CULTIVATION OF A *DUBOISIA* HYBRID. PART B. ALKALOID VARIATION IN A COMMERCIAL PLANTATION: EFFECTS OF SEASONAL CHANGE, SOIL FERTILITY, AND CYTOKININS

O. LUANRATANA and W. J. GRIFFIN

Department of Pharmacy, University of Queensland, Queensland, 4067, Australia

ABSTRACT.—Hybrid plants of *Duboisia myoporoides* R. Br. and *D. leichhardtii* F. Muell., grown in a commercial plantation, were monitored for the major alkaloids, hyoscine and hyoscyamine. In the early months after planting, the hyoscine and hyoscyamine percentages were of the same order of magnitude. Gradually hyoscine became the dominant alkaloid with a maximum of 2.2% in Spring, then decreasing with a minimum in late Autumn. Soil fertilization had no effect on alkaloid yield. After harvesting, the plants were monitored as before on regrowth material. A similar pattern was established in which an increase in hyoscine was accompanied by a decrease in hyoscyamine and vice versa, thus supporting alkaloid interconversion. When plants were sprayed with a commercial seaweed extract, Maxicrop®, there was an 18% increase in leaf yield and a 16% increase in hyoscine content as compared to that of the controls. There was no significant increase in total alkaloid content.

Part A of this sequence reported observed variation in the alkaloid yield of *Duboisia* hybrid plants grown in sand culture under glasshouse conditions. The plants were 'spindlelike' with long internodes compared with the 'bushy' nature of field grown hybrids and had a lower percentage of alkaloids than that of the field grown hybrids.

MATERIALS AND METHODS

PLANT MATERIAL.—Cuttings were taken from a group of hybrid crosses between *Duboisia myoporoides* R. Br. and *D. leichhardtii* F. Muell., which had originally been derived from a single hybrid parent tree. In the development of a new plantation near Murgon, S.E. Queensland, containing 45,000 trees, cuttings were set in such a manner that the plantation could be divided into distinct, identifiable plots labelled A to G inclusive. With the exception of plot B, which was the last to be prepared and thus contained the youngest trees, the plots were planted progressively, such that plot A was planted first, C next, and then D and so on. Commercial harvesting began in September 1976 with plot A, which contained trees 12 months old. Other plots were cropped in subsequent months (table 1). On a fixed day (21st) and time (0900 hrs) in every month, a sample from the commercial bulk harvest was taken from the

TABLE 1. Seasonal variation of alkaloid content of commercial *Duboisia* hybrid samples.

Plot	Harvest date	Age (Months)	Mean percentage alkaloid from 3 replicates (dry weight basis)		
			Hyoscyamine	Hyoscine	6-Hydroxyhyoscyamine
A	Sept. 21, 76	12	0.28±0.01[1]	1.63±0.05	0.34±0.04
C	Nov. 21, 76	13	0.18±0.01	1.44±0.05	0.18±0.03
D	Dec. 21, 76	14	0.43±0.01	1.25±0.04	0.32±0.05
E	Jan. 21, 77	14	0.45±0.01	1.23±0.04	0.34±0.05
F	Feb. 21, 77	14	0.15±0.01	1.55±0.05	0.17±0.03
B	Mar. 21, 77	10	0.59±0.01	1.33±0.04	0.31±0.06
G	Apr. 21, 77	15	0.69±0.01	1.47±0.04	0.30±0.05
A	May 21, 77	20	0.13±0.01	0.78±0.02	0.14±0.02
C	June 21, 77	20	0.49±0.02	0.77±0.05	0.33±0.06

[1]Mean and 95% confidence limits.

drying plant and annotated commercial sample A, C, etc. Individual experimental trees within the plots were tagged. Three trees were designated 'controls' (C) and three trees which received 64 g of ammonium sulfate per month were tagged 'fertilized' (F). None of the tagged trees were included in the commercial bulk harvest. Samples from the tagged experimental trees were taken at monthly intervals and analyzed by gas liquid chromatography (1). The data was subjected to statistical analysis as in Part A. Soil samples from every plot were collected from areas adjacent to the experimental trees and assayed for nitrogen, potassium, sulfur and phosphorus (table 2).

TABLE 2. Elemental analysis of soil samples collected from area adjacent to experimental trees.

