

AN ABSTRACT OF THE THESIS OF

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Title: MONOTERPENE COMPOSITION: RESPONSE TO STRESS IN LODGEPOLE PINE
SEEDLINGS

Abstract approved: Signature redacted for privacy.
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Lodgepole pine seedlings were used to test a general hypothesis of how water and nitrogen stress affect a plants' monoterpene composition. Four treatments, consisting of high and low levels of both water and nitrogen, were applied to the trees. Growth measurements, consisting of shoot and needle lengths, needle fresh weights and areas, foliar nitrogen contents, and pre-dawn plant moisture potentials, were made to determine the effects of the treatments. Significantly greater growth occurred in the high water, high nitrogen treatment. Both low water treatments resulted in the least growth.

Monoterpene composition of current years' needles was analyzed monthly during the five months following budburst. Although many qualitative differences were found among the individual compounds each month, no definite pattern in the changes could be found. However, when monoterpene composition was quantified by totaling the integrator peak areas for a sample, definite seasonal and treatment effects became evident. Total amounts of monoterpenes reached a maximum as shoot and needle growth slowed. The treatments averaged nearly a five-fold

increase in peak area over the growing season. Trees in the high water, low nitrogen treatment were often lower in peak area than the other treatments. The effects of water stress and high nitrogen nutrition led to the highest amounts of monoterpenes. Monoterpenes have implications in tree defense from insect attack and possibly in storage of metabolically active compounds. Both functions are alluded to in this study.

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Monoterpene Composition: Response to
Stress in Lodgepole Pine Seedlings

by

Steven Coolidge Hyland

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MONOTERPENE COMPOSITION: RESPONSE TO STRESS IN LODGEPOLE PINE SEEDLINGS

INTRODUCTION

Monoterpene composition in some plants is thought to vary in such a way as to attract or repel a number of insects. This basic tenet has received substantial support recently as biochemical analyses become more refined (Wright et al., 1979; Sturgeon, 1979). Monoterpenes are primarily plant products and are well known as characteristic components of many essential oils (Croteau and Loomis, 1975). Where monoterpenes are present in plants, their function has largely been implicated in defense mechanisms mediating allelochemic interactions. Also, these compounds probably act as sinks for storage of metabolically active compounds (Croteau and Loomis, 1975; Seigler and Price, 1976; Smith, 1977).

In addition to the most likely role these compounds play in the plants' biochemistry, considerable information has been gained on the mechanism of inheritance (Smith, 1977; Squillace, 1977). The occurrence of monoterpenes in ponderosa pine, for example, as determined by age, season, within, and between trees has been studied extensively (Smith, 1964a, 1964b, 1964c, 1968, 1977). As with ponderosa pine, von Rudloff (1967) found little variation in monoterpene composition among spruce trees varying in age from three to fifty years old. Hanover (1966), using genotypically identical western white pine trees growing in three different environments, showed negligible differences in monoterpene levels. Squillace (1977) also showed only slight environmental

effects in the monoterpene content of slash pine.

Hunt and von Rudloff (1977), however, reported that leaf oils of certain conifers contain a much larger variety of monoterpenes than the oils of the cortex or xylem, thus suggesting effects of position within the plant. Another study, involving effects of nitrogen fertilization on oil composition of Scots pine phloem, shoots, and needles (Hiltunen, Schantz, and Loyllyniemi, 1975), showed total and individual terpenes increasing with the addition of nitrogen. This fertilization effect was most pronounced during the dormant season, suggesting a seasonality of terpene composition.

Composition of monoterpenes may also change when plants are subjected to water stress. Hodges and Lorio (1975), studying three loblolly pine stands differing in age, soil types, and growth characteristics, found that moisture stress significantly increased the concentration of monoterpenes present in the xylem oleoresin. A corresponding decrease in resin acids was also seen. In another study involving loblolly pine trees, Gilmore (1977) found that alpha pinene concentration increased in moisture-stressed trees whereas beta pinene, myrcene, and limonene decreased.

Severe stress induced by nearly any cause often results in increased respiration and reduced net photosynthesis (Whitmore and Zahner, 1967). A reduction in available carbohydrates may in turn affect monoterpene synthesis as they are derived primarily from "head-to-tail" fusion of C_5 units via several possible pathways (Mooney and Chu, 1974; Croteau and Loomis, 1975). Monoterpene production is energetically costly to a plant (Croteau et al., 1972) since it may require

Krebs cycle intermediates important in protein synthesis. A plant, therefore, with low energy reserves should produce less and possibly different monoterpenes (Burbott and Loomis, 1967; Wright et al., 1979).

These studies suggest that secondary compounds such as monoterpenes respond quantitatively and qualitatively to the environment in which the plant is growing. To test this hypothesis of how stressing and luxurious environments affect these groups of compounds, and other phenological events, lodgepole pine seedlings were grown under four different nutrient and moisture regimes and observed for five months before and five months after budburst.

METHODS

Growth Conditions

Lodgepole pine seedlings two years old or less (2-0), obtained from a nursery located near Bonanza, Oregon at an elevation of 1,680 m, were lifted from the field in late October and stored in a cold room at 10°C for five days until planting in a sand box 1.22 m wide, 2.44 m long, and 20 cm deep. Four equal partitions in the box provided for the application of four treatments: high water with high or low nitrogen, and low water with high or low nitrogen. All treatments were provided nutrients biweekly; high water treatments were watered weekly with twenty liters of distilled water, whereas the low water treatments received ten liters at the time of each nutrient application. The low nitrogen solution contained 1.12 ppm of nitrogen whereas the high nitrogen solution contained 112 ppm. Nutrient solutions used were similar to Swan (1960). See Table 1 for composition of the nutrient solutions. In this and Swans' study, the low nitrogen treatment was in the ammonium forms while the high nitrogen treatment received both nitrate and ammonium nitrogen. The sand box was located in an open-air lathe-house, and covered by a clear plastic tarp to shield the trees from rainfall. Each partition was lined with plastic with drainage holes. Seedlings were planted in a matrix, seven by sixteen, providing 112 seedlings per treatment.

Table 1. Nutrient solutions.

<u>cc molar soln</u>		<u>approximate ppm</u>							
	<u>liter feed soln</u>	K	Ca	Mg	NH ₄	NO ₃	P	S	pH
<u>High Nitrogen</u>									
Ca(NO ₃) ₂ ·4H ₂ O	3		120			84			
Mg(SO ₄)·7H ₂ O	2			48				64	
KH ₂ PO ₄	2	78					62		
NH ₄ H ₂ PO ₄	2				28		62		6.4
<u>Low Nitrogen</u>									
Ca(H ₂ PO ₄) ₂	3		120				62		
MgSO ₄ ·7H ₂ O	2			48				64	
KH ₂ PO ₄	2						62		
NH ₄ H ₂ PO ₄	.08				0.28		62		6.5

Micro-nutrients - added to both nutrient solutions

		<u>g/liter stock</u>	<u>approx.</u>	<u>cc molar soln</u>
		<u>soln</u>	<u>ppm</u>	<u>liter stock soln</u>
Boron	H ₃ BO ₃	2.5	0.44	.04
Manganese	MnCl ₂ ·4H ₂ O	0.8	0.22	.004
Zinc	ZnCl ₂	0.10	0.048	.0007
Copper	CuCl ₂ ·2H ₂ O	0.05	0.018	.0003
Molybdenum	MoO ₃	0.05	0.033	.0003
Iron	Fe ₂ O ₃	14.3	5	.09

Sampling

After waiting one month to assure adjustment for transplanting shock, monthly sampling was initiated. Ten seedlings from each treatment were collected and their pre-dawn water potentials, foliar weight, area, and nitrogen content, height and needle growth, and monoterpene composition were measured. After 40 of the most recent years' needles were removed for further analysis, plant water potentials were measured on the main stem of the shoot using a pressure chamber (Waring and Cleary, 1967). The 40 fresh needles from each of ten trees per treatment were pooled and stored at -40°C for later analysis. From these pooled samples of freshly frozen needles, monoterpene composition was determined, as was weight, length, and surface area of representative subsamples. Needle surface areas were measured using a Licor model LI-3000 portable area meter. This tissue was then dried 48 hours at 70°C . The dried tissue was then ground in a Wiley mill, using a size 30 mesh, and analyzed for total nitrogen using the micro-Kjeldahl technique.

Extraction and Analysis of Monoterpenes

Fresh needle subsamples of the pooled sample were weighed, and then crushed while immersed in two ml of pentane (at a pressure of $20,000 \text{ lbs/in}^2$) using a Carver laboratory press. A special crushing vesicle was constructed from stainless steel, which provided a reservoir for the extract displaced by the crushing. The pentane extract was pipetted off, and the extraction procedure repeated twice more to

ensure a more complete extraction of monoterpenes. Anhydrous sodium sulfate was added to each sample to absorb water. The combination of the three extractions yielded about six ml of solvent. The sample extracts were then concentrated to 0.1 ml under a stream of nitrogen gas at room temperature. Monoterpene evaporation is negligible if the solvent is not removed completely (Burbott and Loomis, 1967).

The pooled treatment extracts were analyzed on a Hewlett-Packard 5700A gas chromatograph equipped with dual flame ionization detectors. Monoterpene analysis was conducted on a 60 m long glass capillary column coated with polyethylene glycol 20 M (Carbowax 20 M). The gas flow rates were: helium 3 ml/min; make-up 27 ml/min; hydrogen 25 ml/min; and oxygen 250 ml/min. The oven was run isothermally at 75°C for four minutes, then programmed at 4°C/min to 130°C, and then isothermally at 130°C to the termination of sample elution (about 45 min). Detector and injector temperatures were both 200°C. Samples usually were one μ l in size and were injected using a fixed needle Hamilton micro-liter syringe.

Peak areas were integrated by means of a Hewlett-Packard 3380A integrator. Integration factors and retention times for the individual monoterpenes were determined using known terpene standards and by peak enrichment. Since the changes of individual compounds relative to each other, rather than the absolute amounts of the components are of the most significance in this study, the amounts were measured in terms of percentage of total peak area accounted for by each compound.

Since percentage data is often of a bimodal or skewed nature (Snedecor and Cochran, 1967; Sokal and Rohlf, 1969), an arc sine

transformation of the percentage data was made for data normalization. The percentages were transformed using the following equation (Sokal and Rohlf, 1969): $p' = \arcsin \sqrt{p}$, where p is the proportion of each compound. This equation yields data in radian units.

To analyze the data, three computer programs were developed: (1) calculation of treatment means and ranges of percent composition for each compound for each date; (2) conversion of percentage data to arc sine data, and calculation of means, significant F statistics, and upper and lower 95 percent confidence levels; (3) calculation of significant F statistics, and upper and lower 95 percent confidence intervals for the percentage data. Summation of total integrator peak areas for each treatment on each sampling date was also done. Total peak areas approaches a quantitative estimate of the amount of various compounds present. When these peak areas are totaled over all compounds in a sample, and adjusted for sample injection size and sample fresh weight in grams, fairly quantitative data for each treatment on each sampling date can be obtained.

RESULTS

Significant increases in growth occurred with the high water, high nitrogen (HWHN) treatment. In comparing the ratio of current years' leader growth to the previous years', this treatment differed statistically using Tukeys' multiple pairwise comparisons at $\alpha = 0.05$ from the low water, high nitrogen (LWHN) treatment. The two low nitrogen treatments fell between the growth of the high nitrogen treatments, but did not differ from either high nitrogen treatment. Trees in the HWHN treatment also resulted in the longest needles; again LWHN resulted in the shortest. Leader growth ratios and needle length differences are summarized in Table 2.

When needle weight was compared to needle surface area, a seasonal pattern resembling a sine curve developed (Figure 1). Few significant differences among treatments were found, although late in the season LWHN exhibited heavier leaves than the other treatments. By the final sampling period, both high nitrogen treatments were significantly heavier than the low nitrogen treatments. The effects of nitrogen during the last sampling period appeared to be independent of moisture.

