CULTIVATION OF A *DUBOISIA* HYBRID. PART A. NUTRITIONAL REQUIREMENTS AND EFFECTS OF GROWTH REGULATORS ON ALKALOID CONTENT

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ABSTRACT.—Hybrid plants of *Duboisia myoporoides* R. Br. and *D. leichhardtii* F. Muell, were grown in hydroponic culture, and the nutritional effects of nitrogen, potassium and phosphorus were observed. Double the amount of nitrogen in standard nutrient gave a significant increase in the size of plants. The total alkaloid content was about 27% lower than that found in control plants. Any further increase in nitrogen had an adverse effect and total alkaloid was reduced by 62%. Increased potassium showed a significant increase in the percentage of hyoscine. Plants treated with 6-dimethylaminopurine (21P), 6-benzylaminopurine (BA) and a commercial seaweed extract, Maxicrop®, all exhibited an increase in the percentage of total alkaloid. A significant increase in the percentage of hyoscine was observed in plants treated with a strong solution of Maxicrop®. A weaker solution of Maxicrop® gave a highly significant increase in the percentage of hyoscyamine. Hyoscyamine was also increased highly significantly when plants were treated with BA at 5 ppm and 21P at 1 ppm.

The genus *Duboisia* is a major source of the anticholinergic alkaloids hyoscine (also known as scopolamine) and hyoscyamine (1). Because hyoscyamine has undesirable actions on the central nervous system, hyoscine as hyoscine-*N*-butyl bromide is the preferred parasympatholytic agent.

Cultivation of *Duboisia*, initially *D. leichhardtii* F. Muell, is located in the South Burnett region of S.E. Queensland, which is its natural habitat. *D. myoporoides* R. Br. is more widely distributed along the Eastern Seaboard of Australia and has chiefly been collected annually, the bulk being processed in Japan and West Germany. Initially, plantations of *D. leichhardtii* were made with naturally set seedlings as the seed was very difficult to germinate. Later this 'seed barrier' was broken by a pretreatment with gibberellic acid (2). Little success was had with the vegetative propagation of *D. leichhardtii* and thus the plantations remained genetically heterogenous. In more recent years a hybrid of *D. leichhardtii* and *D. myoporoides* has been cultivated. It is difficult to give specific identification to the hybrid under cultivation. There are no morphological differences between hybrids in the various established plantations. It must be assumed that identity of the hybrid originated from an earlier investigation of Hills, Bottomley and Mortimer, whose hybrids of *D. leichhardtii* x *D. myoporoides* were established in the region (3).

A most recent investigation of *Duboisia* interspecific hybridization showed in general that higher alkaloid yields were observed in crosses where the female parent was *D. myoporoides* (4). The alkaloid mixture is less complex than the parent species (5, 6) and contains only hyoscine, hyoscyamine and 6-hydroxyhyoscyamine with traces of valtropine and butropine (7). The hybrid is readily propagated by cuttings; thus it is possible to obtain a plantation which has been derived entirely from clonal material. Finally, the development of an accurate analytical method made large cultivation trials feasible (8).

MATERIALS AND METHODS

PLANT MATERIAL.—Cuttings of the *Duboisia* hybrid were taken from a single tree[1] and after

[1]A voucher specimen has been lodged with the Queensland Herbarium (BRI 237629).

being dusted with a proprietary hormone rooting powder[2] were propagated in a mist propagater. All test and control plants were similarly propagated. After roots were formed, the cuttings were transferred to sand culture pots in a glasshouse and nourished with a quarter strength Hoaglands solution (9). After 4 months, some plants were transferred to sand culture containing double the amount of nitrogen (2N), quadruple nitrogen (4N), double potassium (2K), and double phosphorus (2P) concentrations compared with the controls.

PLANT TREATMENT.—The plants were sampled at various time intervals (table 1) and assayed as described previously (8). Other plants having been established in control sand culture for a period of six months were periodically sprayed with 50 ml of various cytokinin solutions (10): kinetin, 6-benzylaminopurine (BA), 6-γγ-dimethylaminopurine (21P), and a strong (10 ml per liter) and a weak (5 ml per liter) dilution of a commercial seaweed extract, Maxi-

TABLE 1. Alkaloids of *Duboisia* hybrid plants grown in sand culture.

