

AN ABSTRACT OF THE THESIS OF

Thomas Arthur Ayres for the degree of Master of Science

in AGRONOMIC CROP SCIENCE presented on March 20, 1975

Title: YIELD, QUALITY CHARACTERISTICS, AND COMPOSITION OF LIMNANTHES

ALBA SEED AS AFFECTED BY RATE, SOURCE, AND DATE OF NITROGEN

APPLICATION

Redacted for privacy

Abstract Approved: _____

Dr. David O. Chilcote

The effect of nitrogen (N) application on seed yield of Limnanthes alba was studied for two years at two locations. Seeding following alfalfa (Medicago sativa L.) was compared to establishment after wheat (Triticum aestivum L.). Treatments used were a control (no N applied), and two rates of N (50 and 100 kg/ha) applied at two dates (March and May). Comparisons were also made of ammonium sulfate (AS) and ammonium nitrate (AN) forms of N applied in March the second year. Seed samples were taken for analysis of protein, oil content, and fatty acid composition of oil. From the second year studies, seed weight, germination, and seedling growth determinations were made. Relationships of relevant parameters were also studied.

Most all applications of N resulted in greater protein content, with 100 kg N as AS applied in March producing the greatest increase. Oil content was reduced by almost every rate of N at either date and in either form.

Seed yields were affected by N application in the first year of

study, but not in the second. March application of N increased seed yields whereas the May application decreased yields when L. alba followed wheat. When following alfalfa, seed yields were decreased with the high rates of N at both dates of application.

Protein yield was increased by 100 kg N in March when L. alba followed wheat. After alfalfa, protein yields were decreased when high rates of N were applied. Oil yields were reduced in all three studies from N application.

The fatty acid composition of seed oil was influenced by N application. C20 monoene content was reduced from N treatments, while C22 monoene content increased the first year but not in the second. The C22 diene content was decreased by N application in the first year, but increased in the second. Percent of oil other than C20 and C22 fatty acids was affected by N application, but no obvious trends were observed in these results.

Seed weight was not influenced by either N form or rate of application, but a trend towards a reduction was recorded. Seed weight was positively correlated to oil content and negatively correlated to protein content.

Germination percent was increased from plots receiving the 100 kg N as AS treatment. Protein was positively and oil negatively correlated to germination percent.

Seedling root and shoot growth from the seed from treated plots was increased by both rates of AN. Applications of AS either had no effect, or a negative effect (100 kg N as AS) on seedling growth. No relationships of seedling growth to oil or protein content were recorded.

YIELD, QUALITY CHARACTERISTICS, AND COMPOSITION
OF LIMNANTHES ALBA SEED AS AFFECTED
BY RATE, SOURCE, AND DATE OF
NITROGEN APPLICATION

by

Thomas Arthur Ayres

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed March 20, 1975

Commencement June 1976

APPROVED:

Redacted for privacy

Professor of Agronomic Crop Science
in Charge of Major.

Redacted for privacy

Head of Department of Agronomic Crop Science.

Redacted for privacy

Dean of Graduate School.

Date Thesis is Presented:

3-20-75

ACKNOWLEDGEMENTS

Many individuals have made this thesis possible. Those who deserve special recognition for their assistance are listed.

Dr. David Chilcote, my major professor, whose counseling and suggestions on experimentation techniques and analysis were invaluable.

Dr. David L. Stamp, formerly Assistant Professor of Crop Science and my major professor before becoming Associate Professor of Agronomy, Texas Tech. University, Lubbock, Texas, for suggesting the thesis topic.

Jim Crane, Research Assistant, Hyslop Agronomy Farm, who has done previous field work with Limnanthes alba, made data readily available, and provided many valuable suggestions of importance on preparation, interpretation, and completion of this thesis.

F. R. Earle, formerly Acting Chief (now retired), Industrial Crops Laboratory, Northern Regional Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois, for all the biochemical analysis performed.

Drs. Don Grabe, Frank Conklin, and Dave England, Department of Agronomic Crop Science, Agricultural Economics, and Animal Science, respectively, for assuming the responsibility and expenditure of their time as members of my graduate committee.

Fellow graduate students for their discussion of the results and assistance on statistics and computer programing.

And lastly, and most importantly, to my wife, Mary, and son, Todd, for their patience, understanding, and love.

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
I. GENERAL INTRODUCTION.....	1
Literature Cited.....	5
II. OIL, PROTEIN, AND YIELD OF <u>LIMNANTHES ALBA</u> SEED AS AFFECTED BY RATE, FORM, AND TIME OF NITROGEN APPLICATION.....	7
Abstract.....	7
Introduction.....	8
Materials and Methods.....	10
Results and Discussion.....	13
Protein Content.....	13
Oil Content.....	17
Seed Yield.....	21
Protein Yield.....	23
Oil Yield.....	26
Literature Cited.....	31
III. FATTY ACID COMPOSITION OF <u>LIMNANTHES ALBA</u> SEED OIL AS AFFECTED BY RATE, FORM, AND TIME OF NITROGEN APPLICATION.....	33
Abstract.....	33
Introduction.....	34
Materials and Methods.....	36
Results and Discussion.....	38
C20 Monoene Fatty Acids.....	38
C22 Monoene Fatty Acids.....	42
C22 Diene Fatty Acids.....	45
Fatty Acids other than C20 and C22.....	49
Literature Cited.....	53
IV. INFLUENCE OF RATE AND FORM OF NITROGEN APPLICATION ON SEED WEIGHT, GERMINATION, AND SEEDLING GROWTH OF <u>LIMNANTHES ALBA</u>	54
Abstract.....	54
Introduction.....	55
Materials and Methods.....	56
Seed Weight Determinations.....	57
Germination Studies.....	57
Seedling Growth Determinations.....	58
Results and Discussion.....	59
Seed Weight.....	59
Germination.....	59
Seedling Growth.....	63
Literature Cited.....	69
V. SUMMARY AND CONCLUSIONS.....	70
APPENDIX TABLES.....	73

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. The Effect of Rate, Date, and Form of Nitrogen Application on the Protein Content of <u>L. alba</u> Seed.	14
2. The Effect of Rate, Date, and Form of Nitrogen Application on Oil Content of <u>L. alba</u> Seed.	18
3. Simple Correlation Coefficients for Oil, Protein, and Yield of <u>L. alba</u> Seed, 1973 and 1974.	20
4. The Effect of Rate, Date, and Form of Nitrogen Application on Seed Yield (kg/ha) of <u>L. alba</u>	22
5. The Effect of Rate, Date, and Form of Nitrogen Application on Protein Yield (kg/ha) from <u>L. alba</u> Seed.	24
6. Total Protein Yield Components of <u>L. alba</u> as Influenced by Nitrogen Fertility Treatments, 1973 and 1974.	25
7. The Effect of Rate, Date, and Form of Nitrogen Application on Oil Yield (kg/ha) from <u>L. alba</u> Seed.	27
8. Total Oil Yield Components of <u>L. alba</u> as Influenced by Nitrogen Fertility Treatments, 1973 and 1974.	29
9. The Effect of Rate, Date, and Form of Nitrogen Application on the Percent of <u>L. alba</u> Seed Oil Composed of C20 Monoene Fatty Acids.	39
10. Simple Correlation Coefficients for Oil Content and Fatty Acid Components of Oil from <u>L. alba</u> , 1973 and 1974.	41
11. The Effect of Rate, Date, and Form of Nitrogen Application on the Percent of <u>L. alba</u> Seed Oil Composed of C22 Monoene Fatty Acids.	43
12. The Effect of Rate, Date, and Form of Nitrogen Application on the Percent of <u>L. alba</u> Seed Oil Composed of C22 Diene Fatty Acids.	46
13. The Effect of Rate, Date, and Form of Nitrogen Application on the Percent of <u>L. alba</u> Seed Oil Composed of other than C20 and C22 Fatty Acids.	50
14. The Effect of Rate and Form of Nitrogen Application on Seed weight, Germination, and Seedling Growth of <u>L. alba</u> , 1974.	60
15. Simple Correlation Coefficients for Some Seed Quality and Seedling Growth Characters of <u>L. alba</u> , 1974.	62

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Seed Protein Content of <u>L. alba</u> as Affected by Rate, Date, and Form of Nitrogen Application.	15
2. Seed Oil Content of <u>L. alba</u> as Affected by Rate, Date, and Form of Nitrogen Application.	19
3. C20 Monoene Content of <u>L. alba</u> Seed Oil as Affected by Rate, Date, and Form of Nitrogen Application.	40
4. C22 Monoene Fatty Acid Content of <u>L. alba</u> Seed Oil as Affected by Rate, Date, and Form of Nitrogen Application.	44
5. C22 Diene Fatty Acid Content of <u>L. alba</u> Seed Oil as Affected by Rate, Date, and Form of Nitrogen Application.	47
6. Content of Other than C20 and C22 Fatty Acids in <u>L. alba</u> Seed Oil as Affected by Rate, Date, and Form of Nitrogen Application.	51
7. The Effect of Rate and Form of Nitrogen Application on Seed Weight of <u>L. alba</u> , 1974.	61
8. The Effect of Rate and Form of Nitrogen Application on the Percent Germination of <u>L. alba</u> Seed, 1974.	61
9. The Effect of Rate and Form of Nitrogen Application on Seedling Shoot Growth of <u>L. alba</u> Seed at 14 and 16 Days.	64
10. The Effect of Rate and Form of Nitrogen Application on Seedling Root Growth of <u>L. alba</u> Seed at 14 and 16 Days.	65

LIST OF APPENDIX TABLES

<u>Table</u>	<u>Page</u>
1. Weather Summary for Crop Year 1972-1973, Corvallis, Oregon.	73
2. Weather Summary for Crop Year 1972-1973, Corvallis, Oregon.	74
3. Maximum, Minimum, Average, and Standard Error of the Means Values for Correlation Analysis Factors of <u>L. alba</u> Seed Characters for Two Years.	75
4. Minimum, Maximum, Average, and Standard Error of the Mean Values for Correlation Analysis Factors of <u>L. alba</u> Seed Soil and Fatty Acid Constituents of Seed Oil, 1973 and 1974.	76
5. Maximum, Minimum, Average, and Standard Error of the Mean Values for Correlation Analysis Factors of <u>L. alba</u> Seed Characters, 1974.	77

YIELD, QUALITY CHARACTERISTICS, AND COMPOSITION
OF LIMNANTHES ALBA SEED AS AFFECTED
BY RATE, SOURCE, AND DATE OF
NITROGEN APPLICATION

GENERAL INTRODUCTION

Species of the genus Limnanthes, a member of the Limnanthaceae family, are native to the West coast of North America, appearing as spring annuals from Central California to Southern Oregon. These plants have long been associated as annual weeds in grain fields of Central and Northern California. Besides being a native annual, plants of this genus have been grown as ornamentals in America and Northern Europe (11).

During the late 1950's and early 1960's, huge surpluses of feed and food grains prompted a search for new industrial oil seed crops which could be grown in cereal producing areas of the United States (8). The Northern Regional Research Laboratory at Peoria, Illinois was one agency of the United States Department of Agriculture involved in this task.

At this laboratory, in an initial screening program of 400 seed oils representing 24 plant families containing glyceride fatty acids, Earle and Associates (4) found that Limnanthes douglasii had the highest percent (97) of total fatty acids C20 or greater in length. It was found with further investigations of the seed oil that three of the four major fatty acids C20 or greater in length had never been recorded as occurring in any natural sources (2,15).