Tissue nitrogen was compared both as a percent of tissue dry weight and as the amount per unit of leaf area (Figures 2 and 3, respectively). Both graphs depict a similar trend of foliar nitrogen increasing during the growing season in the high nitrogen treatments. Interestingly, the HWLN, which showed the second best overall growth, contained the smallest percentage and amounts of total nitrogen over most of the season. HWHN, which showed the best overall growth,

Table 2. Growth results for four treatments, July 1979. Means with same significance letter do not differ at $\alpha = .05$.

	HWHN	HWLN	LWHN	LWLN
<u>Shoot Growth</u>				
current year (cm)	14.81	16.13	12.46	14.26
last year (cm)	12.83	14.98	13.08	13.42
current/last	1.20	1.10	0.97	1.12
sample size	40	40	40	40
standard error	0.06	0.05	0.04	0.06
significant subsets	b	ab	a	ab
Tukey F statistic = 3.32				
<u>Needle Growth</u>				
average length (cm)	4.92	4.29	3.89	3.54
sample size	40	40	40	40
standard error	0.17	0.14	0.16	0.12
significant subsets	c	b	ab	a
Tukey F statistic = 14.55				

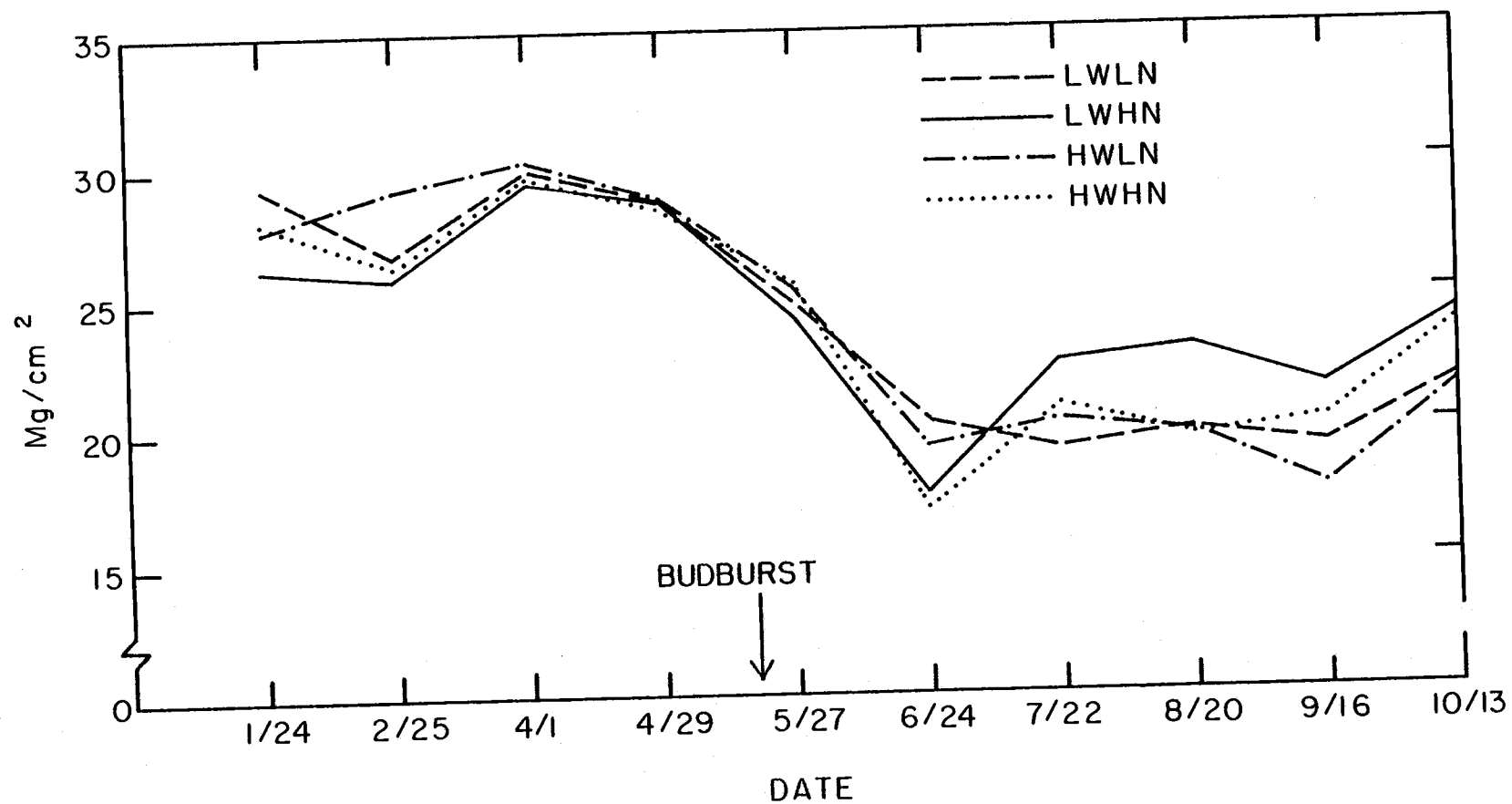


Figure 1. Needle dry weights per unit of surface area over ten sampling dates. Results of ANOVA are listed in Table 4.

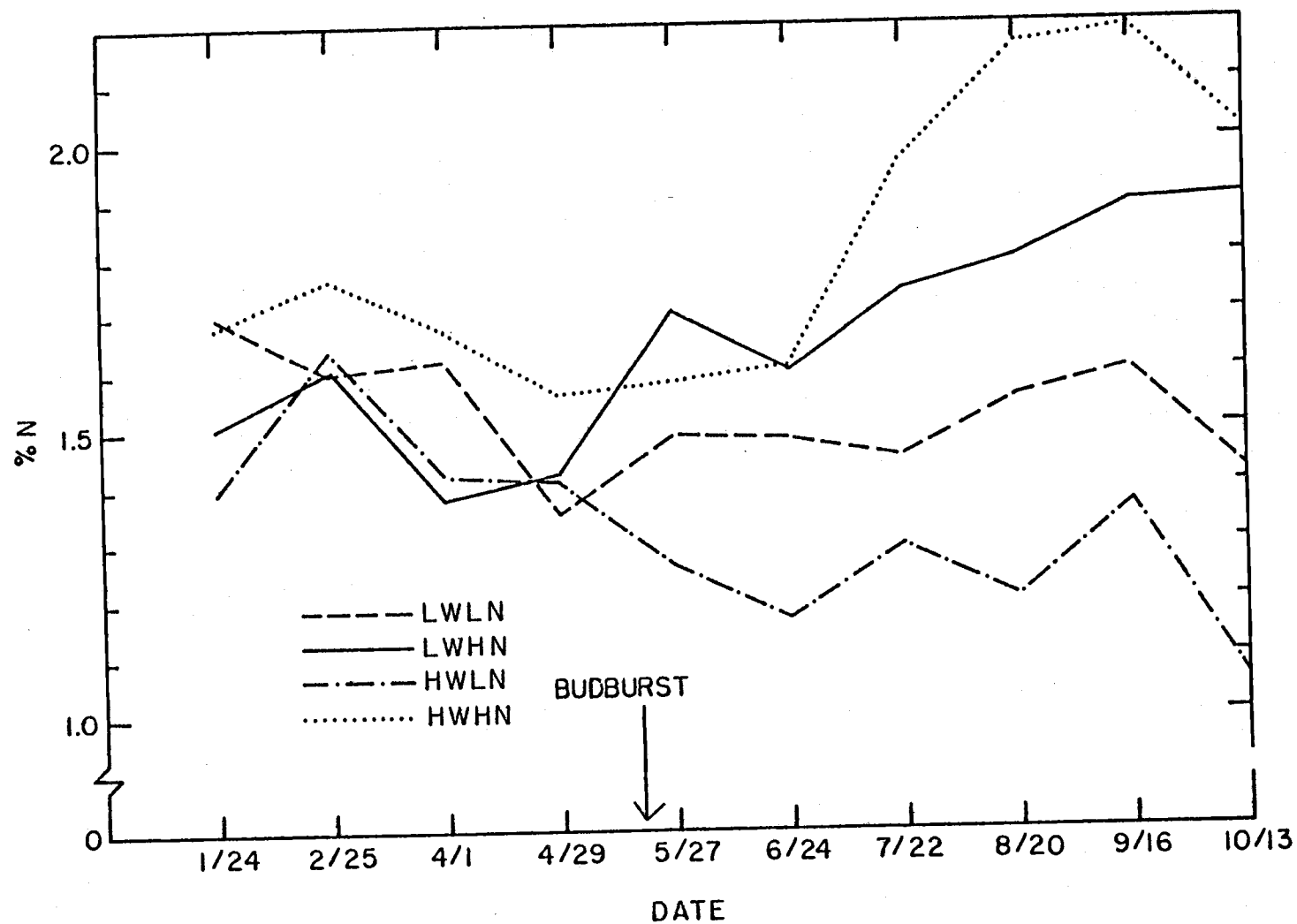


Figure 2. Percentage of needle dry weights in nitrogen over ten sampling dates. Results of ANOVA are listed in Table 2.

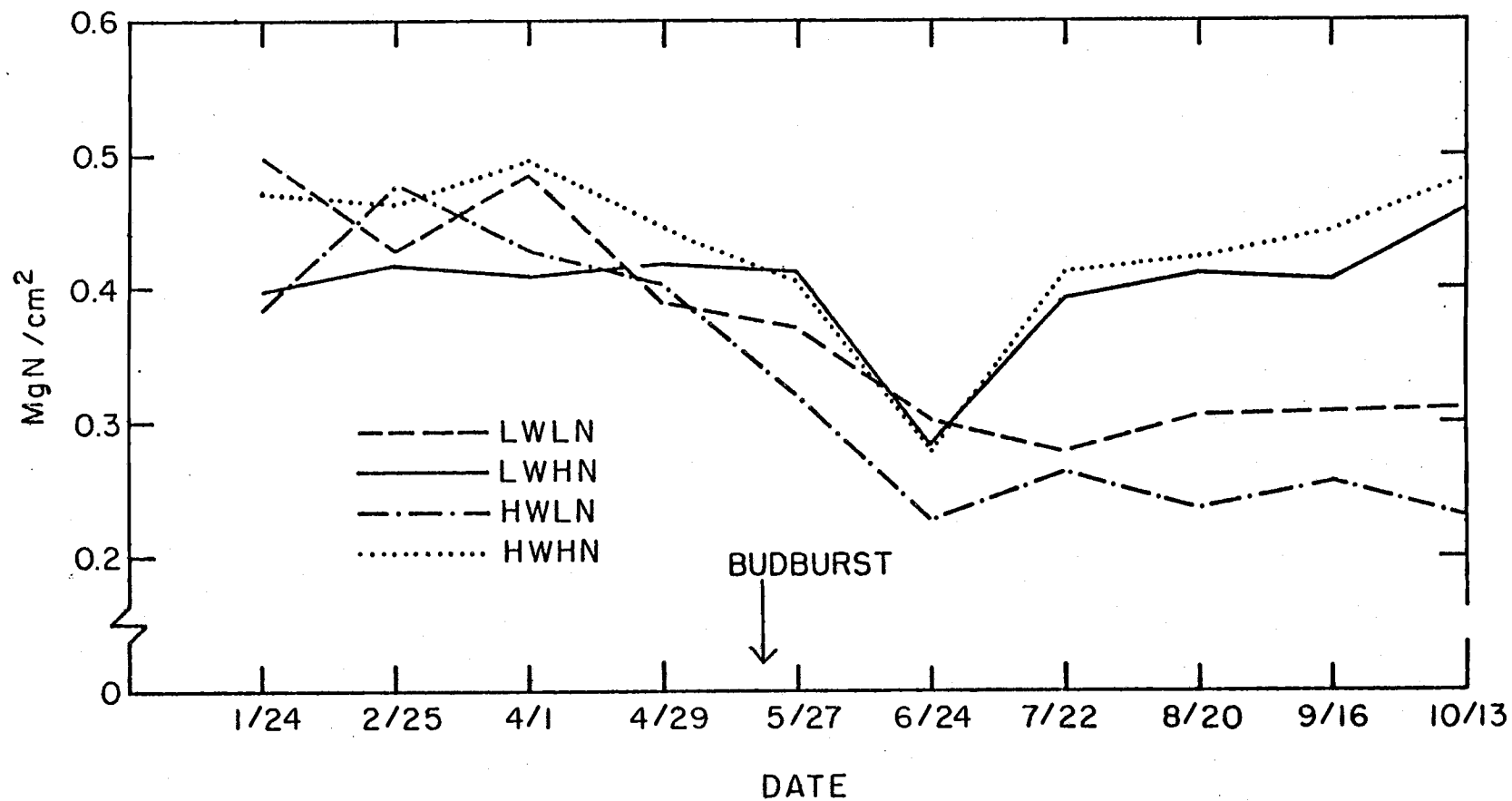


Figure 3. Amounts of nitrogen per unit of needle surface area over ten sampling dates.

contained the highest percentage and amounts of total nitrogen over the same period. Similarly, LWHN was often higher in foliar nitrogen than the LWLN.

