for Coca and 25 °C for Novo. The amount of cocaine produced by a plant is a function of leaf mass and leaf cocaine concentration. Coca plants had higher concentrations of cocaine in their leaves (Fig. 1 B) but Novo plants were vegetatively more vigorous (Fig. 1 A). This resulted in a cocaine content per plant that was not significantly different between species for any given temperature (Fig. 1 C). The optimum temperature for cocaine production per plant mirrored that of leaf mass, demonstrating that cocaine production is more a function of leaf mass than of leaf cocaine concentration.

PPFD. Coca and Novo plants grown under 250 or 400 μmol m^{-2} s^{-1} had greater leaf dry weight than those under 155 μmol m^{-2} s^{-1} (Table 1). However, no significant difference in leaf dry weight was found between 250 and 400 μmol m^{-2} s^{-1} treatments.

Coca plants consistently produced less leaf mass than Novo for any given PPFD treatment (Table 1). When the most favourable temperature and PPFDs were compared

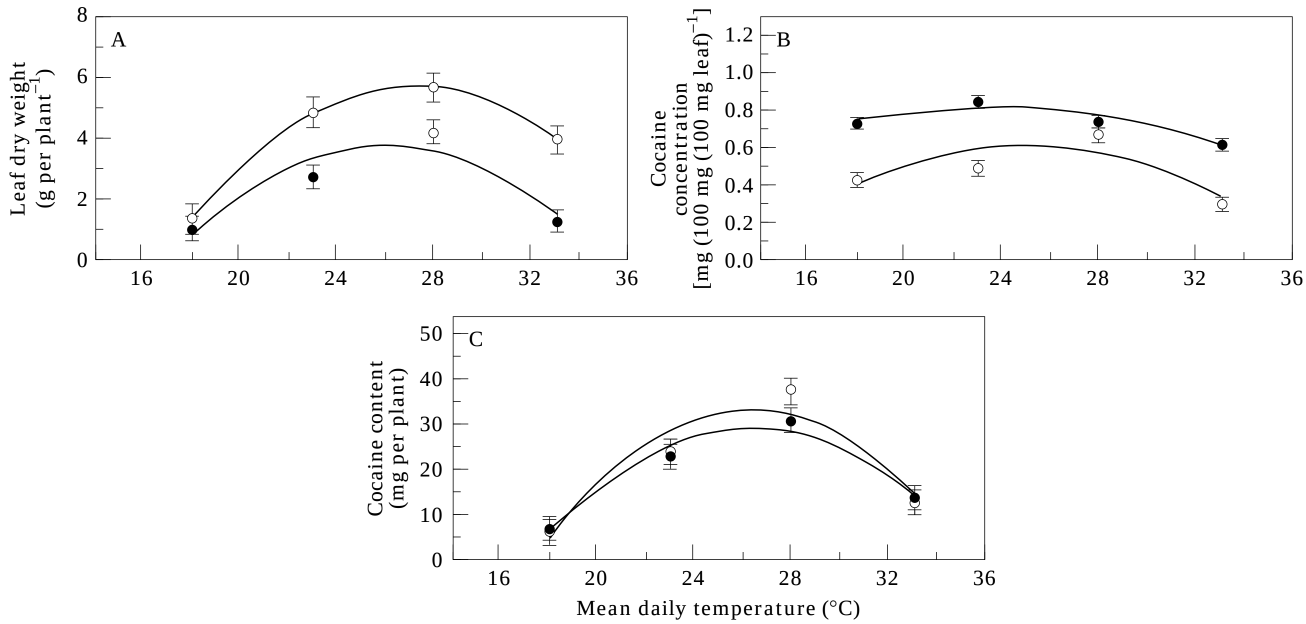

FIG. 1. Leaf dry weight and cocaine concentration for 13·5-month-old *Erythroxylum* plants (cv. Coca and Novo) as a function of mean daily temperature at a photosynthetic photon flux density of 400 μmol m^{-2} s^{-1} at the top of the plant canopy. Vertical bars represent s.e. ($n = 9$). (○) Novo, (●) Coca. A, Leaf dry weight. Equations of the fitted lines: Coca, $y = -27{\cdot}602 + 2{\cdot}408x - 0{\cdot}046x^2$, $r^2 = 0{\cdot}49$. Novo, $y = -32{\cdot}477 + 2{\cdot}806x - 0{\cdot}052x^2$, $r^2 = 0{\cdot}58$. B, Cocaine concentration. Equations of the fitted lines: Coca, $y = -0{\cdot}533 + 0{\cdot}114x - 0{\cdot}0024x^2$, $r^2 = 0{\cdot}30$. Novo, $y = -2{\cdot}081 + 0{\cdot}215x - 0{\cdot}0043x^2$, $r^2 = 0{\cdot}36$. C, Cocaine content. Equations of the fitted lines: Coca, $y = -199{\cdot}79 + 17{\cdot}31x - 0{\cdot}327x^2$, $r^2 = 0{\cdot}59$. Novo, $y = -258{\cdot}10 + 22{\cdot}14x - 0{\cdot}420x^2$, $r^2 = 0{\cdot}62$.