Due to the unique character of the seed oil and the three new fatty acids reported, a botanical survey of the genus in its native habitat and a collection of seed from wild plants was initiated by the Crops Research Division, ARS, USDA, and later reported by Gentry and Miller (6).

From the wild plant seed collections of the genus, Miller et al. (12) analyzed the seed and oil of most of the known species of Limnanthes. It was found that all of the species analyzed contained the same unique fatty acid constituents of seed oil earlier reported for L. douglasii (2,4,15), but in varying proportions. It was then concluded (6,12) that due to genetic variation and adaptability observations, plant breeding could rapidly develop improved selections for agronomic production.

L. alba Benth. was one such species analyzed in these two studies (6,12). Due to uprightness, low water requirements, seed retention ability, pre-adaptation to cultivated lands (a weed in small grain and fallow fields in its native habitat), and slightly higher oil content, it was one of the species chosen for plant selection and agronomic studies (6).

Cultural studies and plant selection efforts, which have been carried out in California, Oregon, and elsewhere since those reported by Higgins et al. (7), have improved the prospect of Limnanthes as an oil seed crop through increased yields, seed retention ability, and management information. The program to develop a new industrial oil seed crop was initiated as an expected partial substitute for the surplus cereal grain production situation of the last decade. Although surplus cereal grain production no longer concerns the United States, the efforts appear not to have been in vain, as this plant may prove to serve another purpose.

Studies of Limnanthes seed oils have revealed that they are similar to jojoba (Simmondsia chinensis) seed oil when prepared as wax esters (9, 12). Both jojoba and Limnanthes are currently being evaluated as potent-

ial substitutes for sperm whale oil (3,17). Utilization studies have also revealed other uses for Limnanthes seed oil (5,13).

Sperm whale oil is used for extreme pressure lubricant additives for automobile transmission fluid, differential fluid and lubricants for automobiles, trucks, tractors, heavy industrial equipment, and aircraft (1). Supplies are being depleted since the United States Congress passed the Endangered Species Conservation Act of 1969 and agreed on an international embargo prohibiting the importation of oil, meat, or any product of whales in an effort to conserve the rapidly diminishing numbers of these mammals (3).

Since the passage of this Act and the subsequent embargo, U.S. producers of extreme pressure lubricants have been relying solely on stockpiled sperm whale oil, and consequently the price has risen. Industry expects to find suitable substitutes for some of the specialized lubricants, possibly through chemical synthesis, but not for all of the lubricants. Limnanthes seed oils are unique, in that the location of the unsaturation on the fatty acid molecule give the oil a viscosity and stability under heat and pressure which appears desirable for substituting these remaining industrial lubricants (D. L. Stamp, Personal Communication).

What appears pertinent at the present time is what factors will influence the feasibility of Limnanthes production as an oilseed crop.

Market demand, the resulting price for the seed, its relation to cost of production, and profit margin relative to other cropping alternatives in areas where Limnanthes is culturally adapted will of course be of prime consideration and in the final analysis of vital importance

(10). Additionally, value of the seed meal for utilization by livestock or poultry may be important from an economic standpoint. Limnanthes seed meal has been shown to be approximately equal in protein value to some legumes (16). Although, an anti-quality component, m-methoxybenzyl isothiocyanate produced by enzymatic hydrolysis of a thioglucoside, if not removed may have deleterious effects on livestock (12). But, a process developed for mustard seed meal that eliminates volatile allyl isothiocyanate might be used (14).

Acreage, which is determined by the profitability of production compared to other cropping alternatives, sponsorship by industry, production under contract, advisory services, and promotion are other considerations which ultimately influence the success of a new oil seed crop (10).

The Limnanthes genus may have potential as an oilseed crop, but successful production will in the final analysis be dependent upon the above mentioned considerations. Agronomic information relating to management practices will serve an important role.

LITERATURE CITED

1. Anonymous. 1964. Sea Hunt for Specialties Oils. Chemical Week. July 4. pp. 27-32.
2. Bagby, M. O., C. R. Smith, T. K. Miwa, R. L. Lohmar, and I. A. Wolff. 1961. A Unique Fatty Acid from Limnanthes douglasii Seed Oil: The C22 Diene. Jour. of Organic Chem. 26: 1261-1265.
3. Cook, A. A. 1971. Thar She Grows. The Farm Index. Oct. pp. 4-6.
4. Earle, F. R., E. H. Melvin, L. H. Mason, C. H. Van Etten, and I. A. Wolff. 1959. Search for New Industrial Oils I. Selected Oils from 24 Plant Families. Jour. of Amer. Oil Chem. Soc. 36: 304-307.
5. Fore, S. P., and G. Sumrell. 1966. Some Derivatives of 5-Eicosenoic Acid. Jour. of Amer. Oil Chem. Soc. 43: 581-584.
6. Gentry, H. S., and R. W. Miller. 1965. The Search for New Industrial Crops IV. Prospectus of Limnanthes. Econ. Bot. 19: 25-32.
7. Higgins, J. J., W. Calhoun, B. C. Willingham, D. H. Dinkel, W. L. Raisler, and G. A. White. 1971. Agronomic Evaluation of Prospectus New Crop Species II. The American Limnanthes. Econ. Bot. 25: 44-54.
8. Jones, Q., and I. A. Wolff. 1961. Using Germ Plasm for New Products. Germ Plasm Resources. AAAS, Washington, D. C. pp. 265-277.
9. Knoepfler, N. B., and H. I. E. Vix. 1958. Review of Chemistry and Research Potential of Simmondsia chinensis (Jojoba) Oil. Agri. and Food Chem. 6: 118-121.
10. Knowles, P. F. 1960. New Crop Establishment. Econ. Bot. 14: 263-275.
11. Mason, C. T. 1952. A Systematic Study of the Genus Limnanthes R. Br. Univ. of Calif. Publ. Bot. 25: 455-512.
12. Miller, R. W., M. E. Daxenbichler, and F. R. Earle. 1964. Search for New Industrial Oils VIII. The Genus Limnanthes. Jour. of Amer. Oil Chem. Soc. 41: 167-169.
13. Moreau, J. P., R. L. Holmes, F. G. Dollear, and G. Sumrell. 1966. Preparation of Vinyl Esters of Some Chlorinated New Oilseed Crops Fatty Acids. Jour. of Amer. Oil Chem. Soc. 43: 94-96.

14. Mustakas, G. C., L. D. Kirk, V. E. Sohns, and L. E. Griffin. 1965. Mustard Seed Processing: Improved Methods for Isolating the Pungent Factor and Controlling Protein Quality. Jour. of Amer. Oil Chem. Soc. 42: 33-37.
15. Smith, C. R., M. O. Bagby, T. K. Miwa, R. L. Lohmar, and I. A. Wolff. 1960. Unique Fatty Acids from Limnanthes douglasii Seed Oil: The C20 and C22 Monoenes. Jour of Organic Chem. 25: 1770-1774.
16. Van Etten, D. M., R. W. Miller, I. A. Wolff, and Q. Jones. 1961. Amino Acid Composition of Twenty- Seven Selected Seed Meals. Agri. and Food Chem. 9: 79-82.
17. Yermanos, D. M. 1973. Jojoba...a Brief Survey of the Agronomic Potential. California Agriculture 27(9): 10-14.

OIL, PROTEIN, AND YIELD OF LIMNANTHES ALBA
SEED AS AFFECTED BY RATE, FORM, AND
TIME OF NITROGEN APPLICATION

ABSTRACT

The effect of nitrogen (N) application on seed yield of Limnanthes alba was studied for two years at two locations. Seeding following alfalfa (Medicago sativa L.) was compared to establishment after wheat (Triticum aestivum L.). Treatments used were a control (no N applied), and two rates of N (50 and 100 kg/ha) applied at two different dates (March and May). In the next year, comparisons were made of ammonium sulfate (AS) and ammonium nitrate (AN) applied in March. Seed samples were taken for analysis of oil and protein content.

Seed protein content was increased by N application, with seed from the 100 kg of N as AS in March treatment having the highest content. Protein yield was influenced by N fertilization in 1973. Protein yield was greatest when 100 kg of N in March was applied to L. alba following wheat. Following alfalfa, 100 kg N in March and May decreased protein yield. Protein yield was well correlated to seed yield both years.

Oil content was reduced by all rates and forms of N application. Oil content was negatively correlated to protein content. Oil yield was somewhat differently affected by N application both years. Following wheat and alfalfa, oil yield was reduced by May application of N, while in the next year a reduction of oil yield occurred from all N applications.

Seed yields were affected by N fertilizer in 1973, but not in 1974. Following wheat, March application of N increased yields, whereas the May applications decreased seed yield. After alfalfa, 100 kg N in March and May reduced seed yields.

INTRODUCTION

Limnanthes seed oil has unique characteristics similar to sperm whale oil, and this fact has stimulated an interest in the genus as a potential industrial oilseed crop. Limnanthes alba is one species which has been shown to have merit for agronomic evaluation due to characteristics which would lend it well to production and mechanization.

Research on Limnanthes seed oil and protein is limited. Miller et al. (11) identified oil and protein content of 13 species and varieties of Limnanthes. Van Etlén et al. (13) analyzed amino acid composition of L. douglasii seed meal protein and found lysine and methionine in amounts comparable to some legumes. But, research has not been done on influences of N fertility on these biochemical components (protein and oil) from an agronomic standpoint.

Yield data has been reported for the genus, but it concerns mostly the evaluation of various species and varieties for agronomic potential (5). Higgins and Associates (7) reported seed yield results from various locations in the United States and examined seeding and harvesting methods of varieties of Limnanthes. Reports on N fertilization research have not appeared in the literature.

For commercial oilseed crops, N fertility reports concerning seed yield and oil content are numerous. Although, reports on the influence of N applications on seed protein content of non-legume oilseed crops are uncommon.

Yermanos et al. (14) reported that increasing rates of N fertilizer caused increased yields of safflower, but also caused a depression in

seed oil content. However, oil yields were largest from the fertilized safflower. On the other hand, Hoag et al. (8) and Gilbert and Tucker (6) reported that oil content of safflower was not affected by rate of N fertilizer. Gilbert and Tucker (6) also found that oil content of safflower was not influenced by source or time of N application.

Reporting work with castorbeans, Kittock et al. (9) found that high N levels (180 kg/ha) reduced oil content in a two year study. Dybing (2) reported similar reductions in oil content of seed flax from various N levels, both in the greenhouse and under controlled environment.

Massey (10) reported limited yield responses to N fertilizer with sunflowers, whereas D'Yakor (1) recorded increases of seed and oil yield with a slight depression in oil content.

If L. alba proves to be economically feasible for production in the Willamette Valley of Oregon, there will be a demand for agronomic information relating to management practices, especially N fertilization. It was the objective of this study to determine what rate, date, and form of N application will be most favorable for seed production from the standpoint of the influence of these management practices on seed oil and protein content.