Plot	Potassium (per cent)	Sulfur (ppm)	Phosphorus (ppm)	Nitrogen (per cent)
A	0.11	430	590	0.26
B	0.08	400	620	0.25
C	0.07	530	660	0.29
D	0.13	490	700	0.27
E	0.08	460	730	0.31
F	0.10	370	510	0.24
G	0.09	340	640	0.25

RESULTS AND DISCUSSION

During the warm summer months (November to February) the hyoscine content of the commercial samples was reasonably stable between 1.2–1.6% (table 1). A sharp fall occurred in May and June corresponding with cooler weather and, although this was accompanied by a slight increase in hyoscyamine and 6-hydroxyhyoscyamine, the total alkaloid content decreased. Then samples from control and fertilized trees from each plot, which had been collected along with and from the same plots as the commercial samples, were assayed (table 3). Inspection of

TABLE 3. Seasonal variation of alkaloid content of experimental plants during 1976–1977.

Plot, treatment and harvest date		Age (Months)	Mean percentage alkaloid from 3 replicates (dry weight basis)		
			Hyoscyamine	Hyoscine	6-Hydroxyhyoscyamine
AC[1]	Sept. 21, 76	12	0.27 ± 0.00[2]	1.63 ± 0.03	0.30 ± 0.02
AF			0.30 ± 0.01	1.36 ± 0.02	0.27 ± 0.03
BC	Oct. 21, 76	5	0.67 ± 0.01	0.95 ± 0.01	0.20 ± 0.03
BF			0.75 ± 0.01	1.04 ± 0.02	0.22 ± 0.02
CC	Nov. 21, 76	13	0.11 ± 0.00	1.24 ± 0.03	0.12 ± 0.02
CF			0.24 ± 0.00	1.45 ± 0.03	0.14 ± 0.01
DC	Dec. 31, 76	14	0.26 ± 0.01	1.84 ± 0.06	0.18 ± 0.04
DF			0.28 ± 0.01	1.69 ± 0.03	0.16 ± 0.03
EC	Jan. 31, 77	14	0.42 ± 0.01	1.88 ± 0.04	0.17 ± 0.02
EF			0.50 ± 0.01	1.83 ± 0.03	0.21 ± 0.03
FC	Feb. 21, 77	14	0.34 ± 0.01	1.53 ± 0.03	0.19 ± 0.02
FF			0.01 ± 0.00	1.41 ± 0.02	0.08 ± 0.02
GC	Mar. 21, 77	14	0.30 ± 0.01	1.94 ± 0.04	0.30 ± 0.03
GF			0.32 ± 0.01	1.55 ± 0.03	0.26 ± 0.03
AC	Apr. 21, 77	19	0.53 ± 0.01	1.15 ± 0.02	0.17 ± 0.02
AF			0.46 ± 0.01	1.22 ± 0.02	0.21 ± 0.02
BC	May 21, 77	11	0.86 ± 0.01	0.81 ± 0.02	0.41 ± 0.03
BF			0.82 ± 0.01	1.07 ± 0.02	0.37 ± 0.03

[1]The first letter represents the individual plot and the second letter represents control or fertilized tree.
[2]Mean and 95% confidence limits.

data showed that there was little difference between the alkaloid content of control and fertilized plants. In each case there was an initial rise in hyoscine content to a maximum of 2.0% followed by a fall to 0.8% which was accompanied by an increase in hyoscyamine and 6-hydroxyhyoscyamine.

In observations made so far, the variation may be made more complex by differences between the plots. Three plots, A, B and E were examined in great detail. Plots A and E had given rise to high yielding total alkaloid commercial samples. The trees in plot B were younger than those in plots A and E and had much higher hyoscyamine levels ($p \ll 0.001$). Trees representing plot E showed a variation with a reducing hyoscine content as was experienced with the analysis of the commercial samples. Plot A demonstrated a similar reduction and B had a more stable hyoscine level. Fertilization seemed to have little effect on alkaloid yield (table 4, figure 1). The hyoscine content for plots A, B and E varied highly

TABLE 4. Seasonal variation of alkaloid content of selected plots during 1976–1977.