Harvest number and date treatment began Feb. 18, 77	Percentage of alkaloid[1] (dry weight basis)				
	Control	2N[2]	4N[2]	2K	2P
1. April 19, 77					
Hyoscine	0.44±0.02[3]			0.52±0.02	0.46±0.01
Hyoscyamine	0.24±0.02			0.34±0.02	0.35±0.01
6-Hydroxy-hyoscyamine	0.06±0.01			0.08±0.01	0.11±0.00
2. May 2, 77					
Hyoscine	0.50±0.02			0.66±0.02	0.41±0.02
Hyoscyamine	0.30±0.02			0.37±0.02	0.40±0.02
6-Hydroxy-hyoscyamine	0.11±0.01			0.04±0.01	0.09±0.01
3. May 16, 77					
Hyoscine	0.53±0.01			0.66±0.03	0.48±0.02
Hyoscyamine	0.30±0.02			0.37±0.02	0.47±0.02
6-Hydroxy-hyoscyamine	0.22±0.01			0.13±0.01	0.12±0.01
4. June 7, 77					
Hyoscine	0.62±0.03			0.74±0.03	0.49±0.02
Hyoscyamine	0.42±0.02			0.34±0.02	0.42±0.02
6-Hydroxy-hyoscyamine	0.22±0.01			0.17±0.01	0.20±0.01
5. July 7, 77					
Hyoscine	0.53±0.02	0.37±0.02	0.33±0.02	0.61±0.03	0.44±0.02
Hyoscyamine	0.39±0.02	0.31±0.02	0.05±0.02	0.30±0.02	0.40±0.02
6-Hydroxy-hyoscyamine	0.11±0.01	0.07±0.01	0.01±0.01	0.03±0.01	0.19±0.01

[1]The analytical sample consisted of a combined sample from 2 replicates with the exception of control, which comprised of 6 replicates.

[2]After inspection of the alkaloid yield in the last harvest, which showed lower alkaloid content than controls, combined with inferior growth in the case of quadruple nitrogen treated plants and previous experience with nitrogenous fertilizer which did not improve alkaloid yield, further analysis was considered unnecessary.

[3]Mean and 95% confidence limits.

crop®[3]. The cytokinin activity of seaweed extracts has been demonstrated by the promotion of growth *in vitro* of carrot explants in a cytokinin free medium containing the seaweed extract (11). All spray solutions contained 0.01 percent Tween 80 to ensure wetting. The spraying was continued until all leaves were saturated, shown by dripping of the solution from the leaves. Similarly, plants were sprayed with other plant growth substances[4] which included

[2]Pyco Products, St. Mary, New South Wales.

[3]Bell Booth Ltd., Johnsonville, New Zealand.

[4]Plant growth substances—organic compounds which at low concentrations promote, inhibit, or qualitatively modify growth. Their effects do not depend on their caloric value or content of essential elements (12).

TABLE 2. The effect of plant growth substances on alkaloid yield of *Duboisia* hybrid plants in sand control culture. Percentage alkaloid (dry weight basis).[1]

Harvest number and date	Control	Kinetin 1 ppm	Kinetin[2] 5 ppm	2IP 1 ppm	2IP 5 ppm	BA 1 ppm	BA 5 ppm	Maxicrop® Strong	Maxicrop® Weak	GA[2] 25 ppm	GA[2] 100 ppm	NAA[2] 25 ppm	IAA[2] 100 ppm
1. Apr. 19, 77													
Hyoscine.....	0.44±0.02[3]	0.55±0.01		0.55±0.02	0.48±0.03	0.38±0.02	0.42±0.02	0.50±0.03	0.54±0.02		0.53±0.02		
Hyoscyamine..	0.24±0.02	0.27±0.02		0.33±0.02	0.23±0.03	0.35±0.02	0.35±0.02	0.33±0.02	0.46±0.02		0.19±0.03		
6-Hydroxy-hyoscyamine..	0.06±0.01	0.09±0.01		0.10±0.01		0.11±0.01	0.08±0.01	0.08±0.01	0.15±0.00		0.09±0.01		
2. May 2, 77													
Hyoscine.....	0.50±0.02	0.52±0.01		0.61±0.02	0.55±0.02	0.59±0.03	0.54±0.02	0.92±0.03	0.67±0.02		0.55±0.02		
Hyoscyamine..	0.31±0.02	0.38±0.02		0.42±0.02	0.26±0.02	0.34±0.02	0.35±0.02	0.31±0.02	0.53±0.03		0.13±0.03		
6-Hydroxy-hyoscyamine..	0.11±0.01	0.13±0.01		0.15±0.00	0.11±0.00	0.16±0.01	0.12±0.01	0.07±0.01	0.24±0.01		0.10±0.00		
3. May 16, 77													
Hyoscine.....	0.53±0.02	0.63±0.01		0.69±0.02	0.53±0.02		0.64±0.03	0.75±0.02	0.61±0.02		0.68±0.03		
Hyoscyamine..	0.30±0.02	0.40±0.02		0.53±0.02	0.30±0.02		0.58±0.02	0.42±0.02	0.52±0.02		0.17±0.03		
6-Hydroxy-hyoscyamine..	0.11±0.01	0.18±0.00		0.20±0.01	0.13±0.01		0.17±0.01	0.13±0.01	0.23±0.01		0.11±0.01		
4. June 30, 77													
Hyoscine.....	0.62±0.03	0.51±0.01		0.48±0.02	0.50±0.02		0.57±0.02	0.54±0.02	0.67±0.02		0.52±0.02		
Hyoscyamine..	0.42±0.02	0.40±0.02		0.50±0.02	0.42±0.02		0.50±0.02	0.35±0.02	0.62±0.03		0.10±0.03		
6-Hydroxy-hyoscyamine..	0.22±0.01	0.18±0.00		0.09±0.01	0.16±0.01		0.18±0.01	0.13±0.01	0.24±0.01		0.09±0.00		
5. July 6, 77													
Hyoscine.....	0.53±0.02	0.56±0.01	0.30±0.01	0.40±0.02	0.60±0.01		0.61±0.02	0.48±0.01	0.59±0.02	0.45±0.01	0.46±0.02	0.38±0.01	0.62±0.02
Hyoscyamine..	0.39±0.p2	0.46±0.02	0.30±0.02	0.73±0.03	0.37±0.02		0.61±0.03	0.27±0.02	0.71±0.02	0.17±0.02	0.17±0.02	0.31±0.02	0.12±0.01
6-Hydroxy-hyoscyamine..	0.11±0.01	0.19±0.00		0.21±0.01	0.13±0.01		0.25±0.01	0.17±0.00	0.15±0.01	0.09±0.01		0.13±0.01	0.10±0.01