MATERIALS AND METHODS

Three nitrogen (N) fertility experiments with L. alba Benth. var. alba (PI 283-704 1/) were conducted at two locations for two years. In 1973, two trials were conducted on a Woodburn silty clay loam soil at Hyslop Agronomy Farm, Corvallis, Oregon. In 1974, an experiment was located on a Dayton series soil at Jackson Farm 2/, Lebanon, Oregon. A weather summary for the two crop years is presented in Appendix Tables 1 and 2:

In 1973, at the Hyslop Farm site, an experiment was established on an area previously cropped with soft white winter wheat (Triticum aestivum L.), presumably leaving the soil low in readily available N. Also at the Hyslop site, a second experiment was planted in an area previously cropped with alfalfa (Medicago sativa L.) for two years.

After initial land preparation, plots of 1.83 by 6.10 m in size were seeded with row spacings of 15.2 cm using a Planet Junior drill (hole # 14) at a rate of 17 kg/ha or approximately 3.5 seeds per dm of row. Plots were planted October 9th in moist soil, and a post-plant, pre-emergence herbicide treatment of propachlor at 4.5 kg active ingredient per ha applied two days later.

The experimental design was a randomized complete block with five treatments and four replications for both experiments in 1973. Treatments included a control (no N applied), and two rates of N (50 and 100 kg/ha) at two dates of application (March 5 and May 4). N was supplied in the ammonium sulfate form (21% N) and applied by a hand-moved spreader.

1/ The plant introduction number assigned a new crop species by USDA.

2/ Leased to Oregon State University by Pacific Power and Light Co.

On the March 5th application date, plants were primarily at a vegetative stage of growth. However, on the May 4th application date, flowering was approximately 50% completed. Flowering has been observed to usually commence during the last week of April.

The 1974 experiment was established on soil previously cropped with soft white winter wheat. Seedbed preparation was similar to 1973. An entire range was seeded solid, using a standard grain drill set at 17 kg/ha, with row spacings of 17.8 cm, or approximately 4 seeds per dm of row. Planting took place October 15th. During the first week of March, plots of the same size as 1973 were staked out and N treatments were applied using a hand-moved spreader. The March date of application was chosen as a standard from preliminary interpretation of 1973 results.

The experimental design for the 1974 study was again a randomized complete block, with five treatments and four replications. Treatments included the same rates as 1973, but instead of dates, two forms of N, ammonium sulfate (21% N) and ammonium nitrate (33% N), were used. As in the previous year, plants were primarily in a vegetative stage of growth.

For both years and locations, plots were harvested using a small plot combine. The harvested area was 7.43 m² (1.2 x 6.1 m). An area 1 m² from each plot was vacuumed to establish a shattering estimate, and to obtain total yield (harvested plus shattered seed). Both seed lots were separately run through a stationary thresher, then dried, cleaned, weighed, and recorded.

Once yields were recorded, one 15 g sample from each treatment replicate was taken and sent to the Industrial Crops Laboratory, Northern Regional Research Laboratory, Northern Utilization Research and Development

Division, ARS, USDA, Peoria, Illinois for oil and protein determinations.

At the Peoria laboratory, methods essentially like those of the American Oil Chemist's Society Official Methods for nitrogen (Aa 5-38, Rev. 1957) and oil (Bb 3-38, Rev. 1954) were used, since there are none established for Limnanthes spp. For nitrogen, the standard Kjeldahl method was varied by receiving the NH_3 in boric acid solution and titrating with HCl . For oil, petroleum ether and the standard Butt apparatus were used and extractions were run for six hours. Fertility replicate samples were run in duplicate and the two values averaged for each plot (F. R. Earle, Personal Communication, 1974).

Oil yield (kg/ha) was calculated by multiplying percent of seed oil by total seed yield for each plot. Protein yield (kg/ha) was calculated similarly.

Data were analyzed statistically using the analysis of variance technique. Means having significant F values were subjected to Duncan's multiple range test. Coefficient of variation and standard error of the mean values were also calculated. Linear correlations were calculated between relevant parameters; the following codes indicate levels of significance: *p = .05, **p = .01, and ***p = .001.

RESULTS AND DISCUSSION

Seed protein content was increased by N fertilizer both years (Table 1 and Figure 1). Following wheat in 1973, protein content was increased 21.6% from the 100 kg N in March treatment. Protein content from both May application rates was also increased. Since protein from plots receiving 100 kg N in March was greater than 100 kg N in May, it may be thought that, likewise, protein from 50 kg N in March would be greater than the same rate in May. This was not the case. Protein content as a result of the 50 kg N in March treatment was not different from the control.

Following alfalfa in 1973, seed protein content was greater from 100 N in March and 100 N in May treatments. Protein content from the high rate in May did not differ from the lower rate of N application during the same period. Seed in the control plots had the lowest protein content. N applied in March at 50 kg/ha resulted in higher protein content than the control, but the protein content did not differ from the same rate in May.

In 1974, seed protein content was increased 34.5% by the 100 kg N as AS treatment as compared to unfertilized plots. Protein content was greater from plots receiving 100 kg N as AS than all other treatments. Protein content from the 100N as AN treatment was not different from the 50 N as AS treatment, but both were greater in protein than the control.

As seen in Figure 1, seed protein content in both of the 1973 experiments exhibited similar responses to N application. Surprisingly, the May application of 50 kg N resulted in slightly higher protein content than the 50 kg N rate in March. However, the opposite was true of

Table 1. The Effect of Rate, Date, and Form of Nitrogen ^{1/} Application on the Protein Content of L. alba Seed.

Treatment	1973 ^{2/}		1974 ^{3/}
	After Wheat	After Alfalfa	
CONTROL	18.5 ^{4/} c ^{5/}	18.4 d	18.4 c
50N AS MARCH	18.9 c	19.8 c	20.0 b
100N AS MARCH	22.6 a	22.0 a	23.8 a
50N AN MARCH	-	-	18.9 c
100N AN MARCH	-	-	20.9 b
50N AS MAY	20.4 b	20.4 bc	-
100N AS MAY	21.2 b	21.2 ab	-
Coefficient of Variation	3.1%	3.1%	3.2%
Standard Error of the Mean	0.32	0.32	0.33

^{1/} kg/ha of N, as AS = ammonium sulfate, and AN = ammonium nitrate.

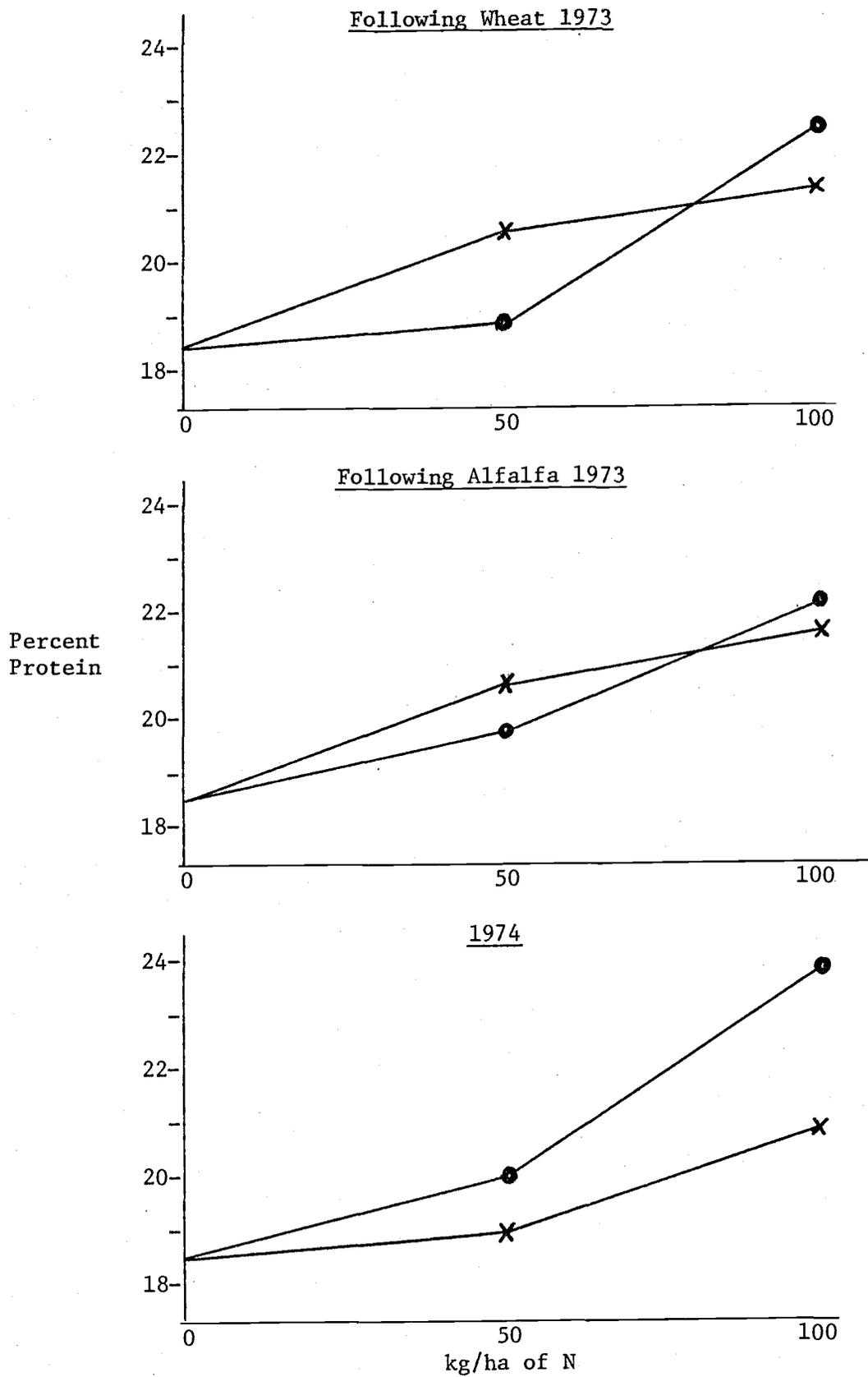
^{2/} Hyslop Agronomy Farm, Corvallis, Oregon.

^{3/} Jackson Farm, Lebanon, Oregon.

^{4/} Means of four replications.

^{5/} Means within columns followed by the same letter(s) are not significant from each other at the .05 level of P according to Duncan's multiple range test.

Figure 1. Seed Protein Content of *L. alba* as Affected by Rate, Date, and Form of Nitrogen Application.



the 100 kg rate of N.

When following a legume, with residual N assumed present, one would expect to observe an increase in seed protein as an effect of rotation in addition to applied N. In this study seed protein content seemed about the same for all N application rates at both dates regardless of the rotation system followed. If a difference were to exist between crop rotations, the plots which received no N would best demonstrate it, due to possible carryover of N from the legume rotation. But, as seen in Table 1, the difference between controls is minute. This discrepancy could either denote a N tie-up in decomposing tap roots and stubble from the previous alfalfa stand or excessive loss of N by leaching prior to the period during which L. alba seed protein was at all responsive to available N in the soil.

In 1974, with such a pronounced difference in response between forms of N, it would seem that the soil was deficient in sulfur, and the AS form of N supplied adequate amounts of sulfur necessary for increased plant growth. High levels of AS, a strong acidifying material, could have reduced the pH of the soil medium and thereby affected the availability of nutrients for plant growth. Although 100 kg of N as AS was not excessive in conventional terms, it might have been enough to affect pH and nutrient availability at a threshold level. Of course, since soil samples were not taken, this is a matter of speculation only.