In the course of applying nitrogen and the other nutrients, only a small, short term plant water stress was achieved (Figure 4). Significant treatment differences were observed especially during the hot, dry summer months. Mean pre-dawn water potentials reached about -13 bars in the low water treatments and were often lower than the other treatments. The LWHN treatment often exhibited the lowest potential.

The monoterpenes which were analyzed are listed in Table 3, with their respective retention times (relative to limonene which eluted from the column in 11.0 to 11.2 minutes), ranges in percent, and average percentage for each compound during the summer months. In addition to the 15 known compounds for which standards were available, 11 compounds of unknown identity were consistently present in the needle extracts and were included in all analyses.

Since it has been shown that seasonal differences in monoterpene composition occur primarily when the plants are actively growing (von Rudloff, 1967; Hunt and von Rudloff, 1974; Gilmore, 1977), analyses were concentrated on data from the period of shoot and needle elongation. This period after budburst also accounted for growth, water potential, and tissue analysis differences. No apparent growth or monoterpene differences were found prior to budburst (mid May). Average monthly composition for each compound for the five months after budburst are listed in Table 3.

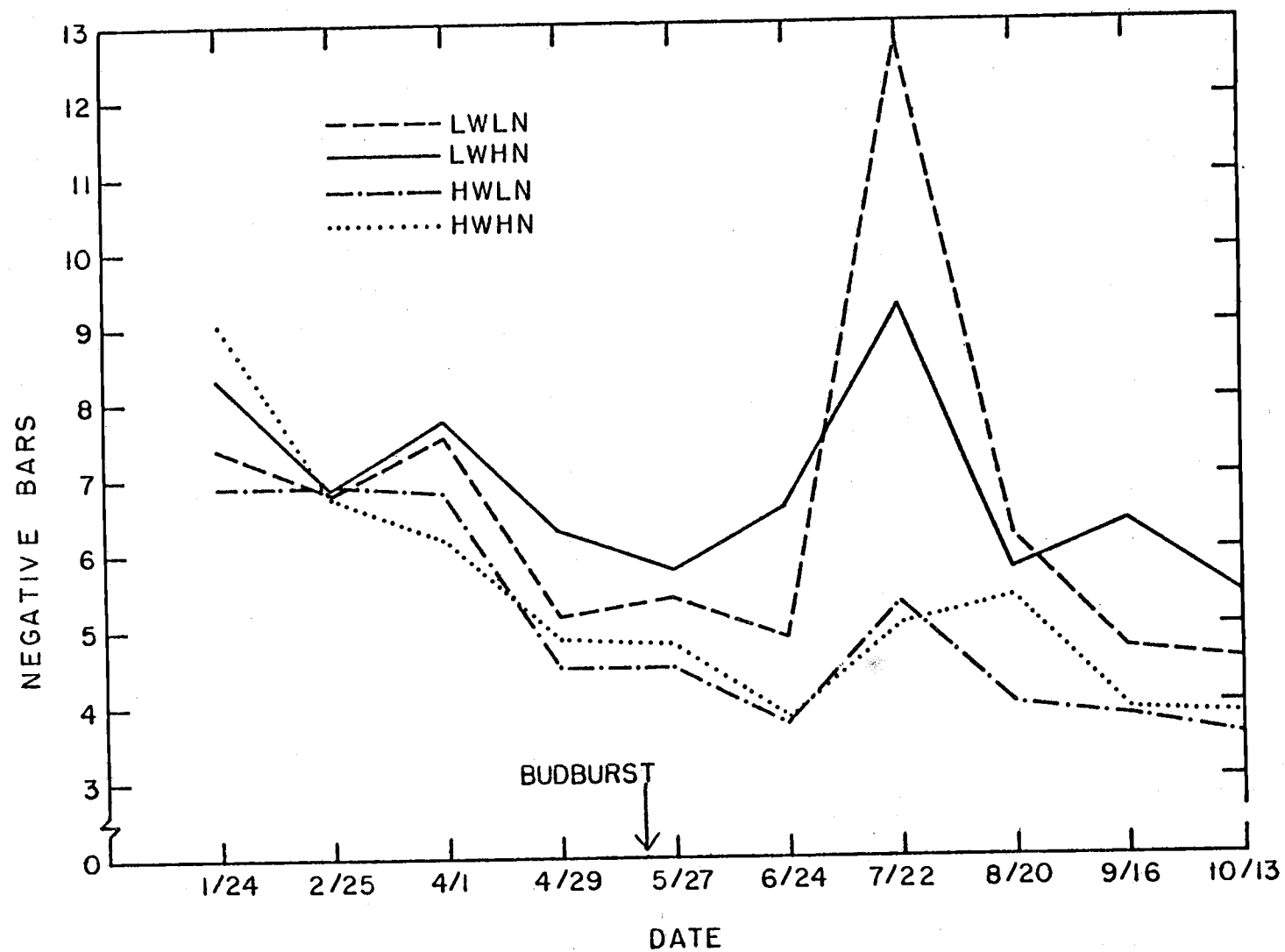


Figure 4. Treatment effects on seasonal water potentials. Results of ANOVA are listed in Table 5.

Table 3. Retention time (relative to limonene = 1.00 which eluted at from 11.00 to 11.2 minutes), monoterpenes studied, range of observed percentages, and average composition of four treatments for summer months.

Relative Retention Time	Monoterpene	Range, %	Average Composition, %				
			June	July	August	September	October
0.57	unknown	0.08-0.24	0.11	0.16	0.16	0.16	0.17
0.58	alpha pinene	4.59-13.35	8.80	8.19	7.43	7.09	6.70
0.67	camphene	0.33-3.61	0.71	1.30	0.61	0.69	0.48
0.69	unknown	0.00-0.64	0.01	0.04	0.13	0.02	0.22
0.76	beta pinene	6.72-30.79	19.69	17.33	24.08	17.95	20.99
0.80	unknown	1.01-3.80	2.24	2.23	2.17	2.33	2.63
0.83	unknown	0.00-2.73	0.12	0.63	1.45	0.59	1.15
0.86	3-carene	0.27-4.29	1.06	0.89	1.28	1.08	1.58
0.88	myrcene	1.24-2.93	1.59	1.78	2.12	2.20	2.33
0.91	alpha phellandrene	1.18-4.16	1.71	2.16	1.62	1.61	1.61
0.96	unknown	0.00-1.74	0.30	0.36	0.23	0.15	0.14
0.98	unknown	0.00-0.82	0.01	0.15	0.05	0.05	0.08
1.00	limonene	1.32-5.47	1.70	2.81	2.30	1.90	1.93
1.03	beta phellandrene	35.63-64.51	53.51	51.70	50.78	56.99	52.29
1.10	unknown	1.09-8.18	2.47	2.53	2.11	2.60	2.86
1.15	unknown	0.05-2.62	0.81	1.36	0.72	0.85	0.84
1.21	p-cymene	0.00-1.42	0.07	0.76	0.29	0.21	0.12
1.25	terpinolene	0.16-1.28	0.31	0.70	0.53	0.31	0.37
2.14	linalool	0.00-0.47	0.01	0.06	0.10	0.09	0.18
2.43	bornyl acetate	0.00-0.21	0.06	0.02	0.07	0.05	0.03
2.47	terpinen-4-ol	0.00-0.49	0.07	0.08	0.12	0.08	0.16
2.52	unknown	0.08-3.07	1.25	1.37	0.81	0.64	0.76
3.13	alpha terpineol	0.00-0.13	0.01	0.02	0.02	0.01	0.03
3.32	unknown	0.06-1.02	0.27	0.47	0.31	0.27	0.22
3.53	unknown	0.04-1.67	0.35	0.54	0.16	0.22	0.17
3.84	geranyl acetate	0.09-4.62	0.63	1.37	0.59	0.93	0.66

Table 4. Carbohydrate balance ANOVA (critical $F(.95,3,8) = 4.07$).

Date	MSE	F	±95% interval
1/24	1.70	2.97	1.48
2/25	2.46	2.58	1.77
4/1	0.95	0.34	1.10
4/29	1.31	0.12	1.29
5/27	5.62	0.18	2.68
6/24	0.78	9.77	1.00
7/22	1.30	4.51	1.29
8/20	0.60	13.29	0.88
9/16	1.19	3.66	1.23
10/13	0.56	9.84	0.85

Table 5. Water potential ANOVA (critical $F(.95,3,36) = 2.89$).

Date	MSE	F	±95% interval
1/24	3.22	2.91	1.09
2/25	0.91	0.03	0.58
4/1	1.09	4.96	0.63
4/29	0.81	8.56	0.55
5/27	0.36	8.57	0.36
6/24	0.55	29.61	0.45
7/22	10.14	13.28	1.93
8/20	1.36	6.08	0.71
9/16	1.10	12.48	0.64
10/13	0.59	11.44	0.47

Table 6. Percent nitrogen ANOVA (critical $F(.95,3,8) = 4.07$).

Date	MSE	F	±95% interval
1/24	0.03	2.07	0.20
2/25	0.10	0.17	0.36
4/1	0.06	1.02	0.28
4/29	0.01	1.92	0.12
5/27	0.03	3.14	0.20
6/24	0.01	19.90	0.12
7/22	0.02	17.10	0.16
8/20	0.005	101.94	0.08
9/16	0.02	17.39	0.16
10/13	0.03	19.55	0.20

In the analysis of variance, transformed means were tested for significant treatment differences using Tukey's multiple comparison test at $\alpha = 0.05$. During the last five dates of the experiment, 31 significant differences were found among the 26 monitored known and unknown compounds. Of these differences, none can be found which follow a trend which could be linked to growth, water potential, or tissue nutrition data. A possible exception may be HWLN trees during the last four dates where percentage of geranyl acetate is greater than HWHN in August and October, and greater than LWHN and LWLN in July and September respectively. More typical of the results, for example, is in July where the HWHN treatment was found to be greater in percent in beta phellandrene than the HWLN, indicating a possible nitrogen effect. But in August, the order was nearly reversed with HWLN being higher in percent than the other three treatments. This variation pattern appeared to be unrelated to moisture or nutrition differences. Appendix 1 summarizes the treatment differences, F statistics, and standard errors for the summer months.

An analysis of variance of the percentage data (Appendix 2) yielded results very similar to that obtained by the arc sine ANOVA. The arc sine transformation yielded only one additional significant difference than the percentage ANOVA.