Plot, treatment and harvest date		Age (Months)	Mean percentage alkaloid from 3 replicates (dry weight basis)		
			Hyoscyamine	Hyoscine	6-Hydroxyhyoscyamine
AC[1]	Sept. 21, 76	12	0.27±0.01[2]	1.63±0.03	0.30±0.02
AF			0.30±0.01	1.36±0.02	0.27±0.03
AC	Oct. 21, 76	13	0.28±0.02	1.74±0.10	0.30±0.06
AF			0.30±0.02	1.57±0.06	0.31±0.05
AC	Nov. 21, 76	14	0.26±0.01	1.30±0.11	0.18±0.03
AF			0.23±0.02	1.04±0.03	0.26±0.01
AC	Dec. 21, 76	15	0.16±0.02	1.27±0.07	0.00
AF			0.27±0.02	1.21±0.04	0.22±0.01
AC	Jan. 21, 77	16	0.39±0.01	1.60±0.09	0.22±0.04
AF			0.24±0.02	1.09±0.03	0.22±0.01
AC	Feb. 21, 77	17	0.28±0.02	1.39±0.08	0.23±0.03
AF			0.22±0.02	1.11±0.04	0.20±0.01
AC	Mar. 21, 77	18	0.32±0.02	1.49±0.09	0.32±0.05
AF			0.19±0.02	0.72±0.02	0.15±0.01
AC	Apr. 21, 77	19	0.53±0.01	1.15±0.03	0.17±0.02
AF			0.46±0.01	1.22±0.02	0.21±0.02
AC	May 21, 77	20	0.38±0.02	1.03±0.06	0.33±0.06
			0.35±0.02	0.71±0.02	0.25±0.01
AC	June 21, 77	21	0.37±0.01	1.17±0.07	0.44±0.04
AF			0.25±0.02	0.96±0.02	0.22±0.01
AC	July 21, 77	22	0.40±0.03	1.11±0.06	0.42±0.06
AF			0.30±0.02	0.69±0.02	0.34±0.01
BC	Sept. 21, 76	4	0.42±0.01	1.17±0.02	0.31±0.03
BF			analytical	sample	unavailable
BC	Oct. 21, 76	5	0.67±0.01	0.95±0.01	0.20±0.02
BF			0.75±0.02	1.04±0.03	0.22±0.01
BC	Nov. 21, 76	6	0.59±0.02	1.12±0.09	0.20±0.03
BF			0.64±0.03	0.78±0.03	0.31±0.01
BC	Dec. 21, 76	7	0.66±0.01	1.11±0.04	0.27±0.05
BF			0.68±0.02	1.26±0.05	0.27±0.04
BC	Jan. 21, 77	8	0.64±0.02	1.29±0.08	0.26±0.04
BF			0.46±0.02	1.14±0.03	0.33±0.01
BC	Feb. 21, 77	9	0.56±0.03	1.16±0.06	0.33±0.06
BF			0.49±0.03	1.29±0.04	0.34±0.01
BC	Mar. 21, 77	10	0.47±0.02	1.35±0.08	0.41±0.07
BF			0.41±0.03	1.06±0.03	0.38±0.01
BC	Apr. 21, 77	11	0.78±0.03	0.88±0.05	0.36±0.06
BF			0.82±0.03	1.07±0.03	0.37±0.01

TABLE 4. Continued.

Plot, treatment and harvest date		Age (Months)	Mean percentage alkaloid from 3 replicates (dry weight basis)		
			Hyoscyamine	Hyoscine	6-Hydroxyhyo-scyamine
BC	May 21, 77	12	0.86±0.01	0.81±0.02	0.41±0.03
BF			0.74±0.03	0.88±0.03	0.33±0.01
BC	June 21, 77	13	0.45±0.02	1.30±0.08	0.41±0.06
BF			0.38±0.02	1.08±0.02	0.45±0.01
BC	July 21, 77	14	0.39±0.02	0.90±0.05	0.26±0.04
BF			0.48±0.02	0.81±0.03	0.28±0.01
EC	Sept. 21, 76	10	0.63±0.01	2.25±0.08	0.35±0.03
EF			0.78±0.01	2.26±0.09	0.44±0.04
EC	Oct. 21, 76	11	0.25±0.01	2.17±0.08	0.15±0.02
EF			0.15±0.01	2.05±0.07	0.13±0.02
EC	Nov. 21, 76	12	0.27±0.01	2.08±0.06	0.04±0.01
EF			0.63±0.01	2.34±0.07	0.41±0.04
EC	Dec. 21, 76	13	0.36±0.01	1.76±0.05	0.00
EF			0.46±0.01	1.97±0.07	0.00
EC	Jan. 21, 77	14	0.42±0.01	1.88±0.04	0.17±0.02
EF			0.50±0.01	1.83±0.03	0.21±0.03
EC	Feb. 21, 77	15	0.36±0.01	1.87±0.09	0.40±0.04
EF			0.43±0.01	1.94±0.07	0.29±0.03
EC	Mar. 21, 77	16	0.22±0.01	1.67±0.06	0.19±0.03
EF			0.34±0.01	1.77±0.06	0.27±0.04
EC	Apr. 21, 77	17	0.41±0.01	1.53±0.05	0.28±0.04
EF			0.57±0.02	1.37±0.08	0.31±0.05
EC	May 21, 77	18	0.26±0.01	1.35±0.08	0.14±0.03
EF			0.36±0.01	1.28±0.07	0.20±0.04
EC	June 21, 77	19	0.27±0.01	1.32±0.08	0.24±0.04
EF			0.47±0.02	1.87±0.12	0.47±0.06
EC	July 21, 77	20	0.35±0.01	1.01±0.06	0.19±0.03
EF			0.45±0.02	1.06±0.06	0.21±0.04