From the results of these studies, it can be seen that N greatly influenced protein content of L. alba seed. March applications of AS had the greatest influence of increasing protein content. Of interest from this study is the response in protein content ; an increase of 21.6 to

34.5%. Protein content of this species can apparently be substantially influenced by fertilization practices. As observed in Table 1, for the three experiments, encompassing two locations and two years, the coefficient of variation ranged from 3.1 to 3.2%, which would suggest that this species responds quite similarly to given N levels under a variety of conditions.

Oil Content

Seed oil content was influenced by applications of N fertilizer in both years (Table 2 and Figure 2). The most evident trend was a reduction in oil content as N application levels increased.

When L. alba followed wheat, the 100 kg N in March treatment caused the greatest reduction in seed oil content. Oil content from the May application of 100 N was not different from the 50 N in March treatment. The May application of 50 N had no effect on oil content.

Following alfalfa, both the March and May applications of 100 N had the greatest effect in reducing seed oil content. March and May treatments of 50 kg N were not different from each other in oil content.

In 1974, 100 N as AS had the greatest influence on decreasing seed oil content. The 50 N as AS and 100 N as AN treatments were not different from each other in oil content. Seed oil content was least affected by 50 N as AN.

In both years, there was a negative correlation between seed oil and protein content (Table 3).

Some reports on other oilseed crops have shown a reduction in seed oil content with increased N levels (1,2,3,14), while others have not

Table 2. The Effect of Rate, Date, and Form of Nitrogen ^{1/} Application on Oil Composition of L. alba Seed.

Treatments	1973 ^{2/}		1974 ^{3/}
	After Wheat	After Alfalfa	
CONTROL	27.5 ^{4/} a ^{5/}	28.3 a	26.8 a
50N AS MARCH	26.0 b	25.7 b	25.1 c
100N AS MARCH	23.2 c	22.7 c	22.2 d
50N AN MARCH	-	-	26.1 b
100N AN MARCH	-	-	24.6 c
50N AS MAY	26.9 a	24.4 b	-
100N AS MAY	25.5 b	22.4 c	-
Coefficient of Variation	2.1%	3.2%	1.6%
Standard Error of the Mean	0.27	0.40	0.19

^{1/} kg/ha of N, as AS = ammonium sulfate, and AN = ammonium nitrate.

^{2/} Hyslop Agronomy Farm, Corvallis, Oregon.

^{3/} Jackson Farm, Lebanon, Oregon.

^{4/} Means of four replications.

^{5/} Means within columns followed by the same letter(s) are not significant from each other at the .05 level of P according to Duncan's multiple range test.

Figure 2. Seed Oil Content of *L. alba* as Affected by Rate, Form, and Date of Nitrogen Application

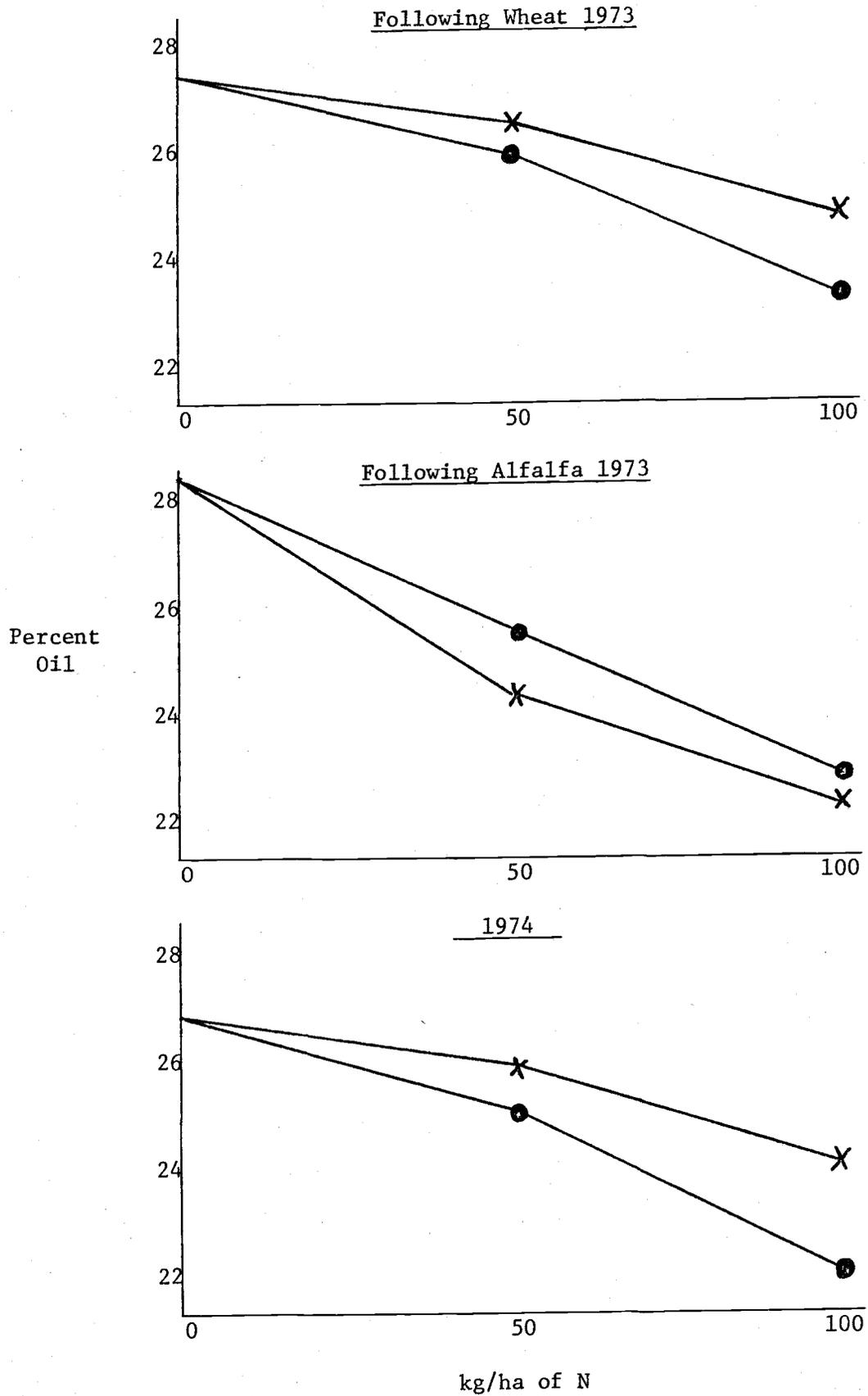


Table 3. Simple Correlation Coefficients for Oil, Protein, and Yield of L. alba from Experiments Conducted for Two Years 1/

Factors	Percent Oil	Seed Yield	Protein Yield	Oil Yield
Percent	-.771 ^{2/} ***	.484**	-.062	-.543**
Protein	-.992 ^{3/} ***	-.391	.176	-.626**
Percent Oil	-----	.264 .446*	.016 -.102	.504** .690**
Seed Yield	-----	-----	.947*** .829***	.960*** .953***
Protein Yield	-----	-----	-----	.841*** .631**

1/ Maximum, Minimum, Average, and standard error of the mean values are presented in Appendix Table 3.

2/ r values of 1973 date (n = 40).

3/ r values of 1974 data (n = 20).

(6,8). In the present study, however, L. alba seed oil content was reduced by all dates of N application as well as forms of N.

Seed Yield

Applications of N fertilizer affected seed yields in 1973, but no differences were recorded in 1974 (Table 4). When L. alba followed wheat, both March application rates increased seed yields. Both rates of N applied in May decreased seed yields.

Following alfalfa, a somewhat different trend was recorded. March and May applications of 100 N decreased seed yields. However, the 50 N rates at both dates of application did not alter seed yields.

Seed yield was correlated to seed protein content in 1973 ($r = .484$ **). As N application rates were increased, protein content and seed yields were increased also.

When N applications were made on L. alba following wheat, it seems as though the most optimum date to apply for seed yield response was in March. When N was applied in May, by the time it was available for plant use, it was too late to influence seed yields.

On the other hand, after alfalfa sufficient N from the previous crop allowed adequate yield without additional N. The results of N treatments after alfalfa are quite different from those after wheat, but this could be expected due to past management practices, and therefore the physical and chemical characteristics of the soil.

On the basis of the results reported here, seed yields can be influenced by crop rotation, N rates and dates of application. Following wheat, the level of response is dependent upon dates of application more

Table 4. The Effect of Rate, Date, and Form of Nitrogen ^{1/} Application on Seed Yield (kg/ha) from L. alba

Treatments	1973 ^{2/}		1974 ^{3/}
	After Wheat	After Alfalfa	
CONTROL	1048 ^{4/} b ^{5/}	1635 a	852 NS ^{6/}
50N AS MARCH	1223 a	1515 a	772
100N AS MARCH	1211 a	1054 b	732
50N AN MARCH	-	-	967
100N AN MARCH	-	-	874
50N AS MAY	914 c	1400 a	-
100N AS MAY	812 c	1036 b	-
Coefficient of Variation	7.7%	14.1%	14.0%
Standard Error of the Mean	39.8	93.7	58.7

^{1/} kg/ha of N, as AS = ammonium sulfate, and AN = ammonium nitrate.

^{2/} Hyslop Agronomy Farm, Corvallis, Oregon.

^{3/} Jackson Farm, Lebanon, Oregon.

^{4/} Means of four replications.

^{5/} Means within the same column followed by the same letter(s) are not significant from each other at the .05 level of P according to Duncan's multiple range test.

^{6/} Not significant at the .05 level of P.

than rate of N applied. When following alfalfa, low rates of N had no effect, while high rates of N decreased seed yields.

It must be pointed out that a possible reason for no differences being recorded in the 1974 experiment is that no weed control measures were employed. Plots with N applications were observed to have had an abundant growth of annual bluegrass (Poa annua L.), and this may have had a deleterious effect on seed yield.

Protein Yield

Protein yield was affected by applications of N in 1973, but not in 1974 (Table 5). When L. alba followed wheat, March applied N at 100 kg increased protein yield. The 50 N in March treatment was recorded as having the second highest protein yield. Both May application rates did not effect protein yield.

Following alfalfa, March and May rates of 100 N decreased protein yield. The March application of 100 kg N was not different in protein yield from the 50 N in May treatment.

Protein yield was well correlated to seed yield both years (Table 3). This would follow, since seed yield is a component of protein yield. There was no relationship between protein yield and protein content recorded, as protein yield was more of an expression of seed yield.

Following wheat, protein yield was increased from both March application rates of N, because both seed protein content and seed yield were also increased (Table 6). At the May date of application, protein yield from both rates of N was not different from the control; which was due to seed yield levels being lower but protein levels being higher than

Table 5. The Effect of Rate, Date, and Form of Nitrogen ^{1/} Application on Protein Yield (kg/ha) from L. alba

Treatments	1973 ^{2/}		1974 ^{3/}
	After Wheat	After Alfalfa	
CONTROL	194 ^{4/} c ^{5/}	300 a	158 NS ^{6/}
50N AS MARCH	231 b	299 a	155
100N AS MARCH	273 a	232 bc	173
50N AN MARCH	-	-	182
100N AN MARCH	-	-	183
50N AS MAY	186 a	286 ab	-
100N AS MAY	177 c	220 c	-
Coefficient of Variation	8.7%	14.2%	16.4%
Standard Error of the Mean	9.1	18.9	13.9

^{1/} kg/ha of N, as AS = ammonium nitrate, and AN = ammonium sulfate.