Each compound peak can also be represented by peak area, which is representative of the peaks' magnitude. As shown in Figure 5, the peak areas reach a minimum in the current needles just before and during budburst, and reach a maximum near the period of needle maturity. All four treatments show a rapid increase in terpene areas during needle

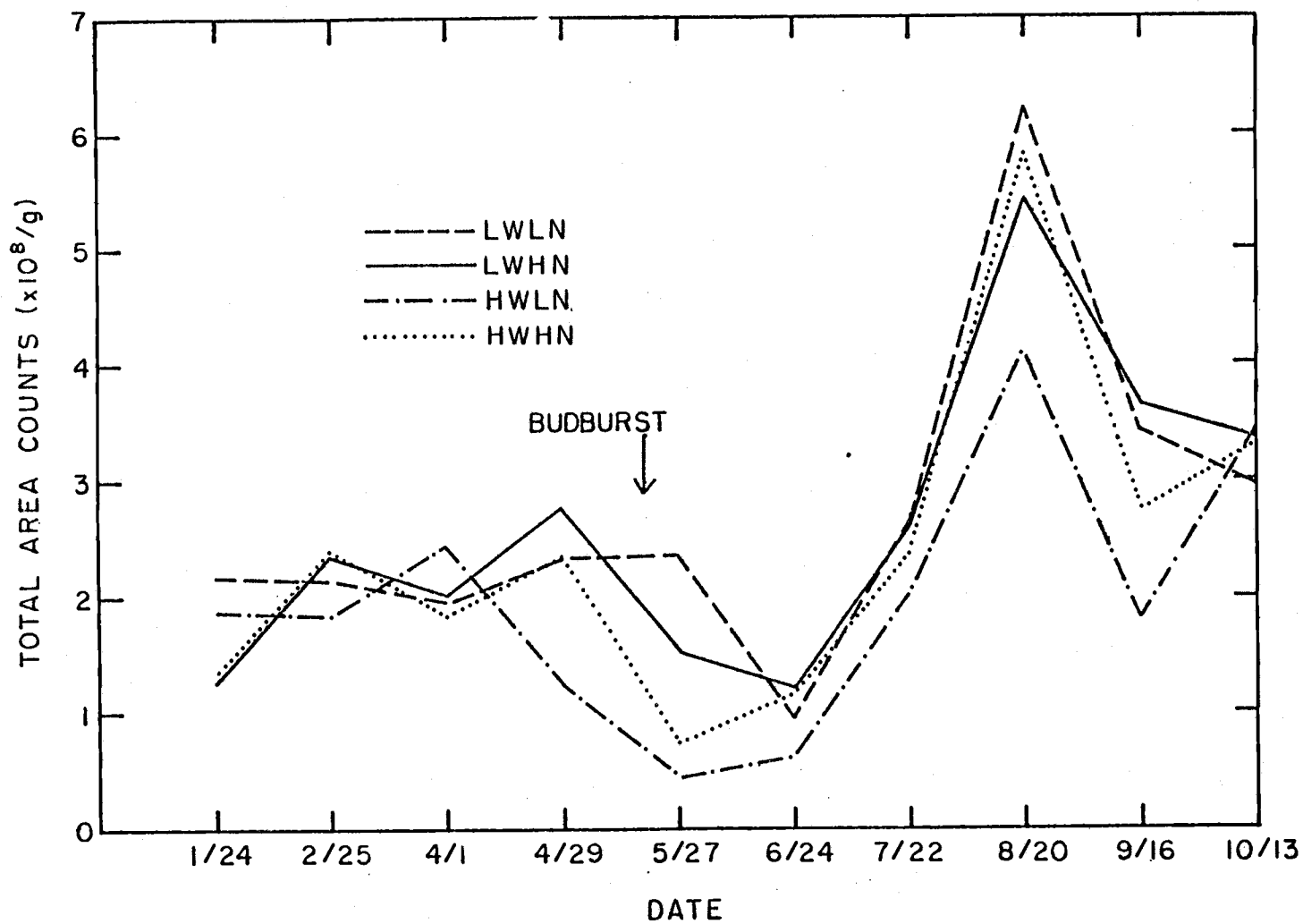


Figure 5. Volatile composition of the four treatments, expressed as total peak areas per sample. Results of ANOVA are listed in Table 7.

Table 7. Total area counts ANOVA (critical $F(.95,3,8) = 4.07$).

Date	Average counts per gram of tissue				MSE ($\times 10^{10}$)	F	$\pm 95\%$ interval ($\times 10^7$)
	HWLN	HWHN	LWLN	LWHN			
1/24	1.887223	1.341861	2.177866	1.23772	13.6247	4.18	4.17999
2/25	1.826910	2.401758	2.145347	2.378266	19.9792	1.07	5.06175
4/1	2.429207	1.855102	1.956923	2.096370	28.9934	0.65	6.09764
4/29	3.001839	2.351165	2.343951	2.795744	6.80382	0.48	9.34089
5/27	0.457149	0.776505	2.356004	1.539330	8.21615	26.24	3.24598
6/24	0.633463	1.175956	0.971828	1.211263	2.19765	9.59	1.67877
7/22	2.056799	2.388757	2.634379	2.617813	9.74093	2.24	3.53437
8/20	4.138584	5.854327	6.209452	5.419979	20.9963	11.68	5.18899
9/16	1.802244	2.747139	3.460205	3.676201	8.90081	24.08	3.37852
10/13	3.410369	3.350671	2.964505	3.374689	6.82301	1.91	2.95801

and shoot elongation. HWLN, which resulted in the second best overall growth, contained the least amounts of terpenes during this period. The other three treatments showed the best (HWHN) and least (LWHN, LWLN) growth, but resulted in the greatest amounts of terpenes. All treatments fall rapidly in total areas after the August peak and tended to group at one level at the end of the experiment.

DISCUSSION

In response to the four treatments applied, quantitative changes in the secondary compounds of the trees were found. Since these secondary compounds, of which monoterpenes are one group, are dependent upon carbohydrates for their synthesis, a change in carbohydrate storage will likely result in changed monoterpene compositions. Since carbohydrates seem to be at the root of any growth or terpene changes, a discussion of carbohydrate utilization and storage is necessary.

The carbohydrate economy of a tree is usually regarded as an integrated system of sources and sinks (Wareing and Patrick, 1975). The sources are generally the green organs of the plant, since these produce the photosynthates. When the production of assimilates exceeds the demand of the sinks, the surplus is converted into reserve substances. These stored reserves, mainly starch, are later hydrolyzed and used for growth, respiration, and differentiation or maturation. The reserves could therefore be seen as a second type of source, which is utilized when required. This second type of source is well known for evergreen conifers, which store large amounts of starch during spring and early summer (Kozlowski and Keller, 1966; Rutter, 1957). These reserves are later used when growth of the tree resumes in the spring (Ericsson, 1978).

Since the sink strength of roots, stem, and shoots was probably weak during the early spring, elongation or growth was minimal, the accumulation of assimilates in the needles may have been the result of low translocation of photosynthates from the needles in comparison with

the photosynthetic production. Evident from Figure 1, all treatments exhibited heavier foliage prior to budburst than afterward when sink strengths presumably increase and reserves are moved from the leaves. Low translocation rates during this period of the year have also been demonstrated by Ericsson in two separate studies (1978, 1979).

When shoot elongation was rapid (June), a decreased rate of starch accumulation or a reduction in starch content probably occurred in the needles. The low values obtained after budburst related to this period of rapid shoot and needle growth. The shoots are of course not the only sinks during this period, but rapid shoot elongation probably reflects favorable environmental conditions for growth in other parts of the tree as well.

The significant rise in tissue weight in the LWHN treatment (Figure 1) during the summer possibly indicates a decline in translocation due to inadequate water supplies (Figure 4), or more likely, an increase in osmotically active compounds to offset the low water supply. This low translocation, or increase in osmotic regulators, coincides with the shoot growth exhibited by the LWHN trees (Table 2) where the LWHN trees grew less than the HWHN trees. A possible inhibiting effect of high nutrient concentrations on seedling root growth, resulting in decreased top growth, was suggested by McClain and Armson (1974). Therefore, the period of most intense water stress, June to August, affected the growth of LWHN trees to a greater degree than the LWLN possibly due to the high concentration of nutrients in the LWHN, causing possible root inhibition problems. This does not explain, however, why the LWLN trees exhibited a lower water potential

than the LWHN in July (Figure 4). Perhaps the greater amounts of nitrogen in the LWHN trees acts as osmotic regulators and partially offsets the low moisture contents.

Fertilization increases the sink activities of a tree (Ericsson, 1979) and thus causes an increased utilization of starch reserves in the needles. Results of Ericssons' study indicated a decline in percent of dry weight of starch in fertilized, and fertilized and irrigated plots. Results presented here (Figure 1), reported on a leaf area basis, indicate a significant increase in October in the carbohydrate balance of the needles in the fertilized treatments. Since the water potentials of all treatments were less negative during the last three months (August, September, and October) of the study (Figure 3), water was not likely to be limiting. Since it has been shown that photosynthetic assimilation of carbon increases when the water and nutrition status of a tree is good (Brix, 1972), an explanation of the increased weights in the high nitrogen or high water treatments could therefore be that demands for carbohydrates is satisfied by increased production. Therefore, more photosynthate is used for storage and growth. This also could explain the good height and needle growth (Table 2) exhibited by the HWHN trees.

As expected, the trees of the high nitrogen treatments showed a steady increase in total foliar nitrogen contents (Figures 2 and 3), expressed either as a percent of dry weight or weight per unit of leaf surface area. Smith et al. (1970) suggest that the best time to characterize differences in foliar nitrogen in plants whose growth may be limited by nitrogen is during periods of rapid growth after reserves

are depleted and demands for nitrogen are large. This would explain the significant departures of all treatments from each other following budburst. All the trees contained roughly the same amount of foliar nitrogen until about the time of budburst. The period of growth after budburst appears to be the first real expression of sink strength, since it is then that foliar nitrogen contents begin to differ. The total foliar nitrogen content of the low nitrogen trees remained nearly the same or decreased slightly after budburst. The high nitrogen trees, on the other hand, appear to be accumulating excess or luxurious nitrogen. One possibility for the trees in the LWHN being lower in total nitrogen content than the HWHN is the failure to take up as much nitrogen due to root inhibition. Schomaker (1969) found with white pine seedlings that moisture-fertility interactions generally resulted in foliar nitrogen increasing as fertility increases and moisture supply decreases. A possible reason the HWHN treatment resulted in higher nitrogen content than LWHN is the lack of leaching loss in the high water treatment that was experienced in Schomakers' experiment. Another possible reason for HWHNs' higher nitrogen content could be its' higher water content which aids translocation and up-take. The high growth, and therefore, large cell size, of the HWLN trees may have served to dilute what little nitrogen there was available in these tissues.

Several studies show that even a small water stress can alter physiological processes and growth. At about the fairly extreme water potential of -18 bars, stomates close and growth ceases in Douglas-fir saplings (Waring, 1971). Water potentials of between -5 and -7 bars

have been shown (Sucoff, 1972) to reduce growth in several species of pine. Two separate studies (Miller, 1965; Kaufman, 1968) showed that needle extension in Pinus taeda decreased about 30 percent as leaf water potential decreased to -20 bars. Whitmore and Zahner (1967), in cambial tissue cultures of Pinus sylvestris, found an approximately 30 percent reduction in the incorporation of labeled glucose into tracheid cell walls when the water potential of the media was reduced from -3.1 bars to -5.9 bars. This gives further credence to Hsaios' suggestion (1973) that two sensitive processes, protein synthesis and cell expansion, may be inhibited at stress levels as little as -5 bars. Thus, it is likely that water stress played a major role in both low water treatments' reduced overall growth, even when the average water potential for both treatments over the duration of the study was -6.7 bars.

The above discussion points out that significant physiological and morphological changes were found in response to the four treatments applied to the seedlings. The dynamic state of monoterpenes in plants and their rapid rate of turnover (Loomis, 1967; Seigler and Price, 1976) suggests that these so-called secondary metabolic products are intimately involved with primary metabolic functions in the plants. Thus, changes in physiological processes and growth, brought on by changes in the plants' nutrition or water relations, should be accompanied by changes in secondary products, in this case the monoterpenes.

The Growth-Differentiation Balance theory of W. E. Loomis (1932) defines growth as cell division and cell enlargement for the benefit of increasing plant size. If plants are grown under favorable temperatures, moisture, nutrients, and illumination regimes, rapid

vegetative growth results. Under these conditions the sugars produced by the plant are used in the synthesis of proteins and to supply the high energy requirements of active meristematic tissues. As differentiation, or maturation, approaches a minimum, stems are succulent, cell walls are thin, and the accumulation of gums, alkaloids, or essential oils are reduced to a minimum. This agrees well with the pattern of total monoterpene peak areas (Figure 5), where the minimum amounts are evident during the period of maximum shoot and needle elongation for all treatments.