TABLE 1. *Effects of light intensity on leaf dry weight and cocaine concentration for 13·5-month-old* Erythroxylum *plants (cv. Coca and Novo) grown at a mean daily temperature of 28 °C*

	Photosynthetic photon flux density (μmol m^{-2} s^{-1})			
	155	250	400	l.s.d.$_{0{\cdot}05}$†
Leaf dry weight (g per plant)				
Coca	2·51‡	4·33	4·14	0·93
Novo	3·93	5·48	5·61	1·18
Coca *vs.* Novo§	*	*	*	
Cocaine concentration [mg (100 mg leaf)$^{-1}$]				
Coca	0·71	0·80	0·74	0·10
Novo	0·68	0·60	0·67	0·16
Coca *vs.* Novo	NS	*	NS	
Cocaine content (mg per plant)				
Coca	17·81	34·81	30·68	7·95
Novo	27·67	31·91	37·11	9·00
Coca *vs.* Novo	NS	NS	NS	

† Mean separation within rows by l.s.d. at $P < 0{\cdot}05$.
‡ Each value is a mean of nine observations.
§ NS, * Nonsignificant or significant at $P < 0{\cdot}05$, respectively.

TABLE 2. *Effects of temperature on leaf dry weight and cocaine concentration for 19·5-month-old* Erythroxylum *plants (cv. Coca and Novo) grown with a photosynthetic photon flux density of 400* µ*mol m^{-2} s^{-1}*

	Mean daily temperature (°C)			
	19	23	27	l.s.d.$_{0{\cdot}05}$†
Leaf dry weight (g per plant)				
Coca	3·44‡	10·90	14·98	4·56
Novo	12·79	18·42	20·95	3·43
Coca *vs.* Novo§	*	*	*	
Cocaine concentration [mg (100 mg leaf)$^{-1}$]				
Coca	1·22	0·87	0·80	0·19
Novo	0·86	0·86	0·88	0·26
Coca *vs.* Novo	*	NS	NS	
Cocaine content (mg per plant)				
Coca	43·0	92·1	120·8	42·6
Novo	107·9	157·4	179·6	39·6
Coca *vs.* Novo	*	*	*	

† Mean separation within rows by l.s.d. at $P < 0{\cdot}05$.
‡ Each value is a mean of five observations.
§ NS, * Nonsignificant or significant at $P < 0{\cdot}05$, respectively.

for each species (that is, 30/26 °C and 250 μmol m^{-2} s^{-1} for Coca and 30/26 °C and 400 μmol m^{-2} s^{-1} for Novo), Novo produced 29·5% more leaf mass than Coca. Under the least favourable growing conditions (20/16 °C and 155 μmol m^{-2} s^{-1} for both species), Novo outproduced Coca by 118%. Novo is clearly the more vegetatively vigorous of the two species.

Leaf cocaine concentration was not affected by differences in PPFD between 155 and 400 μmol m^{-2} s^{-1} for either species (Table 1). Also there were no differences in total

TABLE 3. *Effects of light intensity on leaf dry weight and cocaine concentration for 19·5-month-old* Erythroxylum *plants* (*cv. Coca and Novo*) *grown with a mean daily temperature of 27 °C*

	Photosynthetic photon flux density (μmol m^{-2} s^{-1})			
	155	250	400	l.s.d.$_{0·05}$†
Leaf dry weight (g per plant)				
Coca	8·07‡	11·08	14·98	6·73
Novo	10·72	18·69	20·95	4·86
Coca *vs*. Novo§	NS	*	*	
Cocaine concentration [mg (100 mg leaf)$^{-1}$]				
Coca	0·78	0·89	0·80	0·15
Novo	1·01	0·89	0·88	0·20
Coca *vs*. Novo	*	NS	*	
Cocaine content (mg per plant)				
Coca	65·0	103·1	120·8	69·6
Novo	101·3	166·1	179·6	40·6
Coca *vs*. Novo	NS	NS	*	

† Mean separation within rows by l.s.d. at $P < 0·05$.
‡ Each value is a mean of five observations.
§ NS, * Nonsignificant or significant at $P < 0·05$, respectively.

plant cocaine content for both Coca and Novo under various PPFDs except that the Novo plants grown under 400 μmol m^{-2} s^{-1} had a higher cocaine content than those under 155 μmol m^{-2} s^{-1}.