[1]The analytical sample consisted of a combined sample from 2 replicates with the exception of control which comprised of 6 replicates.

[2]After inspection of the alkaloid yield in the last harvest which showed lower alkaloid content than controls combined with inferior growth in the case of quadruple nitrogen treated plants and previous experience with nitrogenous fertilizer which did not improve alkaloid yield, further analysis was considered unnecessary.

[3]Mean and 95% confidence limits.

TABLE 3. Alkaloid variation in leaves within some treated *Duboisia* hybrids. Percentage alkaloid (dry weight basis).

Alkaloid	2IP (5 ppm)			2IP (1 ppm)			Maxicrop® (weak dilution)		
	Top[2]	Middle[2]	Lower[2]	Top[2]	Middle[2]	Lower	Top[2]	Middle[2]	Lower
Hyoscine	0.50±0.01[1]	0.60±0.01	0.70±0.01	0.37±0.02	0.42±0.02	0.41±0.01	0.43±0.01	0.55±0.02	0.66±0.02
Hyoscyamine	0.28±0.02	0.41±0.02	0.41±0.02	0.70±0.04	0.76±0.04	0.74±0.04	0.91±0.03	0.78±0.02	0.74±0.03
6-Hydroxyhyoscyamine	0.17±0.00	0.19±0.01	0.03±0.01	0.19±0.01	0.20±0.01	0.24±0.00	0.11±0.02	0.11±0.00	0.13±0.00
Total alkaloid	0.95	1.20	1.14	1.26	1.38	1.39	1.45	1.44	1.53

[1]Mean and 95% confidence limits.

[2]Position of leaves on plant.

gibberellic acid (GA), 1-napthylacetic acid (NAA) and 3-indoleacetic acid (IAA). Plants were sampled at 0930 hrs, 2 weeks after each spray period to allow any positive effect from the subsequent spray to eventuate. The sampling was completely random to allow for a possible variation of alkaloid yield with leaf position. Finally the last collection was made in a manner to test the hypothesis by collecting the upper third (T), middle (M) and lower third (L) of the plant separately. Two sets of control plants which had been in culture for 6 months were randomly sampled at 0800 hrs., 1200 hrs., and 1800 hrs., to detect any possible effect the time of harvesting may have on alkaloid yield.

DATA ANALYSIS.—Statistical analysis was done on the assumption that the plants were not different from each other before the commencement of the experiment. And from experience with field work, it was assumed that there was no difference between replicates in each treatment or in controls. Therefore, the leaf samples from replicates were combined and analyzed for alkaloid content. The other reason was to increase the bulk of the sample.

Generally, analysis of variances was applied to every group of data; the significant difference from the control was then detected by the use of the Priori test. Analysis of variances was based on the method of Sokal and Rohlf (15).