As the growth phase of the plants slows or is checked in a manner which does not appreciably reduce the photosynthetic activity of the plant, for example by gradually reducing the moisture or nutrient supply available to the top, the carbohydrates formerly used in growth now accumulate and serve as the stimulus and raw materials for differentiation. Cell walls are thickened, cuticle and cork develop, conducting elements become more abundant in the new tissues, and essential oils accumulate (Loomis, 1932). If stress occurs early in the growing season, the accumulation of secondary compounds may aid the plant in warding off pathogens or predators.

This differentiation is evident in Figure 5, where the total peak areas increase as the growth of the seedlings slows. HWLN does not approach the magnitude of the other treatments since those trees experienced a summer water potential deficit of only about -5 bars; perhaps not enough to induce a moisture stress response in those trees. Both low water treatments experience enough water stress to slow their growth, especially in July (Figure 4), and produce high quantities of

compounds one month later in August. Since the trees were sampled monthly, a lag of up to a month was seen in the monoterpene composition in response to the water stress.

This and other studies (Seigler and Price, 1976; Wright et al., 1979) suggest that secondary oils may act as stores of carbon, perhaps stimulated by luxurious amounts of nitrogen. Since the trees of the HWHN treatment are neither water nor nitrogen stressed, and photosynthetic production approached a maximum, storage of carbon in the form of terpenes may explain this treatment's high total peak areas (Figure 5).

The rapid drop in peak areas in all treatments in September and stabilization in October may be in response to the lessening of water stress, or possibly could be in preparation for cold hardiness. Some of the terpenes could be utilized for the physiological processes involved in hardiness and dormancy. Plant photosynthetic rate would also fall during this time period as temperatures begin to decline and incoming sunlight is less intense (Emmingham and Waring, 1977).

Although the qualitative make-up of the oil did not appear to change in response to stress, the quantity of the total monoterpene fraction does appear to respond. This differs from Gilmore's study (1977) where he showed the percentages of alpha pinene increasing while those of beta pinene, myrcene, and limonene decreasing when loblolly pine trees were moisture stressed. Quantitative changes were not followed in that study.

CONCLUSIONS

Although most work reported in the literature involving monoterpenes is reported in terms of area percentages, and some significant findings have been obtained by them, the results of this study indicate that quantitative results are important in describing a plants' response to stress. If the plants' major monoterpene, in this case beta phellandrene, falls in quantity and the others remain the same, area percentage results would report the activity as the major monoterpene falling in proportion while the others rise in proportion. Future efforts at accurately quantifying monoterpene fluctuations should aid the interpretation of plants' responses to stress of any form. Unpublished work by Waring and Pitman (1980) tentatively shows a correlation between total peak areas of phloem monoterpenes and vigor of lodgepole pine trees. This relationship was not borne out when results were studied in their percentage form. It is also becoming more clear via these studies, why bark beetles, or most any chemoreceptive predator, select certain trees, both by their vigor which may be an expression of the trees' nutrition and moisture status, and their monoterpenes, which may be an expression of the plants' vigor.

As reported earlier, the work of Hodges and Lorio (1975) showed resin acids decreasing in proportion to total monoterpenes in response to water stress. With so much recent emphasis on monoterpenes, and their selective attractiveness to predatory insects, it seems also possible that resin acids, or other resin components, could be just as important either in proportion to monoterpenes or by themselves.

BIBLIOGRAPHY

- Brix, H. 1972. Nitrogen fertilization and water effects on photosynthesis and earlywood-latewood production in Douglas-fir. *Can. J. For. Res.* 2:467-478.
- Burbott, A. J. and W. D. Loomis. 1967. Effects of light and temperature on the monoterpenes of peppermint. *Plant Physiol.* 42:20-28.
- Croteau, R. and W. D. Loomis. 1975. Biosynthesis and metabolism of monoterpenes. *Int. Flavours (Sept./Oct.):*292-296.
- Croteau, R., A. J. Burbott, and W. D. Loomis. 1972. Apparent energy deficiency in mono- and sesquiterpene biosynthesis in peppermint. *Phytochemistry* 11:2437-2948.
- Emmingsham, W. H. and R. H. Waring. 1977. An index of photosynthesis for comparing forest sites in western Oregon. *Can. J. For. Res.* 7:165-174.
- Ericsson, A. 1978. Seasonal changes in translocation of ^{14}C from different age-classes of needles on 20-year-old Scots pine trees (*Pinus sylvestris* L.). *Physiol. Plant.* 43:351-358.
- _____. 1979. Effects of fertilization and irrigation on the seasonal changes of carbohydrate reserves in different age-classes of needles on 20-year-old Scots pine trees (*Pinus sylvestris*). *Physiol. Plant.* 45:200-280.
- Gilmore, A. R. 1977. Effects of soil moisture stress on monoterpenes in loblolly pine. *J. Chem. Ecol.* 3:667-676.
- Hanover, J. W. 1966. Environmental variation in the monoterpene of *Pinus monticola* Dougl. *Phytochemistry* 5:713-717.
- Hiltunen, R., M. W. Schantz, and K. Loyttyniemi. 1975. The effect of nitrogen fertilization on the composition and the quantity of volatile oil in Scots pine (*Pinus sylvestris* L.). *Commun. Inst. Forest. Fenn.* 85:1-14.
- Hodges, J. D. and P. L. Lorio, Jr. 1975. Influence of moisture stress on the composition of xylem oleoresin of loblolly pine. *For. Sci.* 21:283-290.
- Hsaio, T. C. 1973. Plant responses to water stress. *Ann. Rev. Plant Physiol.* 24:519-570.
- Hunt, R. S. and E. von Rudloff. 1974. Chemosystematic studies in the genus *Abies*. I. Leaf and twig oil analysis of alpine and balsam firs. *Can. J. Bot.* 52:477-487.

- Hunt, R. S. and E. von Rudloff. 1977. Leaf-oil-terpene variation in western white pine populations of the Pacific Northwest. *Forest Sci.* 23:507-516.
- Kaufman, M. R. 1968. Water relations of pine seedlings in relation to root and shoot growth. *Plant Physiol.* 43:281-288.
- Kozlowski, T. T. and T. Keller. 1966. Food relations of woody plants. *Bot. Rev.* 32:293-382.
- Loomis, W. D. 1967. In *Terpenoids in Plants* (edited by J. B. Pridham). Academic Press, London, pp. 59-82.
- Loomis, W. E. 1932. Growth-differentiation balance vs. carbohydrate-nitrogen ratio. *Proc. Am. Soc. Hort. Sci.* 29:240-245.
- McClain, K. M. and K. A. Arnson. 1975. Growth responses of pine and spruce seedlings to regimes of soil moisture and fertility. *Soil Sci. Amer. Proc.* 39:140-144.
- Miller, L. M. 1965. Changes in radiosensitivity of pine seedlings subjected to water stress during chronic gamma irradiation. *Health Physics* 11:1653-1662.
- Mooney, H. A. and C. Chu. 1974. Seasonal carbon allocation in Heteromales arbutifolia, a California evergreen shrub. *Oecologia*. 14:295-306.
- Rutter, A. J. 1957. Studies in the growth of young plants of Pinus sylvestris L. I. The annual cycle of assimilation and growth. *Ann. Bot.* 21:399-426.
- Schomaker, C. E. 1969. Growth and foliar nutrition of white pine seedlings as influenced by simultaneous changes in moisture and nutrient supply. *Soil Sci. Soc. Amer. Proc.* 33:614-618.
- Seigler, D. and P. W. Price. 1976. Secondary compounds in plants: primary functions. *Amer. Natur.* 110:101-105.
- Smith, R. H. 1964a. Variations in the monoterpene composition of ponderosa pine wood oleoresin. USDA For. Serv. Res. Pap. PSW-15. 17 p.
- _____. 1964b. Variations in the monoterpenes of Pinus ponderosa Laws. *Science* 143:1337-1338.
- _____. 1964c. Perennial constancy of the monoterpene synthesis in the wood oleoresin of Pinus ponderosa. *Nature* 202:107-108.
- _____. 1968. Intratree measurements of the monoterpene composition of ponderosa pine xylem resin. *Forest Sci.* 14:418-419.

- Smith, R. H. 1977. Monoterpenes of ponderosa pine xylem resin in western United States. USDA For. Serv. Tech. Bull. No. 1532. 48 p.
- Smith, W. H., G. L. Switzer, and L. E. Nelson. 1970. Development of the shoot system of young loblolly pine: 1. Apical growth and nitrogen concentration. Forest Sci. 16:483-490.
- Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods. Sixth edition. pp. 327-329. Iowa State University Press, U.S.A.
- Sokal, R. R. and F. J. Rohlf. 1969. Biometry. pp. 386-387. W. H. Freeman and Co. San Francisco, U.S.A.
- Squillace, A. E. 1977. Use of monoterpene composition in forest genetics research with slash pine. Proc. 14th South. For. Tree Improv. Conf. p. 227-238.
- Sturgeon, K. B. 1979. Monoterpene variation in ponderosa pine xylem resin related to western pine beetle predation. Evolution 33: 803-814.
- Sucoff, E. 1972. Water potential in red pine: soil moisture, evapotranspiration, crown position. Ecology 53:681-686.
- Swan, H. S. D. 1960. The mineral nutrition of Canadian pulpwood species. I. The influence of nitrogen, phosphorus, potassium and magnesium deficiencies on the growth and development of white spruce, black spruce, jack pine and western hemlock seedlings grown in a controlled environment. Pulp and paper research institute of Canada. Woodplants research index No. 116, 66 p.
- von Rudloff, E. 1967. Chemosystematic studies in the genus Picea (Pinaceae). 1. Introduction. Can. J. Bot. 45:890-901.
- Wareing, P. F. and J. Patrick. 1975. Source-sink relations and the partition of assimilate in the plant. In Photosynthesis and Productivity in Different Environments (J. P. Cooper, ed.), Cambridge Univ. Press, New York. pp. 481-499.
- Waring, R. H. 1971. Matching species to site. In Regeneration of ponderosa pine. (R. H. Hermann, ed.), School of Forestry, Oregon State Univ., Corvallis, Ore. pp. 54-61.
- Waring, R. H. and B. D. Cleary. 1967. Plant moisture stress: evaluation by pressure bomb. Science 155:1248-1254.
- Waring, R. H. and G. B. Pitman. 1980. Unpublished data.

Whitmore, F. W. and R. Zahner. 1967. Evidence for a direct effect of water stress on tracheid cell wall metabolism in pine. Forest Sci. 13:397-400.

Wright, L. C., A. A. Berryman, and S. Gurusiddaiah. 1979. Host resistance to the fir engraver beetle, Scolytus ventralis (Coleoptera: Scolytidae). 4. Effect of defoliation on wound monoterpene and inner bark carbohydrate concentrations. Can. Ent. 111:1255-1262.

APPENDICES

Appendix 1. Arc Sine of Percentage Data (6/24). Means with Same Letter Do Not Differ at $\alpha = 0.05$.