^{2/} Hyslop Agronomy Farm, Corvallis, Oregon.

^{3/} Jackson Farm, Lebanon, Oregon.

^{4/} Means of four replications.

^{5/} Means followed by the same letter(s) are not significantly different from each other at the .05 level of P according to Duncan's multiple range test.

^{6/} Not significant at the .05 level of P.

Table 6. Total Protein Yield Components of L. alba as Influenced by Nitrogen 1/ Application Treatments for Two Years.

Treatment	Total Seed Yield kg/ha	Percent Protein	Total Protein Yield kg/ha
<u>Following Wheat 1973 ^{2/}</u>			
CONTROL	1048 ^{3/}	18.5	194
50N AS MARCH	1223	18.9	231
100N AS MARCH	1211	22.6	272
50N AS MAY	914	20.4	186
100N AS MAY	812	21.2	172
<u>Following Alfalfa 1973 ^{2/}</u>			
CONTROL	1635	18.4	300
50N AS MARCH	1515	19.8	299
100N AS MARCH	1054	22.0	232
50N AS MAY	1400	20.4	285
100N AS MAY	1036	21.2	220
<u>1974 ^{4/}</u>			
CONTROL	852	18.4	158
50N AS MARCH	772	19.9	155
100N AS MARCH	732	23.8	173
50N AN MARCH	967	18.9	182
100N AN MARCH	874	20.9	183

1/ kg/ha of N as AS = ammonium sulfate, and AN = ammonium nitrate.

2/ Hyslop Agronomy Farm, Corvallis, Oregon.

3/ Means of four replications.

4/ Jackson Farm, Lebanon, Oregon.

the control. Thus, protein yield from these two treatments was about equal to the control.

After alfalfa, even though the control had the lowest seed protein content, protein yield was the greatest due to a large seed yield. Protein yield from the March and May treatments of 100 kg N was the least, as these rates had such a reducing effect on seed yield that the higher protein content could not compensate for the reduction in seed yield.

From the results of these experiments, it can be seen that protein yield is more dependent on seed yield than protein content, since treatments resulting in greater protein content sometimes had a depressing effect on seed yield. This is evidenced in the lack of a significant correlation between seed protein content and protein yield.

Oil Yield

N applications influenced seed oil yield both years (Table 7). When L. alba followed wheat, oil yield from both rates of application of N in March were not different from the control. The May application of 100 N reduced oil yield the greatest amount. Oil yield was also reduced from the application of 50 N in May.

Following alfalfa, oil yield from both March and May rates of 100N were reduced. The 50 N rate at both dates of application resulted in a higher oil yield than the 100 N rate in March or May. The 50 N in March treatment was not different in oil yield from the control.

In 1974, 100 N as AS had the greatest effect in decreasing oil yield. The 50 N as AN rate of application gave a higher oil yield than the other treatments. Oil yields from all other treatments did not differ from each

Table 7. The Effect of Rate, Date, and Form of Nitrogen ^{1/} Application on Oil Yield (kg/ha) from L. alba Seed.

Treatments	1973 ^{2/}		1974 ^{3/}
	After Wheat	After Alfalfa	
CONTROL	288 ^{4/} a ^{5/}	463 a	288 ab
50N AS MARCH	318 a	389 ab	194 bcd
100N AS MARCH	291 a	239 c	163 d
50N AN MARCH	-	-	252 a
100N AN MARCH	-	-	215 abc
50N AS MAY	246 b	342 b	-
100N AS MAY	208 c	234 c	-
Coefficient of Variation	8.7%	15.1%	13.2%
Standard Error of the Mean	11.7	25.1	13.9

^{1/} kg/ha of N, as AS = ammonium sulfate, and AN = ammonium nitrate.

^{2/} Hyslop Agronomy Farm, Corvallis, Oregon.

^{3/} Jackson Farm, Lebanon, Oregon.

^{4/} Means of four replications.

^{5/} Means within columns followed by the same letter(s) are not significant from each other at the .05 level of P according to Duncan's Multiple range test.

other.

There was a negative correlation recorded between oil yield and percent protein content in both years (Table 3). This would indicate that as protein increases from higher N application levels, oil content and seed yield would decrease. There were a few exceptions to this rule, but overall L. alba seed yield is not greatly increased by N application.

There was a positive correlation between oil content and oil yield. This follows, since oil yield is dependent upon oil content.

Oil yield was positively correlated to seed yield and negatively correlated to protein yield both years. Seed yield is a component of oil yield and therefore a positive correlation would be expected. Protein yield was negatively correlated to oil yield, since as oil decreases protein content increase with higher N application rates.

Following wheat, oil yield from the control, and 50 and 100 N rates in March did not differ from each other, since the latter two treatments had lower oil but greater seed yield than the former (Table 8). Oil yields from May applications of 50 and 100 kg N were less than the control level due to the lower oil content and seed yields from the former treatments.

Following alfalfa, oil yields from plots receiving 100 N in March and May were not different from each other, since both oil content and seed yield were not different from each other. Oil yields from the 50 N rates in March and May were not different, also due to a lack of difference in oil content and seed yield. The control was higher in oil yield than other treatments except 50 N in March, because it had both the highest oil content and seed yield.

Table 8. Total Oil Yield Components of L. alba as Influenced by Nitrogen 1/ Application Treatments for Two Years.

Treatment	Total Seed Yield kg/ha	Percent Oil	Total Oil Yield kg/ha
<u>Following Wheat 1973 ^{2/}</u>			
CONTROL	1048 ^{3/}	27.5	288
50N AS MARCH	1223	26.0	318
100N AS MARCH	1211	23.3	290
50N AS MAY	914	26.9	246
100N AS MAY	812	25.5	208
<u>Following Alfalfa 1973 ^{2/}</u>			
CONTROL	1635	28.3	463
50N AS MARCH	1515	25.7	389
100N AS MARCH	1054	22.7	239
50N AS MAY	1400	24.6	342
100N AS MAY	1036	22.4	236
<u>1974 ^{4/}</u>			
CONTROL	852	26.8	228
50N AS MARCH	772	25.1	194
100N AS MARCH	732	22.2	163
50N AN MARCH	967	26.1	252
100N AN MARCH	874	24.6	215

1/ kg/ha of N, as AS= ammonium sulfate, and AN = ammonium nitrate.

2/ Hyslop Agronomy Farm, Corvallis, Oregon.

3/ Means of four replications.

4/ Jackson Farm, Lebanon, Oregon.

In 1974, oil yield resulting from the application of 50 N as AN was greater than all other treatments except the control due to a higher oil content and seed yield levels of the two. The 100 N as AS rate of application resulted in the lowest oil yield level of all treatments because of a lower seed oil content and seed yield level.

From these results, it appears that unlike other oilseed crops (Dybing et al. with flax), L. alba seed oil content decreases with increased N application rates and is accompanied by a corresponding reduction in oil yield.

Dybing (2) reported that even though N rates decreased seed oil content, the benefit from N use in enhancing seed yield and thus oil yield outweighed this decrease. In this study, however, L. alba seed yields were not stimulated enough by N to outweigh the decrease in seed oil content associated with N application at either date.

LITERATURE CITED

1. D'Yakov, A. B. 1971. Protein Content and the Level of Oil Accumulation in Sunflowers. *Vestnik Sel'skokhozyaistvennoi Nauki, Moskua* 7: 57-63.
2. Dybing, C. D. 1964. Influence of Nitrogen Level on Flax Growth and Oil Production in Varied Environments. *Crop Sci* 4: 491-494.
3. _____, and D. C. Zimmerman. 1964. Temperature Effects on Flax (*Linum usitatissimum* L.) Growth, Seed Production, and Oil Quality in Controlled Environments. *Crop Sci* 5: 184-187.
4. Ford, J. H., and D. C. Zimmerman. 1964. Influence of Time of Flowering on Oil Content and Oil Quality of Flaxseed. *Crop Sci* 4: 653-656.
5. Gentry, H. S., and R. W. Miller. 1965. The Search for New Industrial Crops IV. Prospectus of *Limnanthes*. *Econ Bot* 19: 25-32.
6. Gilbert, N. W., and T. C. Tucker. 1967. Growth, Yield, and Yield Components of Safflower as Affected by Source, Rate, and Time of Nitrogen Application. *Agron J.* 69: 54-56.
7. Higgins, J. J., W. Calhoun, B. C. Willingham, D. H. Dinkel, W. L. Raisler, and G. A. White. 1971. Agronomic Evaluation of Prospectus New Crop Species II. The American *Limnanthes*. *Econ Bot* 25: 44-54.
8. Hoag, B. K., J. C. Zubriski, and G. N. Griszler. 1968. Effect of Fertilizer Treatment and Row Spacing on Yield, Quality, and Physiological Response of Safflower. *Agron J.* 60: 198-200.
9. Kittock, D. L., J. H. Williams, and D. G. Hanway. 1967. Castorbean Yield and Quality as Influenced by Irrigation Schedules and Fertilization Rates. *Agron J.* 59: 463-467.
10. Massey, J. H. 1971. Effect of Nitrogen Rates and Plant Spacing on Sunflower Seed Yields and Other Characteristics. *Agron J.* 63: 137-138.
11. Miller, R. W., M. E. Daxenbichler, F. R. Earle, and H. S. Gentry. 1964. Search for New Industrial Oils VIII. The Genus *Limnanthes*. *Jour of Amer Oil Chem Soc* 41: 167-169.
12. Stamp, D. L. 1973. The Culture of *Limnanthes* spp.: A substitute for Sperm Whale Oil. *Agron. Abst.*, pp. 69.

13. Van Etten, C. H., R. W. Miller, I. A. Wolff, and Q. Jones. 1961. Amino Acid Composition of Twenty-Seven Selected Seed Meals. *Agricultural and Food Chemistry* 9: 79-82.
14. Yermanos, D. M., B. J. Hall, and W. Burge. 1964. Effect of Iron Chelates and Nitrogen on Safflower and Flax Seed Production and Oil Content and Quality. *Agron J.* 56: 582-585.

FATTY ACID COMPOSITION OF LIMNANTHES ALBA
SEED OIL AS AFFECTED BY RATE, FORM,
AND DATE OF NITROGEN APPLICATION

ABSTRACT

The effect of nitrogen (N) application on seed yield of Limnanthes alba was studied for two years at two locations. Seeding following alfalfa (Medicago sativa L.) was compared to establishment after wheat (Triticum aestivum L.). Treatments used were a control (no N applied) and two rates of N (50 and 100 kg/ha) applied at two dates (March and May). Comparisons were also made of ammonium sulfate (AS) and ammonium nitrate (AN) applied in March the second year. Seed samples were taken for analysis of oil content and fatty acid composition.

C20 monoene content was reduced by the application of N at the two dates in either form. C20 monoene content was positively correlated to seed oil content both years.

Both 50 and 100 kg N rates applied in March and May increased C22 monoene content. C22 monoene content was negatively correlated to both seed oil and C20 monoene content.

C22 diene content was decreased by May applications of N and negatively correlated to C22 monoene content in the first year. C22 diene content was increased by higher rates of N application from both sources of N, and was negatively correlated to oil content and C20 monoene content in the second year.