RESULTS AND DISCUSSION

A morphological change was observed, with plants having been treated with gibberellic acid, which produced elongated leaves with an undulating margin. Plants which were subjected to an increase in nitrogen showed a decrease in the percentage of hyoscine and hyoscyamine content (table 1). However, this may not be significant, since there was also a significant increase in the stem diameter ($p=0.01$–0.005) and plant height ($p=0.025$–0.01). A similar observation was made with field trials of *Datura candida* (pers.) Stafford (13). Increased nitrogen merely gave a larger plant. Only increased potassium showed any significant

TABLE 4. Alkaloid variation in control plants harvested at different times of day.[1]

	Percentage alkaloid (dry weight basis).		
	0800 hrs	1200 hrs	1800 hrs
Hyoscine	0.61±0.02[2]	0.71±0.02	0.54±0.01
Hyoscyamine	0.44±0.02	0.47±0.02	0.35±0.02
6-Hydroxyhyoscyamine	0.21±0.01	0.25±0.01	0.20±0.00
Total alkaloid	1.26	1.43	1.09

[1]The sample of each harvest hour consisted of leaves from all parts (top, middle and lower) of 6 plants.

[2]Mean and 95% confidence limits.

increase in hyoscine percentage ($p=0.05$–0.025). Generally there was some increase in height, but statistical analysis suggested it was not significant ($p=0.50$–0.25). Total alkaloid percentage (table 2) was significantly increased ($p=0.025$–0.01) by treatment with 21P (1 ppm), BA (5 ppm) and very significantly with Maxicrop® (weak dilution) ($p<0.001$). Plants treated with BA (1 ppm) showed some increase in diameter ($p=0.05$–0.25) with increases in total alkaloid content of approximately 13% and 18% for the first and second harvests respectively, there being no further analysis due to lack of material. Hyoscyamine was very significantly increased ($p<0.001$) with 21P (1 ppm) and a weak dilution of Maxicrop®. An increase with BA (5 ppm) was less significant ($p=0.005$–0.001). Hyoscine may have been increased by the stronger solution of Maxicrop® ($p=0.05$–0.025). Cytokinins delay plant maturation and leaf senescence and have been shown to have many beneficial effects on crop yields (14). They also occur in

commercial seaweed extracts. Thus in *Duboisia* cytokinins increase alkaloid yield significantly but have no significant effect on growth.

Analysis of leaves collected from various parts of the plant (table 3) showed that the distribution of alkaloids within the plant was irregular ($p < 0.001$) with a tendency of accumulation in the lower leaves. Alkaloid content in the uppermost leaves was significantly less than that found in the middle and lower leaves ($p < 0.001$). The yield of alkaloid from samples collected at different times of day did vary (table 4); however, this variation may not be significant ($p = 0.05$–0.025).

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LITERATURE CITED

1. C. Barnard, *Econ. Bot.*, **6**, 3 (1952).
2. U. Zutsi and K. Atal Chand, *Herba Hung.*, **9**, 51 (1970).
3. K. L. Hills, W. Bottomley and P. I. Mortimer, *Aust. J. Appl. Sci.*, **5**, 283 (1954).
4. T. Ikenaga, M. Abe, A. Itaka and H. Ohashi, *Planta Medica*, **35**, 51 (1979).
5. J. F. Coulson and W. J. Griffin, *Planta Medica*, **15**, 459 (1967).
6. W. J. Griffin, *Aust. J. Pharm. Sci. Suppl.*, **36**, S128 (1968).
7. W. J. Griffin, *Naturwissenschaften*, **62**, 97 (1975).
8. W. J. Griffin, H. P. Brand and J. G. Dare, *J. Pharm. Sci.*, **64**, 1921 (1975).
9. D. R. Hoagland and D. I. Arnon, "The Water-Culture Method for Growing Plants Without Soil", California Agri. Exp. Station Circular 347 (1950).
10. K. V. Thimann, "Hormone Action in the Whole Life of Plants", Univ. of Massachusetts Press, Amherst, U.S.A., 1977, p. 240.
11. K. R. Brain, M. C. Chalopin, T. D. Turner, G. Blunden and P. B. Wildgoose, *Plant Sci. Letters*, **1**, 241 (1973).
12. L. J. Audus, "Plant Growth Substances", Harper and Row, New York, 1972, p. 19.
13. W. J. Griffin, *Econ. Bot.*, **30**, 361 (1976).
14. G. Blunden and P. B. Wildgoose, *J. Sci. Fd. Agric.*, **28**, 121 (1977).
15. R. R. Sokal and F. J. Rohlf, "Biometry", W. H. Freeman & Company, San Fransisco, 1969.