

FINAL REPORT FOR THE NORTHERN LAND USE INSTITUTE
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Project Title: The effect of organic fertilizer on the growth of fibres in industrial hemp
(*Cannabis sativa* L.) in Northern British Columbia

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The results from this research will be used towards the completion of a M.Sc. degree at UNBC by Charlene Forest (Supervisor: Dr. Jane Young). Part of the budget paid the salary of a research assistant for Ms. Forrest, who helped set up experimental plots and collect data in both field and greenhouse trials. Some of the data has been analyzed and will be included in this report.

OBJECTIVES OF STUDY:

- 1) To determine the fertilizer (of four types) that will produce the highest yield of fibre in hemp,
- 2) To present results to the Gitsegukla First Nation regarding the growth of fibre hemp crops using organic fertilizers, and
- 3) To publish results in a Masters of Science thesis and in appropriate scientific journals.

MATERIALS AND METHODS:

1) Field Trial

Kompolti Field Trial - June 7-22, 2000

Cannabis sativa var. *kompolti* was planted by hand at a density of approximately 120 stems m⁻² at the first Gitsegukla field site (this site was to be thinned to the density of 90 stems m⁻²). As of June 22, 2000, there was only 17% germination. This low germination rate may have been due to low seed viability and/or the effects of cool soil and air temperatures. A second Gitsegukla field was tilled in preparation for planting a second crop, but this time the fibre hemp variety, *fedrina*, was used.

Fedrina Field Trial - July 6 – September 11, 2000

Cannabis sativa var. *fedrina* was planted with a tractor disc seed drill and germination was highly successful. The 1 m² plots and surrounding 0.5 m buffers of all four blocks in this heavily over-planted field (approximately 400-500 stems m⁻²) were weeded and thinned to 90 stems m⁻² on July 20-22. Twenty treatments were replicated four times, therefore, there were four

blocks of 20 – 1 m² plots (80 plots in total). The treatments were: control (no addition of fertilizer); control + P; fishmeal @ 75, 150, or 300 kg ha⁻¹ N; fishmeal 75, 150, or 300 kg ha⁻¹ N (all + P); bloodmeal @ 75, 150, or 300 kg ha⁻¹ N; bloodmeal @ 75, 150, or 300 kg ha⁻¹ N (all + P); inorganic N @ 75, 150, or 300 kg ha⁻¹ N; or inorganic N @ 75, 150, or 300 kg ha⁻¹ N (all + P).

Soil analysis of this field showed only trace levels of nitrogen, 0.12 kg ha⁻¹ phosphorus and 263 kg ha⁻¹ potassium. One third of each of the three nitrogen fertilizer types was added one week after 90% germination (July 23) and the remaining two thirds, one month after germination (August 12). Phosphorus levels were brought to 90 kg ha⁻¹ P two weeks after germination (July 30). Potassium levels were sufficient (120 kg ha⁻¹ K for fibre hemp), therefore, no additional fertilizer was added.

Height measurements of 40 sample plants/treatment were taken every two weeks (July 20 – September 11). Phyllotaxic change and flowering became apparent approximately 46 days after planting, which indicated the optimum time to harvest for fibre analysis. The field plots were harvested by hand on September 9-11 (65 days after planting). At harvest, the following measurements were made of the sample plants: plant height, number of internodes, length of the third internode (from the bottom), sex and phyllotaxy. The third internode of each of the plants was taken back to the laboratory for further measurements (see **Laboratory Measurements**).

2) Greenhouse Trial - July 4, 2000

Cannabis sativa var. fedrina was planted by hand and germination was highly successful. The 0.0625 m² plots and surrounding 0.125 m buffers of all four blocks in this over-planted (approximately 144 stems m²) greenhouse were thinned to 90 stems m² on July 12. Twenty treatments were replicated four times, therefore, there were four blocks of 20 - 0.0625 m² plots. The treatments were as above for the field trial except that bloodmeal was used instead of sea star meal.

Soil analysis showed only trace levels of nitrogen and phosphorus and 126 kg ha⁻¹ potassium. One third of each the three nitrogen fertilizer treatments was added one week after approximately 90% germination (July 19) and the remaining two thirds, one month after germination (August 9). Phosphorus levels were brought to 90 kg ha⁻¹ two weeks after germination (July 28). Potassium levels were sufficient (120 kg ha⁻¹) for fibre hemp, therefore, no additional fertilizer was added to the soil.