The percent of seed oil composed of other than C20 and C22 fatty acids was influenced by some N application treatments in the first year, but no definite trends were established.

INTRODUCTION

Limnanthes seed oil has unique characteristics similar to sperm whale oil, and this fact has stimulated an interest in the genus as a potential oilseed crop (3).

Earle and Associates (1959) reported that oil from the seed of Limnanthes douglasii was unusual because 94% of its fatty acids exhibited longer retention times on a gas chromatograph column than linolenic acid, the slowest C18 component of common plant oils.

Subsequently, Smith et al. (1960) isolated and identified three of the four major fatty acid components of L. douglasii seed oil: The C20 (cis-5-eicosenoic acid) and C22 (cis-5-docosenoic and cis-13-docosenoic acids) monoenes. One constituent, a C22 fatty acid of unknown character, was quantified but not identified. In a later report, Bagby et al. (1961) identified and characterized the remaining unknown constituent as a C22 diene: cis-5-cis-13 docosadienoic acid.

The C20 monoene has been described as being unique, in that no other monoethenoid fatty acid with a Δ^5 double bond had been recorded as a triglyceride constituent in either the plant or animal kingdoms. Likewise, cis-5-docosenoic acid is also unique (11). But the other C22 monoene, cis-13-docosenoic (Erusic) acid, is a common constituent of rapeseed oil (6,8).

Similarly, the C22 diene is also unique in that no other dienoic acid with hexylene interrupted unsaturation appears to have been recorded as a triglyceride constituent in natural sources (1).

Research on other than genetic variation of fatty acid constituents of oil from Limnanthes seed has been limited. However, the effect of man-

agement on the fatty acids of other crops have been reported. Oil production and fatty acid constituents of flaxseed (Linum usitatissimum) oil have been shown to be quite responsive to nitrogen levels in varied environments (4). Regional variation, as an effect of environment, has been reported for the fatty acids of sunflower (Helianthus annuus), soybean (Glycine max), and cotton (Gossypium spp.) seed oils (9,2,12). Stansbury et al. (12) reported on cottonseed oil that ranges of iodine values under the influence of different environments were approximately 50% greater than those for the influence of variety alone. Variability of the climate at a given location caused variation as large as that associated with widely separated locations.

Gentry and Miller (6) collected Limnanthes seed from a wide range of geographical and environmental conditions, but the variation in the chemical constituents was attributed primarily to genotypic differences. This study was initiated to ascertain what degree variability of fatty acid composition of seed oil is affected by factors other than genetic differences. The objective was to determine the influence of N rates, forms, and dates of application on the fatty acid composition of an individual accession of Limnanthes alba. In addition, inter-relationships of seed oil and fatty acid components were also examined.

MATERIALS AND METHODS

Three nitrogen (N) fertility experiments with L. alba var. alba (PI 283-704 1/) were conducted at two locations for two years. In 1973, two trials were conducted on a Woodburn silty clay loam soil at Hyslop Agronomy Farm, Corvallis, Oregon. In 1974, an experiment was located on a Dayton series soil at the Jackson Farm 2/, Lebanon, Oregon. A weather summary for the two crop years is presented in Appendix Tables 1 and 2.

In 1973, at the Hyslop Farm site, an experiment was established on an area previously cropped with soft white winter wheat (Triticum aestivum L.), presumably leaving the soil low in readily available N. Also at the Hyslop site, a second experiment was planted in an area previously cropped with alfalfa (Medicago sativa L.) for two years.

The experimental design was a randomized complete block with five treatments and four replications for both experiments in 1973. Treatments included a control (no N applied) and two rates of N (50 and 100 kg/ha) at two dates of application (March 5 and May 4). N was supplied in the ammonium sulfate form (21% N) and applied by a hand-moved spreader.

On the March 5th application date, plants were primarily at a vegetative stage of growth. However, on the May 4th application date, flowering was approximately 50% completed. Flowering has been observed to usually commence during the last week of April.

1/ The plant introduction number assigned a new crop species or variety within a species by USDA.

2/ Leased to Oregon State University by Pacific Power and Light Company, Portland, Oregon.

The 1974 experiment was established on soil previously cropped with soft white winter wheat. During the first week of March, plots were staked out and N treatments applied using a hand-moved spreader. The March date of application was chosen as a standard from preliminary interpretation of the 1973 results.

The experimental design for the 1974 study was again a randomized complete block, with five treatments and four replications. Treatments included the same rates as 1973, but instead of dates of application, two forms of N were used. The forms were ammonium sulfate (AS) and ammonium nitrate (AN). As in the previous year, plants were primarily in a vegetative stage of growth when the N treatments were applied.

For both years and locations, plots were harvested using a small plot combine. Once harvested and cleaned, fifteen gram samples were taken from each plot and sent for analysis of fatty acid content in seed oil to the Industrial Crop Laboratory, Northern Research and Development Division, Agricultural Research Service, USDA, Peoria, Illinois.

At the Peoria laboratory, seed oil was analyzed for fatty acid content by methods described by Smith *et al.* (10) for C20 and C22 monoenes, and by Bagby *et al.* (1) for C22 dienes. The percent of seed oil composed of other than C20 and C22 fatty acids was calculated by subtracting the sum of C20 and C22 fatty acids from 100 percent.

Data were analyzed statistically using the analysis of variance technique. Means having significant F values were subjected to Duncan's multiple range test. Coefficient of variation and standard error of the mean values were also calculated. Linear correlations were calculated between relevant parameters.

RESULTS AND DISCUSSION

C20 Monoene Fatty Acids

Almost without exception, the C20 monoene content was reduced by N application in both years of the study (Table 9 and Figure 3). C20 monoene content was greatest in the control plots.

Analysis of samples from the trial following wheat revealed that the C20 monoene content in the control plots was higher than other treatments except 100 N applied in May. The 100 N March and 50 N May treatment reduced the C20 monoene content. There were no differences observed in the C20 monoene content between the 100 N May and 50 N March treatments, however these were lower than the control. The 50 N March and 100 N May treatments were not different from each other in C20 monoene content.

In a similar fashion, C20 monoene content after alfalfa was reduced with increased rates of N applied at the two dates. The control was again greater in C20 monoene content than all other treatments. N at the low rate in March and May caused a slight reduction in C20 monoene content while the high rate in March created the greatest decrease. The C20 monoene level was reduced by the 100 N rate in May, but this level was not different from other rates and dates.

In 1974, 100 N as both AS and AN resulted in the lowest content of C20 monoene fatty acids. The 100 N as AN, however, was not different in C20 monoene content from either the 50 N as AS or AN treatments.

C20 monoene content was positively correlated to seed oil content both years (Table 10). Seed oil content decreased with higher rates of N application (Chapter I). Therefore, as N rates increase, seed oil and

Table 9. The Effect of Rate, Date, and Form of Nitrogen ^{1/} Application on the Percent of L. alba Seed Oil Composed of C20 Monoene Fatty Acids.

Treatments	1973 ^{2/}		1974 ^{3/}
	After Wheat	After Alfalfa	
CONTROL	62.7 ^{4/} a ^{5/}	62.4 a	61.2 a
50N AS MARCH	61.3 b	61.3 b	60.4 abc
100N AS MARCH	59.7 c	60.4 c	58.7 d
50N AN MARCH	-	-	60.5 ab
100N AN MARCH	-	-	59.4 bcd
50N AS MAY	59.7 c	61.7 b	-
100N AS MAY	61.5 ab	60.9 bc	-
Coefficient of Variation	1.2%	0.8%	1.3%
Standard Error of the Mean	0.29	0.25	0.38

^{1/} kg/ha of N, as AS = ammonium sulfate, and AN = ammonium nitrate.

^{2/} Hyslop Agronomy Farm, Corvallis, Oregon.

^{3/} Jackson Farm, Lebanon, Oregon.

^{4/} Means of four replications.

^{5/} Means with columns followed by the same letter(s) are not significant from each other at the .05 level of P according to Duncan's multiple range test.

Figure 3. C20 Monoene Fatty Acid Content of *L. alba* Seed Oil as Affected by Rate, Date, and Form of Nitrogen Application.

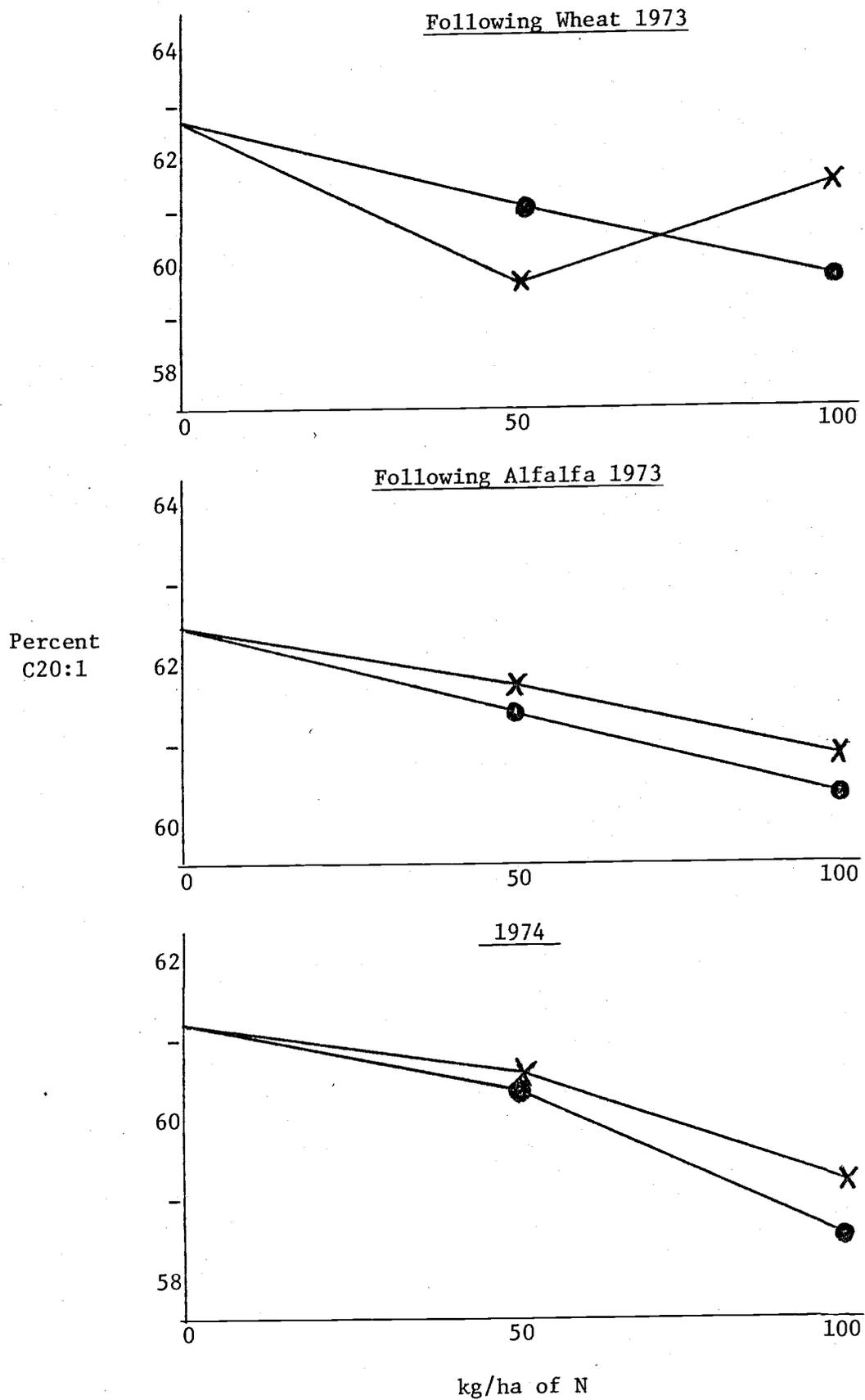


Table 10. Simple Correlation Coefficients of Oil Content and Fatty Acid Components of Oil from L. alba. 1/

Factors	C20:1 Monoene	C22:1 Monoene	C22:2 Diene	Non C20-22
Oil	----- .484 ^{2/} ** .749 ^{3/} **	-.880*** .436	.315 -.886**	.192 -.301
C20:1 Monoene	-----	-.446** .005	.224 -.915***	-.610** -.537*
C22:1 Monoene	-----	-----	-.389* -.337	-.295 -.170
C22:2 Diene	-----	-----	-----	-.371* .449*

1/ Maximum, Minimum, Average, and standard error of the mean values are presented in Appendix Table 4.