The first letter represents the individual plot and the second letter represents control or fertilized tree.
[2]Mean and 95% confidence limits.

significantly with month (p=0.01–0.005) and plot (p<0.001); however, variation of total alkaloid was not significant (p=0.25–0.10). Statistical analysis of plot E showed that there was no significant effect on the alkaloid yield of plot E trees with the application of nitrogenous fertilizer. An inspection of the results of the soil analysis demonstrated no correlation with alkaloid yield and nitrogen, potassium and sulfur. Plot E, the high yielding plot, had the greatest phosphorus value and there may be some connection between phosphorus and alkaloid synthesis.

With the cessation of harvesting in July, 1977, all of the tagged experimental trees were cut with the intention of a repeat experiment in the following season, assaying regrowth material. This would more truly represent the normal commercial pattern since trees are cropped for a number of years before replacement is necessary. No significant difference was found between replicate trees within plots (p>0.75) which suggested that future analysis could be restricted to a single mixed sample obtained from all three replicate trees. Leaves for the three control trees in each plot were collected and pooled, and an analytical sample was taken in the usual way by the U.S.P. method of quartering (table 5, fig. 1). In

TABLE 5. Seasonal variation of alkaloid content of regrowth trees during 1978.

Plot and harvest date	Percentage alkaloid of a combined sample from 3 replicates (dry weight basis)		
	Hyoscyamine	Hyoscine	6-Hydroxyhyoscyamine
A. Jan. 7, 78	analytical	sample	unavailable
Feb. 7, 78	0.35±0.01[1]	1.42±0.05	0.17±0.02
Mar. 7, 78	0.70±0.02	1.69±0.06	0.43±0.04
Apr. 7, 78	0.49±0.01	1.20±0.04	0.24±0.03
May 8, 78	1.11±0.03	1.20±0.04	0.50±0.05
June 9, 78	0.80±0.03	0.78±0.03	0.43±0.05
July 7, 78	0.84±0.03	0.90±0.04	0.19±0.02
Aug. 14, 78	0.69±0.03	1.13±0.05	0.46±0.05
Sept. 14, 78	0.34±0.02	1.30±0.06	0.40±0.05
B. Jan. 7, 78	analytical	sample	unavailable
Feb. 7, 78	0.37±0.01	1.47±0.05	0.23±0.02
Mar. 7, 78	0.58±0.02	1.58±0.05	0.25±0.02
Apr. 7, 78	0.79±0.02	1.32±0.04	0.41±0.04
May 7, 78	analytical	sample	unavailable
June 9, 78	0.78±0.03	0.73±0.03	0.41±0.04
July 7, 78	0.86±0.03	0.90±0.04	0.19±0.02
Aug. 14, 78	0.58±0.03	1.03±0.05	0.33±0.04
Sept. 14, 78	0.33±0.02	1.32±0.06	0.39±0.04
E. Jan. 7, 78	0.16±0.02	2.33±0.17	0.18±0.02
Feb. 7, 78	0.61±0.02	1.37±0.04	0.34±0.03
Mar. 7, 78	0.50±0.02	1.23±0.04	0.27±0.02
Apr. 7, 78	0.58±0.02	1.19±0.03	0.29±0.03
May 8, 78	0.82±0.02	0.92±0.03	0.37±0.03
June 9, 78	0.88±0.03	0.79±0.04	0.44±0.04
July 7, 78	0.82±0.03	0.88±0.04	0.17±0.02
Aug. 14, 78	0.58±0.02	1.02±0.05	0.34±0.04
Sept. 14, 78	0.37±0.02	1.31±0.06	0.44±0.05

[1]Mean and 95% confidence limits.