Plant height, number of internodes and length of the third internode (from the bottom) were measured for each of the six sample plants. Phyllotaxic change and flowering became apparent at approximately 44 days after planting. The greenhouse plants were harvested by hand on September 2-5 (60 days after planting). At harvest, phyllotaxy, sex and root dry weights of each sample plant were recorded. The third internode of each of the plants was taken back to the laboratory for further measurements (see **Laboratory Measurements**).

3) Laboratory Measurements

Cross-sections (and scanning) of all sample plant third internodes were completed by April 28, 2001. Internode cross sectioning began on September 28, 2000, and involved the help of six volunteers in the fall semester and nine volunteers in the winter semester. There was only one field and three greenhouse stems that were not possible to scan (in order to save an image for tissue measurements). All other cross-sections were scanned onto zip disks using Northern Exposure software.

The secondary xylem depth of the third internode of each sample plant was measured with an ocular micrometer at the time of cross sectioning and Toluidene Blue (TBO) staining. A randomly chosen subset of plants from both greenhouse (2 plants/plot; 160 in total) and field (3 plants /plot; 240 in total) trials were used for preliminary tissue measurements. Primary and secondary phloem depths, number of primary phloem cells per area and primary phloem cell wall widths were measured on the scanned images with Corel 10 software. The number of cells per area was counted in a 200 μm x tissue depth area of the primary phloem. Three cell wall widths were measured per sample. All initial measurements were completed by May 6, 2001. There are 7 plants/field plot (560 total) and 4 plants/greenhouse plot (320 total) remaining to be measured and analyzed.

Dry Weights

All sectioned internodes were oven dried and weighed by May 9, 2001. Fresh weight/dry weight ratios will be compared across the treatments.

Summary of Measurements:

- Height
- Number of internodes
- Third internode length
- Third internode diameter
- Third internode fresh and dry weights
- Stem fresh and dry weights
- Greenhouse root weights
- Sex
- Phyllotaxy
- Xylem depth
- Primary and secondary phloem tissue depths
- Number of primary phloem cells/area
- Primary phloem cell wall thickness
- *****Cortex depth*** -will be added to remaining measurements required for remaining stems**

- Temperature for greenhouse and field

4) Data Analysis

Tissue depths were converted to tissue area composition per internode area and were statistically analyzed. The means of each measurement were compared among the treatments using the statistical software package, 'SAS'. A one-way analysis of variance (one-way ANOVA) and the Student-Newman Keuls Multiple Range Test were used to determine any significant effects of treatments on means. Future analysis will be conducted using SPSS software.

RESULTS:

In this initial analysis, five categories: fertilizer nitrogen concentration, addition of phosphorus fertilizer, fertilizer nitrogen level combined with addition of phosphorus fertilizer, block layout and nitrogen fertilizer type, were assessed for effect at a 0.05 alpha level on five characteristics: xylem tissue depth, primary phloem tissue depth, secondary phloem tissue depth, average primary phloem cell wall width and plant harvest height of hemp growth (Table 1). Shaded cells in the ANOVA summary table represent significant effect.

In both the field and greenhouse trials, neither nitrogen concentration level combined with the addition of phosphorus, nor nitrogen fertilizer type produced significant results for any hemp growth category. In the field trial the most consistent effect was produced through the blocking layout, followed by nitrogen fertilizer concentration and finally, the addition of phosphorus to plots. In the greenhouse trial the most consistent effect was produced through the addition of phosphorus to plots, followed by block layout and finally, nitrogen fertilizer concentration level.

In this initial analysis, the four levels of nitrogen fertilizer concentration, i.e., 0, 75, 150 and 300 kg ha⁻¹ of nitrogen fertilizer, were assessed for effect at a 0.05 alpha level on five categories: xylem tissue depth, primary phloem tissue depth, secondary phloem tissue depth, average primary phloem cell wall width and plant harvest height of hemp growth. In the Student-Newman Keuls summary table, significant differences between means are represented by letter (A, B or C) designation (Table 2).

In the field trial, 0 kg ha⁻¹ N was most often significantly different from the other concentrations followed by 75 and 300 kg ha⁻¹ N, while 150 kg ha⁻¹ N maintained the most consistency with other concentration levels. Xylem tissue depth and plant harvest heights, followed by primary phloem tissue depths, were the growth categories most often exhibiting differences across all fertilizer types.

In the greenhouse trial 75 kg ha⁻¹ N was different twice as compared to 0, 150 and 300 kg ha⁻¹ N which were only different once across all comparisons. Only inorganic primary phloem cell

wall width and sea star xylem depths offered differences among concentration.

Table 1. The effect of fertilizer on hemp growth.