2/ r values of 1973 data (n = 40).

3/ r values of 1974 data (n = 20).

C20 monoene content are reduced.

It seems that N availability shortly before or during flowering has an adverse effect on the synthesis of C20 monoene fatty acids. This effect could be caused by metabolic regulation of C20 monoene synthesis, the preferential synthesis of other fatty acids, or by other unexplained events which occur when adequate levels of N are available for increased plant growth and seed development.

C22 Monoene Fatty Acids

N rates and dates of application in 1973 had a significant effect, increasing the C22 monoene content in extracted oil (Table 11 and Figure 4). However, in 1974 no differences were recorded for either the rate or form of N application.

Following wheat, earlier applications of 100 N showed the greatest increase in the percent of C22 monoene fatty acids in oil. The 100 N in May treatment resulted in a lower content of C22 monoenes than the same rate in March, but was higher than the 50 N rate in March. This suggests that the stimulus to C22 monoene fatty acid development was not as great from the May treatment as was the same rate during March. The May application of 50 N did not change the C22 monoene content of oil.

When L. alba followed alfalfa, rates of 100 N during March and May increased the C22 monoene content in extracted oil the greatest amount. The C22 monoene content resulting from the 50 N rate in March was not different from the 50 N rate in May or the control.

A negative correlation ($r = -.880^{**}$) of C22 monoene and seed oil content was recorded in 1973 (Table 10). Since seed oil content decreases

Table 11. The Effect of Rate, Date, and Form of Nitrogen ^{1/} Application on the Percent of L. alba Seed Oil Composed of C22 Monoene Fatty Acids.

Treatments	1973 ^{2/}		1974 ^{3/}
	After Wheat	After Alfalfa	
CONTROL	19.3 ^{4/} d ^{5/}	19.4 c	17.3 NS ^{6/}
50N AS MARCH	20.2 c	20.0 bc	17.1
100N AS MARCH	21.9 a	21.5 a	16.8
50N AN MARCH	-	-	17.6
100N AN MARCH	-	-	17.2
50N AS MAY	19.3 d	20.4 b	-
100N AS MAY	20.7 b	22.1 a	-
Coefficient of Variation	0.9%	2.8%	2.3%
Standard Error of the Mean	0.20	0.29	0.19

^{1/} kg/ha of N, as AS = ammonium sulfate, and AN = ammonium nitrate.

^{2/} Hyslop Agronomy Farm, Corvallis, Oregon.

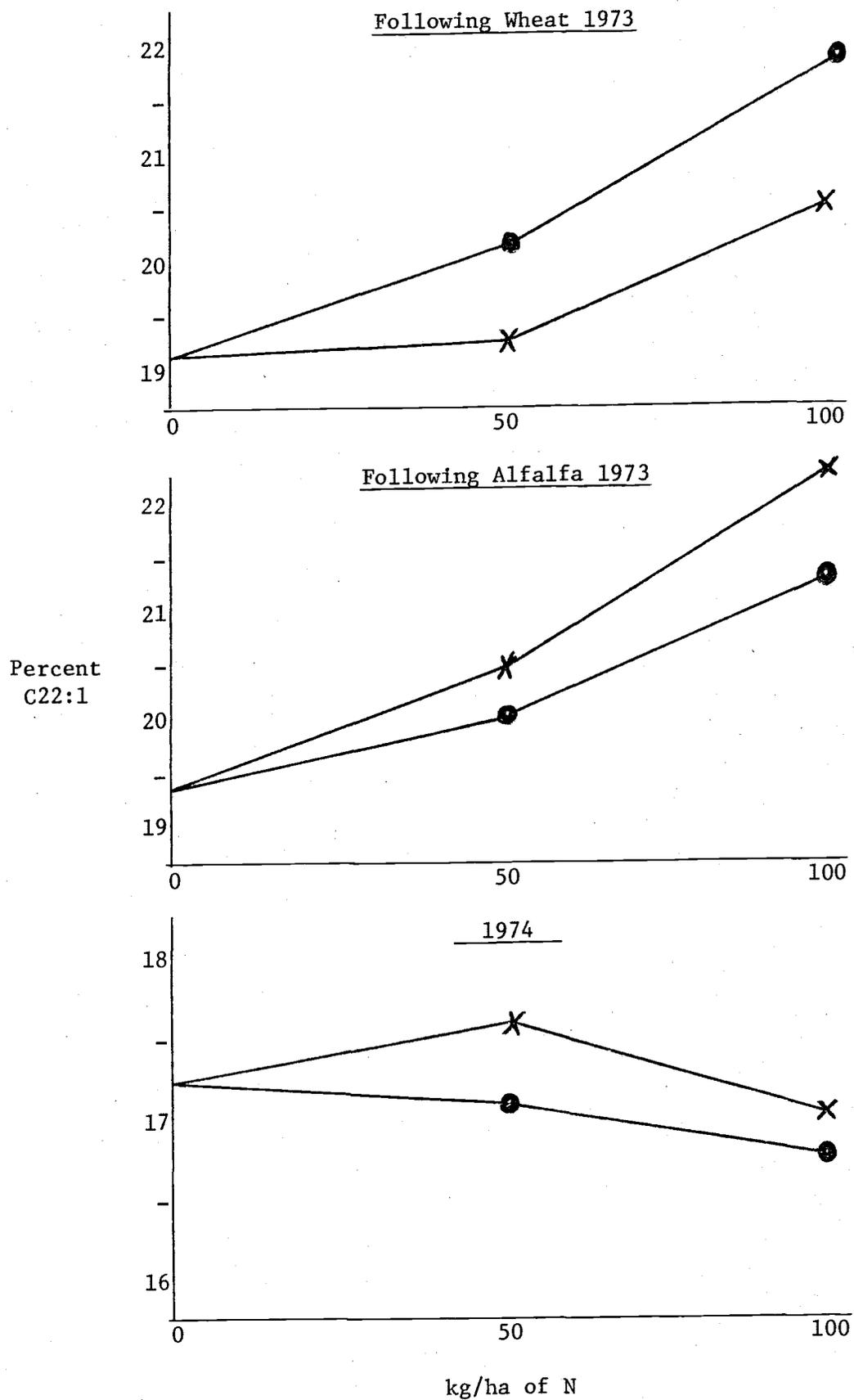
^{3/} Jackson Farm, Lebanon, Oregon.

^{4/} Means of four replications.

^{5/} Means within columns followed by the same letter(s) are not significant from each other at the .05 level of P according to Duncan's multiple range test.

^{6/} Not significant at the .05 level of P.

Figure 4. C22 Monoene Fatty Acid Content of *L. alba* Seed Oil as Affected by Rate, Date, and Form of Nitrogen Application.



with high rates of N fertilization (Chapter I), this type of relationship would be expected because C22 monoene increased with higher rates of N. There was also a negative correlation (-.446**) recorded between C22 monoenes and C20 monoenes in 1973. This type of relationship seems valid, since the former increased while the latter decreased with higher rates of N fertilization.

Since there was a negative relationship recorded between C20 and C22 monoenes in 1973, it could be hypothesized that the increase in C22 monoenes was at the expense of the C20 monoenes; since the former increased with higher rates of N while the latter decreased. Again, it could be reasoned that under adequate levels of N, there is a regulatory shunt of C20 to C22 monoene fatty acids.

C22 Diene Fatty Acids

C22 diene fatty acid content of seed oil was reduced by later dates and higher rates of N application in 1973 (Table 12 and Figure 5). But, in contrast, C22 dienes increased with high rates of N in 1974.

Following wheat, C22 diene content was decreased by both May application rates of N. The control and March application rates were not different from each other, but were higher in C22 diene content than the May applications.

When L. alba followed alfalfa, a reduction in the C22 diene content resulted from the application of 100 N in May. C22 diene content resulting from the March application of 50 N, although not different from the control, was greater than the other treatments. The control did not differ from either the 100N rate in March or 50 N in May treatments in C22

Table 12. The Effect of Rate, Date, and Form of Nitrogen ^{1/} Application on the Percent of L. alba Oil Composed of C22 Diene Fatty Acids.

Treatments	1973 ^{2/}		1974 ^{3/}
	After Wheat	After Alfalfa	
CONTROL	16.7 ^{4/} a ^{5/}	16.9 ab	19.5 c
50N AS MARCH	16.9 a	17.4 a	20.4 bc
100N AS MARCH	16.8 a	16.5 b	22.0 a
50N AN MARCH	-	-	19.9 c
100N AN MARCH	-	-	20.8 b
50N AS MAY	16.0 b	16.7 b	-
100N AS MAY	16.0 b	15.4 c	-
Coefficient of Variation	1.6%	2.0%	2.7%
Standard Error of the Mean	0.13	0.17	0.27

^{1/} kg/ha of N, as AS = ammonium sulfate, and AN = ammonium nitrate.