	HWLN		HWHN		LWLN		LWHN		Tukey	
	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	F ratio	F Prob.
unknown	0.0340	0.0020	0.0315	0.0024	0.0340	0.0022	0.0335	0.0027	0.279	0.8390
alpha pinene	0.3316	0.0275	0.2850	0.0097	0.2846	0.0073	0.2965	0.0128	1.827	0.2203
camphene	0.0768	0.0027	0.0784	0.0044	0.0852	0.0055	0.0926	0.0157	0.687	0.5847
unknown	0.0000	0.0000	0.0033	0.0033	0.0067	0.0033	0.0080	0.0042	1.301	0.3391
beta pinene	0.3468a	0.0430	0.4730b	0.0224	0.4421ab	0.0191	0.5547b	0.0142	10.113	0.0043
unknown	0.1523	0.0230	0.1548	0.0194	0.1416	0.0034	0.1460	0.0046	0.155	0.9235
unknown	0.0211	0.0113	0.0376	0.0035	0.0318	0.0032	0.0380	0.0078	1.173	0.3787
3 carene	0.1300	0.0260	0.0951	0.0086	0.0947	0.0034	0.0778	0.0057	2.417	0.1416
myrcene	0.1345	0.0032	0.1255	0.0058	0.1287	0.0074	0.1160	0.0023	2.301	0.1539
alpha phellandrene	0.1316	0.0052	0.1222	0.0031	0.1340	0.0065	0.1322	0.0215	0.209	0.8874
unknown	0.0348	0.0181	0.0437	0.0068	0.0527	0.0081	0.0636	0.0255	0.557	0.6581
unknown	0.0000	0.0000	0.0033	0.0033	0.0047	0.0047	0.0047	0.0047	0.356	0.7863
limonene	0.1328	0.0082	0.1292	0.0017	0.1301	0.0042	0.1291	0.0079	0.081	0.9687
beta phellandrene	0.8391	0.0351	0.8277	0.0119	0.8414	0.0197	0.7744	0.0166	1.940	0.2017
unknown	0.1985	0.0459	0.1328	0.0026	0.1513	0.0138	0.1243	0.0090	1.847	0.2168
unknown	0.0793	0.0287	0.0929	0.0042	0.0952	0.0042	0.0830	0.0032	0.269	0.8463
p-cymene	0.0320	0.0165	0.0383	0.0042	0.0387	0.0013	0.0432	0.100	0.216	0.8823
terpinolene	0.0594	0.0104	0.0548	0.0040	0.0564	0.0035	0.0502	0.0049	0.364	0.7810
linalool	0.0047	0.0047	0.0105	0.0053	0.0147	0.0050	0.0033	0.0033	1.588	0.2669
bornyl acetate	0.0206	0.0021	0.0167	0.0067	0.0194	0.0097	0.0293	0.0020	0.808	0.5241
terpinen-4-ol	0.0163	0.0011	0.0173	0.0042	0.0400	0.0041	0.0191	0.0108	3.355	0.0759
unknown	0.1195	0.0152	0.1046	0.0136	0.1093	0.0084	0.1082	0.0101	0.278	0.8400
alpha terpineol	0.0000	0.0000	0.0080	0.0042	0.0033	0.0033	0.0000	0.0000	2.018	0.1900
unknown	0.0406	0.0053	0.0322	0.0057	0.0681	0.0188	0.0491	0.0055	2.133	0.1742
unknown	0.0949b	0.0231	0.0284a	0.0055	0.0461ab	0.0026	0.0319a	0.0028	6.489	0.0155
geranyl acetate	0.0568a	0.0045	0.0505a	0.0112	0.1107b	0.0128	0.0808ab	0.0036	9.287	0.0055

Arc Sine of Percentage Data (7/22)

	HWLN		HWHN		LWLN		LWHN		Tukey	
	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	F ratio	F Prob.
unknown	0.0364	0.0020	0.0412	0.0012	0.0379	0.0004	0.0439	0.0023	4.104	0.0490
alpha pinene	0.3319	0.0189	0.2744	0.0176	0.2623	0.0232	0.2835	0.0154	2.586	0.1257
camphene	0.1444	0.0348	0.0976	0.0165	0.0900	0.0096	0.0994	0.0181	1.277	0.3463
unknown	0.0000	0.0000	0.0129	0.0129	0.0162	0.0116	0.0186	0.0139	0.556	0.6584
beta pinene	0.4850	0.0154	0.3286	0.0009	0.4338	0.0659	0.4487	0.0308	3.259	0.0806
unknown	0.1361	0.0108	0.1369	0.0183	0.1746	0.0134	0.1435	0.0155	1.518	0.2827
unknown	0.0075a	0.0075	0.0878b	0.0105	0.0759b	0.0238	0.1014b	0.0068	9.904	0.0045
3 carene	0.0783	0.0175	0.1134	0.0109	0.0956	0.0146	0.0779	0.0045	1.722	0.2395
myrcene	0.1228	0.0052	0.1401	0.0061	0.1350	0.0054	0.1366	0.0043	2.024	0.1891
alpha phellandrene	0.1558	0.0259	0.1587	0.0132	0.1370	0.0099	0.1300	0.0060	0.807	0.5246
unknown	0.0799	0.0276	0.0111	0.0111	0.0532	0.0125	0.0521	0.0099	2.840	0.1057
unknown	0.0058a	0.0058	0.0655b	0.0241	0.0082ab	0.0082	0.0141ab	0.0000	4.703	0.0355
limonene	0.1957	0.0344	0.1567	0.0117	0.1506	0.0109	0.1574	0.0175	0.976	0.4508
beta phellandrene	0.6915a	0.0423	0.8798b	0.0175	0.8284ab	0.0445	0.8098ab	0.0253	5.376	0.0255
unknown	0.1362	0.0142	0.1636	0.0095	0.1833	0.0181	0.1462	0.0159	1.951	0.2001
unknown	0.1041	0.0040	0.1221	0.0073	0.1270	0.0250	0.1068	0.0064	0.692	0.5821
p-cymene	0.0900	0.0173	0.0785	0.0211	0.0804	0.0261	0.0795	0.0174	0.065	0.9767
terpinolene	0.0946	0.0112	0.0756	0.0123	0.0799	0.0175	0.0735	0.0130	0.482	0.7038
linalool	0.0033	0.0033	0.0244	0.0196	0.0149	0.0103	0.0196	0.0096	0.549	0.6629
bornyl acetate	0.0185	0.0053	0.0075	0.0075	0.0105	0.0053	0.0033	0.0033	1.342	0.3277
terpinen-4-ol	0.0294	0.0044	0.0271	0.0091	0.0292	0.0063	0.0223	0.0042	0.271	0.8446
unknown	0.1013	0.0113	0.1143	0.0042	0.0862	0.0078	0.1527	0.0182	6.044	0.0188
alpha terpineol	0.0082	0.0082	0.0047	0.0047	0.0088	0.0088	0.0100	0.0100	0.077	0.9705
unknown	0.0852	0.0038	0.0658	0.110	0.0633	0.0058	0.0530	0.0089	2.890	0.1023
unknown	0.0670	0.0052	0.0901	0.0125	0.0681	0.0072	0.0632	0.0062	2.168	0.1697
geranyl acetate	0.1596b	0.0286	0.0999ab	0.0076	0.1010ab	0.0016	0.0877a	0.0067	4.503	0.0394

Arc Sine of Percentage Data (8/20).

	HWLN		HWHN		LWLN		LWHN		Tukey	
	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	F ratio	F Prob.
unknown	0.0389	0.0030	0.0386	0.0019	0.0402	0.0028	0.0404	0.0043	0.080	0.9693
alpha pinene	0.2468	0.0105	0.2948	0.0178	0.2722	0.0054	0.2860	0.0145	2.634	0.1216
camphene	0.0726	0.0043	0.0792	0.0076	0.0801	0.0164	0.0742	0.0084	0.129	0.9399
unknown	0.0239	0.0191	0.0213	0.0165	0.0193	0.0146	0.0300	0.0252	0.058	0.9803
beta pinene	0.4171	0.0536	0.5321	0.0119	0.5359	0.0154	0.5527	0.0346	3.484	0.0702
unknown	0.1483	0.0022	0.1488	0.0041	0.1420	0.0094	0.1556	0.0094	1.203	0.3691
unknown	0.1129	0.0076	0.1260	0.0010	0.1106	0.0047	0.1256	0.0276	0.317	0.8130
3 carene	0.0985a	0.0115	0.1079a	0.0059	0.1411b	0.0056	0.0996a	0.0023	7.776	0.0093
myrcene	0.1549	0.0086	0.1454	0.0047	0.1395	0.0092	0.1422	0.0069	0.785	0.5351
alpha phellandrene	0.1251	0.0102	0.1194	0.0152	0.1426	0.0152	0.1189	0.0031	1.388	0.3149
unknown	0.0383	0.0092	0.0352	0.0037	0.0634	0.0195	0.0377	0.0024	1.442	0.3012
unknown	0.0262	0.0062	0.0187	0.0031	0.0204	0.0030	0.0182	0.0041	0.733	0.5661
limonene	0.1536	0.0050	0.1378	0.0030	0.1067	0.0138	0.1535	0.0114	1.054	0.4205
beta phellandrene	0.8788b	0.0159	0.7825a	0.0050	0.7490a	0.0057	0.7630a	0.0303	11.176	0.0031
unknown	0.1818b	0.0168	0.1226a	0.0054	0.1396ab	0.0041	0.1296a	0.0116	6.112	0.0181
unknown	0.0954	0.0205	0.0589	0.0057	0.0911	0.0200	0.0741	0.0177	0.959	0.4574
p-cymene	0.0481	0.0167	0.0241	0.0052	0.0721	0.0288	0.0311	0.0092	1.497	0.2877
terpinolene	0.0742ab	0.0170	0.0534ab	0.0034	0.0974b	0.0086	0.0510a	0.0043	4.753	0.0346
linalool	0.0167	0.0120	0.0309	0.0076	0.0229	0.0229	0.0199	0.0109	0.175	0.9106
bornyl acetate	0.0229	0.0046	0.0345	0.0102	0.0153	0.0153	0.0067	0.0033	1.511	0.2842
terpinen-4-ol	0.0342	0.0038	0.0329	0.0113	0.0267	0.0097	0.0334	0.0104	0.139	0.9336
unknown	0.0942	0.0097	0.0842	0.0147	0.0860	0.0113	0.0895	0.0086	0.151	0.9261
alpha terpineol	0.0088	0.0088	0.0088	0.0088	0.0115	0.0071	0.0067	0.0067	0.062	0.9782
unknown	0.0507	0.0037	0.0633	0.0158	0.0579	0.0076	0.0443	0.0020	0.856	0.5017
unknown	0.0489	0.0047	0.0327	0.0039	0.0381	0.0030	0.0387	0.0007	3.902	0.0549
geranyl acetate	0.0963b	0.0078	0.0470a	0.0147	0.0887b	0.0049	0.0617ab	0.0056	6.403	0.0161

Arc Sine of Percentage Data (9/16)

	HWLN		HWHN		LWLN		LWHN		Tukey	
	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	F ratio	F Prob.
unknown	0.0404	0.0008	0.0396	0.0008	0.0374	0.0008	0.0420	0.0014	3.658	0.0632
alpha pinene	0.2553	0.0102	0.2777	0.0057	0.2719	0.0047	0.2723	0.0086	2.303	0.1537
camphene	0.0789	0.0026	0.0932	0.0057	0.0753	0.0010	0.0833	0.0085	2.132	0.1743
unknown	0.0067	0.0033	0.0033	0.0033	0.0000	0.0000	0.0153	0.0153	0.674	0.5917
beta pinene	0.3603a	0.0179	0.4482b	0.0067	0.5020c	0.0093	0.4298b	0.0025	29.720	0.0001
unknown	0.1515	0.0072	0.1435	0.0010	0.1673	0.0075	0.1483	0.0071	2.642	0.1209
unknown	0.0391a	0.0217	0.0661ab	0.0005	0.1068b	0.0015	0.0688ab	0.0269	4.351	0.0428
3 carene	0.0938a	0.0010	0.1400b	0.0091	0.0856a	0.0052	0.0856a	0.0091	14.061	0.0015
myrcene	0.1521	0.0008	0.1495	0.0021	0.1440	0.0019	0.1496	0.0054	1.251	0.3540
alpha phellandrene	0.1290	0.0036	0.1252	0.0016	0.1207	0.0042	0.1330	0.0064	1.497	0.2877
unknown	0.0328	0.0037	0.0420	0.0059	0.0301	0.0038	0.0458	0.0071	1.947	0.2007
unknown	0.0237	0.0014	0.0202	0.0036	0.0223	0.0013	0.0230	0.0015	0.501	0.6920
limonene	0.1421	0.0025	0.1414	0.0065	0.1306	0.0017	0.1381	0.0033	1.761	0.2322
beta phellandrene	0.9098c	0.0140	0.8290ab	0.0160	0.8098a	0.0021	0.8747bc	0.0104	14.439	0.0014
unknown	0.1767	0.0146	0.1591	0.0098	0.1374	0.0172	0.1669	0.0159	1.307	0.3376
unknown	0.0986	0.0106	0.0946	0.0105	0.0772	0.0105	0.0932	0.0058	0.939	0.4657
p-cymene	0.0414	0.0054	0.0545	0.0184	0.0290	0.0036	0.0448	0.0056	1.075	0.4126
terpinolene	0.0544	0.0025	0.0680	0.0078	0.0460	0.0031	0.0512	0.0048	3.564	0.0669
linalool	0.0261	0.0087	0.0185	0.0053	0.0335	0.0047	0.0331	0.0015	1.554	0.2743
bornyl acetate	0.0000a	0.0000	0.0000a	0.0000	0.0297b	0.0029	0.0298b	0.0025	79.974	0.0000
terpinen-4-ol	0.0244ab	0.0059	0.0379b	0.0040	0.0289ab	0.0006	0.0124a	0.0024	7.858	0.0091
unknown	0.0798ab	0.0073	0.0622a	0.0013	0.0932b	0.0058	0.0804ab	0.0053	5.629	0.0226
alpha terpineol	0.0000	0.0000	0.0000	0.0000	0.0033	0.0033	0.0000	0.0000	1.000	0.4411
unknown	0.0601b	0.0054	0.0569ab	0.0038	0.0497ab	0.0056	0.0378a	0.0019	5.055	0.0298
unknown	0.558b	0.0054	0.0529b	0.0041	0.0311a	0.0011	0.0430ab	0.0034	8.605	0.0069
geranyl acetate	0.1036b	0.0024	0.1055b	0.0028	0.1074b	0.0101	0.0609a	0.0005	17.156	0.0008