Phase II. Second harvest

Temperature. Leaf dry weight of plants grown at 23 or 27 °C was greater than those at 19 °C for Coca and Novo at a PPFD of 400 μmol m^{-2} s^{-1} (Table 2). Novo had more leaf dry weight than Coca at all temperatures under this PPFD.

Leaf cocaine concentration of plants grown at a PPFD of 400 μmol m^{-2} s^{-1} was not affected by temperature in Novo but the concentrations were lower at 23 and 27 °C than at 19 °C in Coca (Table 2). However, cocaine content per plant was higher for both Coca and Novo plants grown at 23 or 27 °C than in 19 °C.

Cocaine content per plant was greater in Novo than in Coca (Table 2). This is because leaf cocaine concentration was about the same for Coca and Novo at all temperatures except 19 °C and Novo produced more leaf mass.

PPFD. Novo produced more leaf mass at a PPFD of 250 or 400 μmol m^{-2} s^{-1} than at 155 μmol m^{-2} s^{-1} at the near optimum temperature of 27 °C but Coca did not produce more leaf mass until PPFD increased to 400 μmol m^{-2} s^{-1} (Table 3).

Leaf cocaine concentration was not influenced by PPFDs below 400 μmol m^{-2} s^{-1} in either species at 27 °C (Table 3). The difference in cocaine content per plant under various PPFDs in Novo was a result of more leaf mass (Table 3).

Comparison of cocaine concentration between first and second harvest. Leaf cocaine concentration was higher in 19·5-month-old plants (second harvest) compared with 13·5-month-old plants (first harvest) in Novo at all temperatures and in Coca at a mean daily temperature of 18 °C (Table 4). This difference in cocaine concentration may have been influenced by differences in leaf age at harvest. Mean leaf age on a selected number of plants was calculated on the basis of changes in numbers of growing points during the growth period. At the first harvest, mean leaf age for the whole plant was estimated as 19 d. At the second harvest, mean leaf age was 52 d.

It is possible that some of the differences in leaf cocaine concentration attributed to temperature and species in this study may be an indirect effect of leaf age. This is because temperature and species affect mean leaf age on a plant by influencing the rate of appearance of new growing points. Higher temperatures, higher PPFDs, and more vegetatively vigorous species initiate more growing points and therefore produce canopies with a higher proportion of young leaves. Younger leaves tend to have lower cocaine concentrations. To identify any direct effect of temperature, light, and species on leaf cocaine concentration, it will be necessary to examine cocaine concentration in leaves of similar age.

In conclusion, the optimum temperature for leaf production in Coca and Novo is near a daily mean of 27 °C. Leaf mass in Coca plants did not decrease as PPFDs decreased from 400 to 155 μmol m^{-2} s^{-1} as dramatically as it did in Novo, supporting the assumption that Coca is shade-adapted while Novo is more adapted to grow at higher light intensities.

Leaf cocaine concentration was not affected by PPFDs $\leqslant$ 400 μmol m^{-2} s^{-1}. However, the effect of temperature on leaf cocaine concentration was unstable, the relationship changing from one harvest to the next for both species. Cocaine production on a per plant basis was largely a function of leaf mass, with environmental conditions that stimulated leaf growth resulting in higher cocaine yields. Effects of temperature, PPFD, and species on leaf cocaine concentration could have been direct, or could have resulted from differences in mean leaf age on the plant.

TABLE 4. *Mean leaf cocaine concentration* [*mg* (*100 mg leaf*)$^{-1}$ *of* Erythroxylum *plants* (*cv. Coca and Novo*) *grown with a photosynthetic photon flux density of 400* μ*mol* m^{-2} s^{-1} *at the top of the plant canopy. Mean leaf age for the whole plant was 19 d at the first harvest and 52 d at the second*

Species	Mean daily temperature (harvest 1/harvest 2) 18/19 °C	23/23 °C	28/27 °C
Coca			
Harvest 1	0·73	0·84	0·74
Harvest 2	1·22	0·87	0·80
Harvest 1 *vs*. Harvest 2†	*	NS	NS
Novo			
Harvest 1	0·43	0·49	0·67
Harvest 2	0·86	0·86	0·88
Harvest 1 *vs*. Harvest 2	*	*	*

† NS, * Nonsignificant or significant at $P < 0·05$, respectively.

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