the first experiment during 1976 on plants which had yet to be harvested, the age of the plant appeared to be a significant factor in alkaloid synthesis. The youngest plants in plot B had a significantly low hyoscine level ($p<0.001$) and, relative to other plots, a very significantly high hyoscyamine content ($p<0.001$). Recently we have demonstrated the interrelationship between hyoscyamine and hyoscine in *in vitro Duboisia* hybrid tissue culture (2). This relationship is clearly reflected in the 1978 experiment. In the latter case, samples were of regrowth material and the age of the plant seemed irrelevant. The total alkaloid content did not significantly vary from month to month ($p=0.50$–0.25), but the variation in hyoscine content and the contrasting change in hyoscyamine level are most striking (fig. 1). There was a significant decrease in hyoscine from January to June ($p=0.005$–0.001) and a significant increase from June to September ($p=0.005$–0.001). For hyoscyamine the reverse was true. The level of the other hyoscyamine metabolite, 6-hydroxyhyoscyamine, did not vary significantly ($p=0.10$–0.05). The *Duboisia* hybrid, therefore, under plantation conditions, should not be harvested between May and September if maximum hyoscine yield is to be realized.

In part A it was shown that cytokinins increased the yield of alkaloid. A

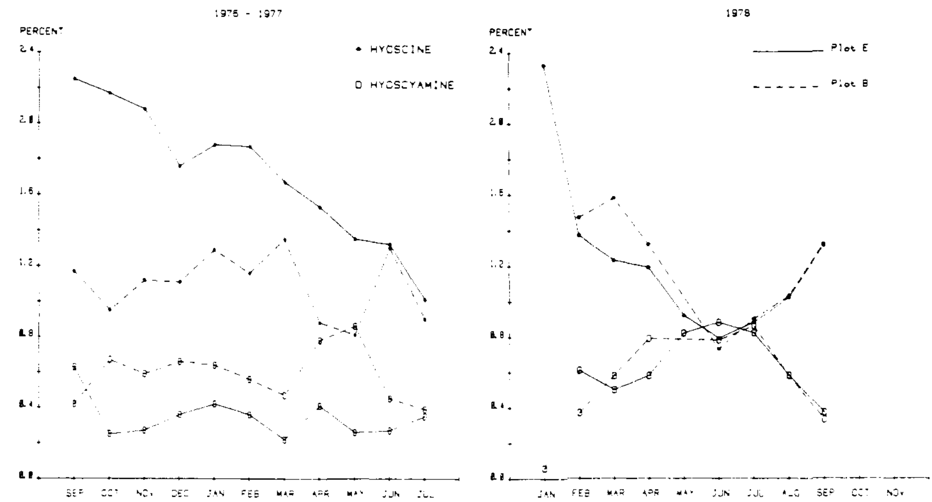

FIG. 1. Seasonal variation of hyoscine and hyoscyamine in control plots E (mature trees) and B (young trees) of a *Duboisia* hybrid plantation. (In 1978 both plots constituted regrowth material).

dilution of a commercial seaweed preparation, Maxicrop®[1], (5 ml per liter) was sprayed over two *Duboisia* hybrid trees under plantation cultivation; two adjacent trees were selected as controls. The solution was sprayed until saturation was indicated by free run-off from the surface of the leaves. The trees were sprayed twice over a two month period. Measurements of the girth and height were made

TABLE 6. Comparison of size, leaf yield and alkaloid yield between *Duboisia* hybrid trees sprayed with a dilution of Maxicrop® and controls.

Date	Control plants		Treated plants	
	C1	C2	M1	M2
Feb. 10,78 Girth (feet)	23	20	23	20
Height (feet)	11	10	12	10
April 3,78 Girth (feet)	26	22	27	23
Height (feet)	12	12	14	12
Leaf yield (kg)	2.58		3.05	
Alkaloid yield (percentage dry weight basis)				
Hyoscine	1.00±0.03[1]		1.16±0.03	
Hyoscyamine	0.72±0.02		0.67±0.02	
6-Hydroxyhyoscyamine	0.36±0.03		0.37±0.03	
Total alkaloid	2.08		2.20	

[1]Mean and 95% confidence limits.

two weeks after the spray treatments. At the time of the last measurement, the trees were fully cropped and the yield of dry leaves recorded (table 6). Although the increase in total alkaloid content was insignificant ($p=0.75–0.5$),

[1]Bell-Booth Ltd., Johnsonville, New Zealand.

there was a relative increase in hyoscine level (16%) in the treated plants as compared with the controls. There was an increase in leaf yield, too (18%). The role of the Maxicrop® spray, rather than increasing the hyoscine content, may be to delay the February to April decline experienced in the experiment discussed previously. If this can be established, then it could point to a method whereby a portion of the plantation could be sprayed to delay maturation and thus permit collection throughout the season without a diminution in hyoscine production.

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