Arc Sine of Percentage Data (10/13)

	HWLN		HWHN		LWLN		LWHN		Tukey	
	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	F ratio	F Prob.
unknown	0.0436	0.0013	0.0436	0.0007	0.0404	0.0011	0.0390	0.0023	2.414	0.1419
alpha pinene	0.2698	0.0087	0.2606	0.0042	0.2581	0.0033	0.2579	0.0018	1.174	0.3786
camphene	0.0765	0.0070	0.0658	0.0018	0.0703	0.0055	0.0622	0.0003	1.825	0.2207
unknown	0.0215a	0.0059	0.0605	0.0025	0.0334ab	0.0104	0.0549b	0.0066	6.893	0.0131
beta pinene	0.4389	0.0215	0.4535	0.0198	0.4979	0.0304	0.5081	0.0083	2.439	0.1394
unknown	0.1637	0.0072	0.1581	0.0020	0.1651	0.0051	0.1637	0.0067	0.306	0.8204
unknown	0.0930	0.0085	0.1121	0.0100	0.1020	0.0041	0.1185	0.0006	2.649	0.1204
3 carene	0.1107a	0.0038	0.1093a	0.0148	0.1773b	0.0195	0.0821a	0.0043	10.401	0.0039
myrcene	0.1561ab	0.0023	0.1549ab	0.0024	0.1586b	0.0041	0.1427a	0.0039	4.592	0.0376
alpha phellandrene	0.1306	0.0029	0.1319	0.0035	0.1250	0.0034	0.1205	0.0031	2.639	0.1212
unknown	0.0362	0.0036	0.0368	0.0029	0.0399	0.0075	0.0350	0.0018	0.213	0.8846
unknown	0.0283ab	0.0010	0.0335b	0.0021	0.0295ab	0.0040	0.0191a	0.0009	6.689	0.0143
limonene	0.1427ab	0.0025	0.1368ab	0.0034	0.1512b	0.0076	0.1244a	0.0045	5.248	0.0271
beta phellandrene	0.8303b	0.0093	0.8414b	0.0079	0.7672a	0.0181	0.7943ab	0.0046	9.293	0.0055
unknown	0.1892	0.0209	0.1586	0.0156	0.1666	0.0022	0.1602	0.0043	1.136	0.3912
unknown	0.0969	0.0155	0.0902	0.0098	0.0866	0.0029	0.0872	0.0105	0.197	0.8954
p-cymene	0.0353	0.0078	0.0358	0.0066	0.0202	0.0036	0.0405	0.0039	2.319	0.1519
terpinolene	0.0573	0.0032	0.0563	0.0083	0.0690	0.0061	0.0559	0.0087	0.832	0.5127
linalool	0.0403	0.0022	0.0401	0.0070	0.0232	0.0122	0.0533	0.0037	2.828	0.1066
bornyl acetate	0.0100ab	0.0100	0.0000a	0.0000	0.0000a	0.0000	0.0299b	0.0045	6.628	0.0146
terpinen-4-ol	0.0416	0.0059	0.0255	0.0028	0.0407	0.0104	0.0426	0.0140	0.758	0.5484
unknown	0.0995c	0.0068	0.0341a	0.0043	0.0673b	0.0062	0.1212c	0.0095	30.007	0.0001
alpha terpineol	0.0283	0.0041	0.105	0.0053	0.0058	0.0058	0.0114	0.0059	3.424	0.0728
unknown	0.0383	0.0009	0.0406	0.0151	0.0509	0.0048	0.0512	0.0023	0.713	0.5709
unknown	0.0333	0.0036	0.0316	0.0042	0.0522	0.0047	0.0400	0.0100	2.285	0.1557
geranyl acetate	0.0833b	0.0012	0.0416a	0.0008	0.0888b	0.0022	0.0967b	0.0146	11.032	0.0032

Appendix 2. Percentage Data (6/24). Means With the Same Letter Do Not Differ at $\alpha = 0.05$.

	HWLN		HWHN		LWLN		LWHN		Tukey	
	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	F ratio	F Prob.
unknown	0.1167	0.0133	0.1000	0.0153	0.1167	0.0145	0.1133	0.0186	0.261	0.8519
alpha pinene	10.7200	1.6636	7.9233	0.5206	7.8933	0.3943	8.6767	0.7220	1.899	0.2082
camphene	0.5900	0.0404	0.6167	0.0694	0.7300	0.0902	0.9033	0.3134	0.720	0.5677
unknown	0.0000	0.0000	0.0033	0.0033	0.0067	0.0033	0.0100	0.0058	1.333	0.3300
beta pinene	11.8433a	2.6203	20.8100bc	1.7843	18.3533ab	1.4752	27.7567c	1.2598	12.518	0.0022
unknown	2.4033	0.7202	2.4500	0.6265	1.9933	0.0956	2.1200	0.1358	0.207	0.8885
unknown	0.0700	0.0436	0.1433	0.0260	0.1033	0.0203	0.1567	0.0536	1.058	0.4190
3 carene	1.8100	0.7315	0.9167	0.1691	0.8967	0.0639	0.6100	0.0850	1.884	0.2108
myrcene	1.8000	0.0854	1.5733	0.1484	1.6567	0.1876	1.3400	0.0529	2.204	0.1652
alpha phellandrene	1.7267	0.1378	1.4867	0.0733	1.7933	0.1738	1.8267	0.6069	0.233	0.8779
unknown	0.1867	0.1068	0.2000	0.0551	0.2900	0.0814	0.5333	0.3883	0.600	0.6328
unknown	0.0000	0.0000	0.0033	0.0033	0.0067	0.0067	0.0067	0.0067	0.407	0.7520
limonene	1.7667	0.2111	1.6600	0.0436	1.6867	0.1084	1.6700	0.2042	0.094	0.9612
beta phellandrene	55.3367	3.4895	54.2200	1.1819	55.5800	1.9558	48.9000	1.6633	1.942	0.2014
unknown	4.2700	1.9563	1.7533	0.0674	2.3067	0.4204	1.5533	0.2284	1.517	0.2828
unknown	0.7900	0.3775	0.8633	0.0767	0.9067	0.0784	0.6900	0.0529	0.228	0.8740
p-cymene	0.1567	0.0869	0.1500	0.0306	0.1500	0.0100	0.2067	0.0967	0.168	0.9153
terpinolene	0.3733	0.1309	0.3033	0.0426	0.3200	0.0404	0.2567	0.0498	0.402	0.7555
linalool	0.0067	0.0067	0.0167	0.0088	0.0233	0.0088	0.0033	0.0033	1.596	0.2650
bornyl acetate	0.0433	0.0088	0.0367	0.0267	0.0567	0.0285	0.0867	0.0120	1.127	0.3942
terpinen-4-ol	0.0267a	0.0033	0.0333a	0.0145	0.1633b	0.0338	0.0600ab	0.0416	5.174	0.0281
unknown	1.4667	0.3620	1.1267	0.2751	1.2033	0.1885	1.1867	0.2218	0.312	0.8162
alpha terpineol	0.0000	0.0000	0.0100	0.0058	0.0033	0.0033	0.0000	0.0000	2.000	0.1927
unknown	1.700	0.0436	0.1100	0.0400	0.5333	0.2603	0.2467	0.0567	1.887	0.2102
unknown	1.0033b	0.4088	0.0867a	0.0328	0.2133ab	0.0240	0.1033ab	0.0176	4.539	0.0387
geranyl acetate	0.3267a	0.0524	0.2800a	0.1097	1.2533b	0.2662	0.6533ab	0.0570	9.053	0.0060

Appendix 2. Percentage Data (7/22).

	HWLN		HWHN		LWLN		LWHN		Tukey	
	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	F ratio	F Prob.
unknown	0.1333	0.0145	0.1700	0.0100	0.1433	0.0033	0.1933	0.0203	4.000	0.0519
alpha pinene	10.6733	1.1380	7.3967	0.9008	6.8167	1.1133	7.8633	0.8127	2.925	0.0999
camphene	2.3033	0.8992	1.0033	0.3012	0.8267	0.1703	1.0500	0.3251	1.781	0.2284
unknown	0.0000	0.0000	0.0500	0.0500	0.0533	0.0484	0.0733	0.0684	0.409	0.7511
beta pinene	21.7600	1.2657	10.4167	0.0570	18.2100	5.1251	18.9367	2.4128	2.801	0.1085
unknown	1.8633	0.3007	1.9267	0.4640	3.0500	0.4480	2.0900	0.4598	1.706	0.2425
unknown	0.0167a	0.0167	0.7767ab	0.1091	0.6867ab	0.3034	1.0333b	0.1393	6.077	0.0185
3 carene	0.6733	0.2917	1.3033	0.2557	0.9533	0.2567	0.6100	0.0709	1.807	0.2238
myrcene	1.5067	0.1278	1.9567	0.1715	1.8167	0.1408	1.8567	0.1167	1.904	0.2074
alpha phellandrene	2.5367	0.8357	2.5300	0.3983	1.8833	0.2590	1.6867	0.1577	0.814	0.5212
unknown	0.7867	0.4855	0.0367	0.0367	0.3133	0.1172	0.2900	0.0964	1.505	0.2857
unknown	0.0100a	0.0100	0.5433b	0.2569	0.0200ab	0.0200	0.0200ab	0.0000	4.172	0.0472
limonene	4.0033	1.2068	2.4600	0.3625	2.2733	0.3123	2.5167	0.5134	1.314	0.3355
beta phellandrene	40.7233a	4.1703	59.3733b	1.7137	54.2600ab	4.4215	52.4367ab	2.5235	5.376	0.0255
unknown	1.8833	0.3954	2.6700	0.2991	3.3833	0.6300	2.1700	0.4513	2.033	0.1878
unknown	1.0833	0.0841	1.4933	0.1761	1.7267	0.5871	1.1433	0.1374	0.915	0.4758
p-cymene	0.8667	0.2826	0.7033	0.3094	0.7800	0.3729	0.6900	0.2450	0.071	0.9740
terpinolene	0.9167	0.2038	0.6000	0.1709	0.6967	0.2546	0.5733	0.1913	0.565	0.6532
linalool	0.0033	0.0033	0.1367	0.1317	0.0433	0.0384	0.0567	0.0467	0.595	0.6357
bornyl acetate	0.0400	0.0208	0.0167	0.0167	0.0167	0.0088	0.0033	0.0033	1.162	0.3824
terpinen-4-ol	0.0900	0.0265	0.0900	0.0557	0.0933	0.0393	0.0533	0.0176	0.254	0.8564
unknown	1.0467ab	0.2313	1.3033ab	0.0961	0.7533a	0.1328	2.3767b	0.5194	5.721	0.0217
alpha terpineol	0.0200	0.0200	0.0067	0.0067	0.0233	0.0233	0.0300	0.0300	0.204	0.8908
unknown	0.7267	0.0639	0.4567	0.1568	0.4067	0.0767	0.2967	0.1020	2.970	0.0970
unknown	0.4533	0.0677	0.8400	0.2287	0.4733	0.1033	0.4067	0.0821	2.148	0.1723
geranyl acetate	2.6800	0.9721	1.0067	0.1500	1.0167	0.0318	0.7767	0.1157	3.153	0.0862

Appendix 2. Percentage Data (8/20).