NOVA	A					
		concentration	phosphorus	concentration X phosphorus	block	fertilizer type
Greenhouse						
Inorganic	xylem depth					
	primary phloem depth					
	secondary phloem depth					
	primary phloem cell wall width					
	harvest height					
Greenhouse						
Fish Meal	xylem depth					
	primary phloem depth					
	secondary phloem depth					
	primary phloem cell wall width					
	harvest height					
Greenhouse						
Sea Star	xylem depth					
	primary phloem depth					
	secondary phloem depth					
	primary phloem cell wall width					
	harvest height					
Greenhouse						
Control	xylem depth					
	primary phloem depth					
	secondary phloem depth					
	primary phloem cell wall width					
	harvest height					
Greenhouse						
All Fertilizers	xylem depth					
	primary phloem depth					
	secondary phloem depth					
	primary phloem cell wall width					
	harvest height					
Field						
Inorganic	xylem depth					
	primary phloem depth					
	secondary phloem depth					
	primary phloem cell wall width					
	harvest height					
Field						
Fish Meal	xylem depth					
	primary phloem depth					
	secondary phloem depth					
	primary phloem cell wall width					
	harvest height					
Field						
Blood Meal	xylem depth					
	primary phloem depth					

	secondary phloem depth				
	primary phloem cell wall width				
	harvest height				
Field					
Control	xylem depth				
	primary phloem depth				
	secondary phloem depth				
	primary phloem cell wall width				
	harvest height				
Field					
All Fertilizers	xylem depth				
	primary phloem depth				
	secondary phloem depth				
	primary phloem cell wall width				
	harvest height				

Table 2. Significant effects of fertilizer on hemp growth.

Student Newman Keuls		Concentration Level			
		0 kg N/ha	75 kg N/ha	150 kg N/ha	300 kg N/ha
Greenhouse					
Inorganic	xylem depth	A	A	A	A
	primary phloem depth	A	A	A	A
	Secondary phloem depth	A	A	A	A
	primary phloem cell wall width	A	AB	A	B
	harvest height	A	A	A	A
Greenhouse					
Fish Meal	xylem depth	A	A	A	A
	primary phloem depth	A	A	A	A
	Secondary phloem depth	A	A	A	A
	primary phloem cell wall width	A	A	A	A
	harvest height	A	A	A	A
Greenhouse					
Sea Star	xylem depth	B	AB	AB	A
	primary phloem depth	A	A	A	A
	secondary phloem depth	A	A	A	A
	primary phloem cell wall width	A	A	A	A
	harvest height	A	A	A	A
Greenhouse					
All Fertilizers	xylem depth	A	A	A	A
	primary phloem depth	A	A	A	A
	secondary phloem depth	A	A	A	A
	primary phloem cell wall width	A	A	A	A
	harvest height	A	A	A	A
Field					
Inorganic	xylem depth	B	A	AB	A
	primary phloem depth	A	A	A	A
	secondary phloem depth	B	A	AB	AB
	primary phloem cell wall width	A	A	A	A
	harvest height	B	A	A	A

Field					
Fish Meal	xylem depth	B	A	A	A
	primary phloem depth	A	A	A	A
	secondary phloem depth	B	AB	A	AB
	primary phloem cell wall width	A	A	A	A
	harvest height	B	A	A	A
Field					
Blood Meal	xylem depth	B	AB	A	A
	primary phloem depth	A	A	A	A
	secondary phloem depth	A	A	A	A
	primary phloem cell wall width	A	A	A	A
	harvest height	B	B	A	B
Field					
All Fertilizers	xylem depth	B	A	A	A
	primary phloem depth	A	A	A	A
	secondary phloem depth	A	A	A	A
	primary phloem cell wall width	A	A	A	A
	harvest height	C	B	A	B

PRESENT AND FUTURE WORK:

Upon completion of all measurements, the data will be subjected to a final analysis of variance (ANOVA) to determine conclusively if there are any significant differences in the measured characters across the treatments. Of the three organic fertilizers, and the inorganic fertilizer, it will be determined which one, if any, produces a higher yield of fibre in hemp under the experimental conditions used (Study Objective 1). The results will be presented to the Gitsegukla First Nation Band once the analysis on the data is complete (Study Objective 2), however, Mr. Dave Ryan, the Project coordinator for the Band, has been updated on the progress regularly over the study period. The results will be published in a Masters of Science thesis (Charlene Forrest's), and will be submitted to appropriate scientific journals (Study Objective 3).

PLEASE NOTE: Much of the substance of this report was taken from a Progress Report written by Charlene Forrest, M.Sc. candidate, UNBC.

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