	HWLN		HWIN		LWLN		LWHN		Tukey	
	MEAN	S _X ⁻	MEAN	S _X ⁻	MEAN	S _X ⁻	MEAN	S _X ⁻	F ratio	F Prob.
unknown	0.1533	0.0204	0.1500	0.0153	0.1633	0.0219	0.1667	0.0367	0.096	0.9603
alpha pinene	5.9900	0.5048	8.4967	0.9878	7.2333	0.2810	7.9933	0.7900	2.460	0.1373
camphene	0.5300	0.0600	0.6367	0.1167	0.6933	0.2885	0.5633	0.1167	0.189	0.9010
unknown	0.1300	0.1250	0.1000	0.0950	0.0800	0.0751	0.2167	0.2117	0.193	0.8979
beta pinene	16.7933	4.0104	25.7567	1.0465	26.0967	1.3485	27.6767	3.0287	3.451	0.0716
unknown	2.1833	0.0636	2.0867	0.1189	2.0200	0.2665	2.4033	0.0689	1.192	0.3725
unknown	1.2800	0.1710	1.5800	0.0252	1.2233	0.1048	1.7167	0.6410	0.497	0.6946
3 carene	0.9933a	0.2368	1.1667a	0.1299	1.9833b	0.1539	0.9900a	0.0451	9.105	0.0059
myrcene	2.3933	0.2702	2.1033	0.1353	1.9500	0.2597	2.0167	0.1934	0.778	0.5384
alpha phellandrene	1.5767	0.2577	1.4200	0.0624	2.0633	0.4332	1.4100	0.0723	1.431	0.3039
unknown	0.1633	0.0784	0.1267	0.0273	0.4767	0.2520	0.1433	0.0176	1.574	0.2700
unknown	0.0767	0.0367	0.0367	0.0120	0.0433	0.0133	0.0367	0.0167	0.754	0.5502
limonene	2.3467	0.1506	1.8900	0.0819	2.5967	0.4368	2.3633	0.3355	1.051	0.4216
beta phellandrene	59.2767	1.5575	49.7100	0.5000	46.3600	0.5667	47.7633	3.0274	11.180	0.0031
unknown	3.3200b	0.6213	1.5000a	0.1309	1.9400ab	0.1150	1.6933a	0.3002	5.367	0.0256
unknown	0.9900	0.3727	0.3533	0.0649	0.9067	0.3233	0.6100	0.2650	1.066	0.4160
p-cymene	0.2867	0.1532	0.0633	0.0233	0.6833	0.4011	0.1133	0.0549	1.684	0.2469
terpinolene	0.6067	0.2717	0.2867	0.0353	0.9600	0.1701	0.2633	0.0426	4.047	0.0506
linalool	0.0567	0.0517	0.1067	0.0463	0.1567	0.1567	0.0633	0.0410	0.275	0.8418
bornyl acetate	0.0567	0.0203	0.1400	0.0600	0.0700	0.0700	0.0067	0.0033	1.357	0.3235
terpinen-4-ol	0.1200	0.0265	0.1333	0.0841	0.0900	0.0603	0.1333	0.0754	0.098	0.9591
unknown	0.9033	0.1822	0.7500	0.2663	0.7633	0.2046	0.8133	0.1530	0.114	0.9495
alpha terpineol	0.0233	0.0233	0.0233	0.0233	0.0233	0.0186	0.0133	0.0133	0.062	0.9784
unknown	0.2600	0.0361	0.4500	0.2150	0.3467	0.0845	0.1967	0.0176	0.879	0.4916
unknown	0.2433	0.0441	0.1100	0.0265	0.1467	0.0233	0.1500	0.0058	4.013	0.0515
geranyl acetate	0.9367b	0.1434	0.2633a	0.1586	0.7900ab	0.0866	0.3867a	0.0717	7.040	0.0124

Appendix 2. Percentage Data (9/16).

	HWLN		HWLN		LWLN		LWLN		Tukey	
	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	F ratio	F Prob.
unknown	0.1633	0.0067	0.1567	0.0067	0.1400	0.0058	0.1767	0.0120	3.486	0.0701
alpha pinene	6.3833	0.2885	7.5200	0.3001	7.2167	0.2431	7.2467	0.4492	2.226	0.1626
camphene	0.6233	0.0406	0.8733	0.1084	0.5667	0.0145	0.7067	0.1417	2.119	0.1761
unknown	0.0067	0.0033	0.0033	0.0033	0.0000	0.0000	0.700	0.0700	0.909	0.4785
beta pinene	12.4767a	1.1853	18.7833b	0.5275	23.1600c	0.7923	17.3667b	0.1866	33.047	0.0001
unknown	2.2867	0.2194	2.0467	0.0273	2.7833	0.2489	2.1933	0.2134	2.612	0.1235
unknown	0.2467a	0.1651	0.4367a	0.0067	1.1367b	0.0186	0.5200ab	0.2326	7.260	0.0114
3 carene	0.8767a	0.0186	1.9633b	0.4452	0.7367a	0.0865	0.7467a	0.1565	14.239	0.0014
myrcene	2.2967	0.0240	2.2200	0.0624	2.0600	0.0529	2.2267	0.1590	1.231	0.3603
alpha phellandrene	1.6567	0.0921	1.5600	0.0400	1.4533	0.0982	1.7667	0.1723	1.451	0.2988
unknown	0.1100	0.0252	0.1833	0.0533	0.0933	0.0240	0.2200	0.0702	1.604	0.2635
unknown	0.0567	0.0067	0.0433	0.0145	0.0500	0.0058	0.0533	0.0067	0.389	0.7643
limonene	2.0067	0.0706	1.9933	0.1856	1.6967	0.0448	1.8967	0.0899	1.654	0.2528
beta phellandrene	62.3067c	1.3579	54.3467ab	1.5922	52.4367a	0.2067	58.8800bc	1.0221	14.527	0.0013
unknown	3.1300	0.5072	2.5267	0.3147	1.9333	0.4486	2.8067	0.5362	1.220	0.3635
unknown	0.9900	0.2166	0.9167	0.2186	0.6167	0.1517	0.8733	0.1102	0.812	0.5223
p-cymene	0.1767	0.0470	0.3633	0.2333	0.0867	0.0203	0.2067	0.0491	0.893	0.4854
terpinolene	0.2967	0.0273	0.4733	0.1102	0.2133	0.0273	0.2667	0.0498	3.149	0.0864
linalool	0.0833	0.0433	0.0400	0.0208	0.1167	0.0296	0.1100	0.0100	1.472	0.2937
bornyl acetate	0.0000a	0.0000	0.0000a	0.0000	0.0900b	0.0173	0.0900b	0.0153	20.250	0.0004
terpinen-4-ol	0.0667ab	0.0291	0.1467b	0.0285	0.0833ab	0.0033	0.0167a	0.0067	6.727	0.0140
unknown	0.6467ab	0.1114	0.3867a	0.0167	0.8733b	0.1068	0.6500ab	0.0819	5.141	0.0285
alpha terpineol	0.0000	0.0000	0.0000	0.0000	0.0033	0.0033	0.0000	0.0000	1.000	0.4411
unknown	0.3667b	0.0669	0.3267ab	0.0441	0.2533ab	0.0524	0.1433a	0.0145	4.102	0.0490
unknown	0.3167b	0.0623	0.2833b	0.0448	0.0967a	0.0067	0.1867ab	0.0291	5.838	0.0206
geranyl acetate	1.0700b	0.0493	1.1100b	0.0586	1.1700b	0.2170	0.3700a	0.0058	10.647	0.0036

Appendix 2. Percentage Data (10/13).

	HWLN		HWHN		LWLN		LWHN		Tukey	
	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	MEAN	$S_{\bar{X}}$	F ratio	F Prob.
unknown	0.1900	0.0115	0.1900	0.0058	0.1633	0.0088	0.1533	0.0186	2.384	0.1450
alpha pinene	7.1167	0.4384	6.6400	0.2098	6.5167	0.1623	6.5067	0.0874	1.224	0.3624
camphene	0.5933	0.1020	0.4333	0.0233	0.5000	0.0802	0.3867	0.0033	1.848	0.2166
unknown	0.0533a	0.0285	0.3667b	0.0296	0.1333ab	0.0664	0.3100b	0.0700	7.858	0.0091
beta pinene	18.1200	1.6404	19.2433	1.5789	22.9033	2.5884	23.6767	0.7024	2.388	0.1466
unknown	2.6667	0.2347	2.4800	0.0624	2.7067	0.1676	2.6633	0.2185	0.305	0.8213
unknown	0.8767	0.1637	1.2700	0.2268	1.0400	0.0808	1.3967	0.0145	2.541	0.1297
3 carene	1.2233a	0.0819	1.2333a	0.3051	3.1833b	0.6654	0.6767a	0.0717	8.848	0.0064
myrcene	2.4167ab	0.0696	2.3800ab	0.0723	2.4967b	0.1281	2.0267a	0.1099	4.487	0.0398
alpha phellandrene	1.6967	0.0769	1.7333	0.0935	1.5567	0.0837	1.4467	0.0731	2.578	0.1264
unknown	0.1333	0.0260	0.1367	0.0219	0.1700	0.0651	0.1233	0.0120	0.297	0.8271
unknown	0.800ab	0.0058	0.1133b	0.0145	0.0900ab	0.0231	0.0367a	0.0033	5.221	0.0274
limonene	2.0233ab	0.0698	1.8633ab	0.0926	2.2800b	0.2303	1.5433a	0.1117	4.815	0.0336
beta phellandrene	54.4833b	0.9254	55.5900b	0.7823	48.1833a	1.8073	50.8933ab	0.4572	9.309	0.0055
unknown	3.6167	0.7907	2.5400	0.5041	2.7500	0.0721	2.5467	0.1335	1.160	0.3830
unknown	0.9833	0.2981	0.8300	0.1833	0.7500	0.0503	0.7800	0.1724	0.278	0.8398
p-cymene	0.1367	0.0504	0.1367	0.0517	0.0433	0.0145	0.1667	0.0328	1.763	0.2318
terpinolene	0.3300	0.0351	0.3300	0.0874	0.4833	0.0819	0.3267	0.1009	0.927	0.4710
linalool	0.1633	0.0176	0.1700	0.0603	0.0833	0.0491	0.2867	0.0384	3.580	0.0662
bornyl acetate	0.0300b	0.0300	0.0000a	0.0000	0.0000a	0.0000	0.0933b	0.0285	4.526	0.0390
terpinen-4-ol	0.1800	0.0458	0.0667	0.0133	0.1867	0.0928	0.2200	0.1358	0.608	0.6281
unknown	0.9967bc	0.1386	0.1200a	0.0306	0.4600ab	0.0839	1.4800c	0.2254	18.363	0.0006
alpha terpineol	0.0833b	0.0240	0.0167ab	0.0088	0.100a	0.0100	0.0200ab	0.0115	5.246	0.0271
unknown	0.1467	0.0067	0.2100	0.1450	0.2633	0.0504	0.2633	0.0233	0.509	0.6869
unknown	0.1133	0.0233	0.1033	0.0285	0.2767	0.0498	0.1800	0.0900	2.137	0.1738
geranyl acetate	0.6933ab	0.0203	0.1733a	0.0067	0.7867ab	0.0384	0.9733b	0.2885	5.512	0.0239