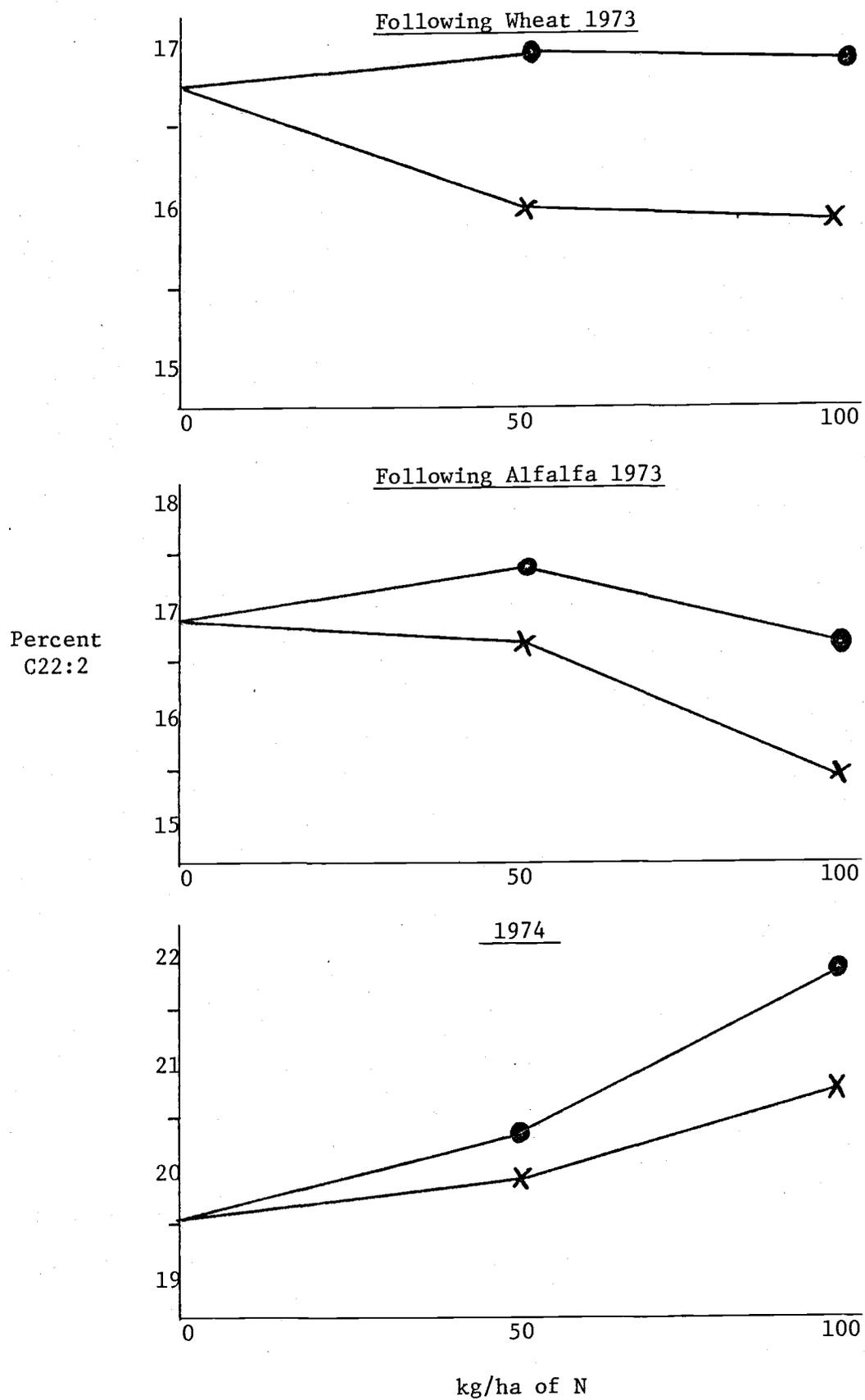
^{2/} Hyslop Agronomy Farm, Corvallis, Oregon.

^{3/} Jackson Farm, Lebanon, Oregon.

^{4/} Means of four replications.

^{5/} Means within columns followed by the same letter(s) are not significant from each other at the .05 level of P. according to Duncan's multiple range test.

Figure 5. C22 Diene Fatty Acid Content of *L. alba* Seed Oil as Affected by Rate, Date, and Form of Nitrogen Application.



diene content.

In 1974, C22 diene content was increased the greatest amount from 100 N as AS. The 50 N rates as AS or AN were not different in C22 diene content from the control. The 50 N as AS and 100 N as AN treatments were not different from each other in C22 diene content.

Seed oil and C22 diene content were negatively correlated in 1974 (Table 10). Seed oil was reduced by both rates and forms of N, and C22 diene content increased with high rates of N. Also in 1974, a negative correlation of C22 diene and C20 monoene fatty acids was recorded. C20 monoenes were reduced and C22 dienes were increased with high rates of N. In 1973, a negative correlation was recorded between C22 monoenes and dienes. As N application rates were increased, C22 monoene content increased and C22 diene content was reduced.

Results of the 1973 experiments, being in agreement with other reports (4,12), suggests that the longer chain, higher unsaturated fatty acids are reduced with late N applications. Dybing (4), studying flax, found that even in controlled environments excessive rates of N reduced the percentage of linoleic and linolenic acid content of seed oil. Oleic acid, a C16 monoene, increased at the expense of the two former acids.

In the 1973 study, as C22 diene fatty acids decreased, C22 monoenes increased. A negative relationship between these two fatty acids suggest that synthesis of C22 dienes was not favored physiologically over the C22 monoene fatty acid.

However, in 1974 content of C22 diene fatty acids was much higher than the previous year, and N application resulted in higher rather than lower C22 diene content of seed oil. These results do not agree with

those reported by Dybing (4). Possibly weather conditions during flowering and seed maturation in 1974 could account in part for this discrepancy. Plots fertilized with the higher rates of N exhibited delayed flowering. Other researchers have reported that when seed development in soybeans, cotton, and sunflowers occurs during cooler periods, longer chain, higher unsaturated fatty acids are increased (7,12,9). In this study, with delayed and prolonged flowering in 1974 from high N rates, seed developed later in the spring and were subjected to warmer weather conditions than seeds which developed earlier (Appendix Tables 1 and 2). This did not result in a lower level of unsaturation, but rather a higher level. This suggests that either C22 diene fatty acid formation does not follow pattern of nutritional response as observed in other oilseed crops or there was an environmental or physiological factor not accounted for.

Fatty Acids Other Than C20-22

Rate and date of N application had a mixed effect on the percent of seed oil composed of fatty acids other than C20 and C22 (NC20-22) in 1973 (Table 13 and Figure 6). In 1974, no differences were recorded.

The NC20-22 component of seed oil was increased from the May application of 50 N when L. alba followed wheat. Other treatments did not differ from each other or the control in NC20-22 content. However, after alfalfa the 100 N rate in March and May increased the NC20-22 content of seed oil. The other treatments were not different from the control.

The NC20-22 component of seed oil was negatively correlated to C20 monoene content both years (Table 10). The latter component decreased with higher N rates while the former increased. A negative and positive

Table 13. The Effect of Rate, Date, and Form of Nitrogen ^{1/} Application on the Percent of L. alba Seed Oil Composed of Other Than C20 and C22 Fatty Acids.

Treatment	1973 ^{2/}		1974 ^{3/}
	After Wheat	After Alfalfa	
CONTROL	1.28 ^{4/} b ^{5/}	1.28 b	2.43 NS ^{6/}
50N AS MARCH	1.63 b	1.30 b	2.13
100N AS MARCH	1.58 b	1.68 a	2.58
50N AN MARCH	-	-	1.90
100N AN MARCH	-	-	2.70
50N AS MAY	5.00 a	1.28 b	-
100N AS MAY	1.88 b	1.55 ab	-
Coefficient of Variation	41.3%	12.5%	18.0%
Standard Error of the Mean	0.47	0.09	0.21

^{1/} kg/ha of N, as AS = ammonium sulfate, and AN = ammonium nitrate.

^{2/} Hyslop Agronomy Farm, Corvallis, Oregon.

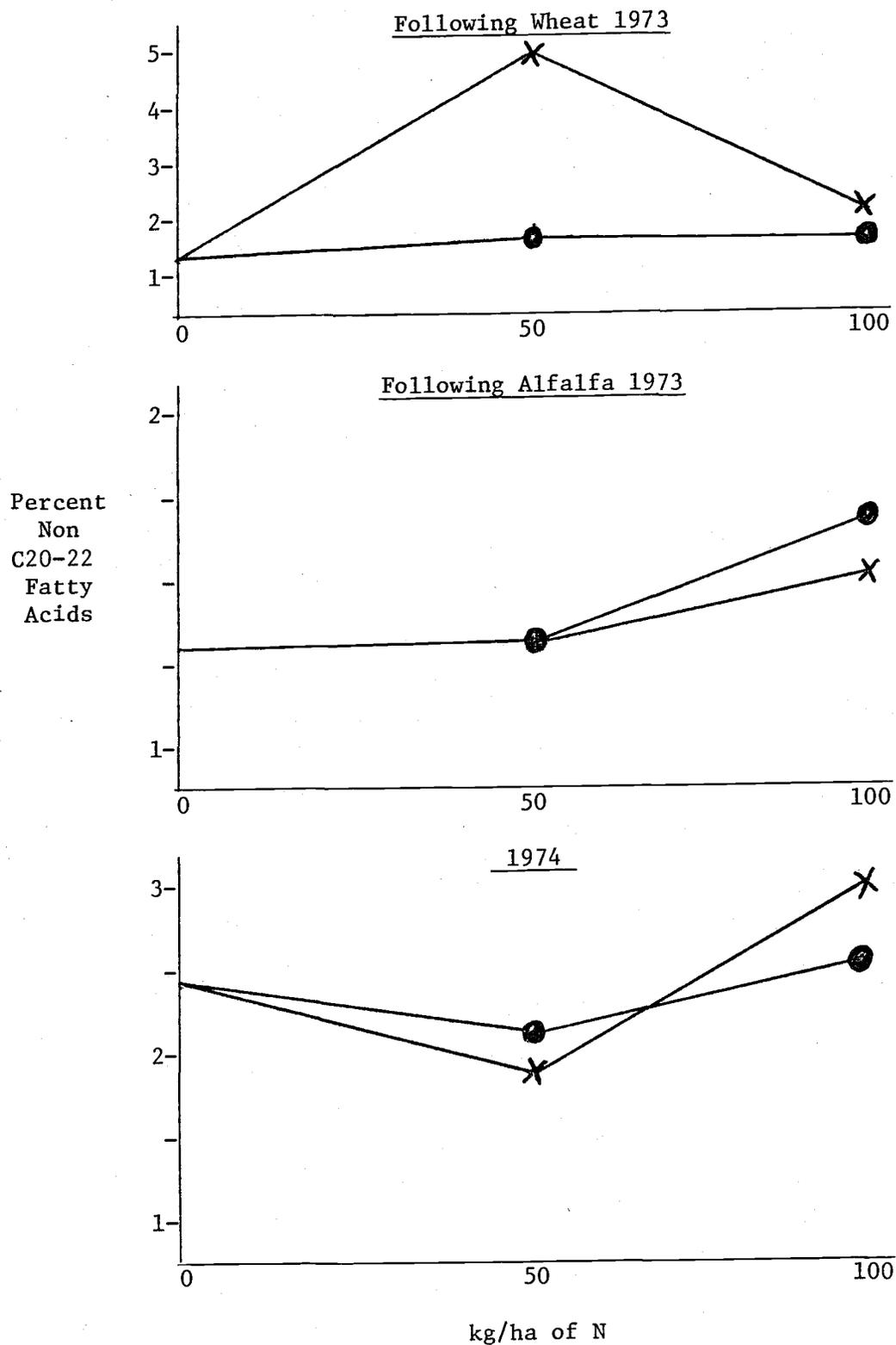
^{3/} Jackson Farm, Lebanon, Oregon.

^{4/} Means of four replications.

^{5/} Means within columns followed by the same letter(s) are not significant from each other at the .05 level of P., according to Duncan's multiple range test.

^{6/} Not significant at the .05 level of P.

Figure 6. Content of Other Than C20 and C22 Fatty Acids in *L. alba* Seed Oil as Affected by Rate, Date, and Form of Nitrogen Application.



correlation was recorded between the NC20-22 content and C22 diene content in 1973 and 1974 respectively.

It is indicated from these results that the NC20-22 component of seed oil can be influenced by N rates and dates of application. But, from an agronomic standpoint the practical significance of these small differences is questionable.

Results of these investigations demonstrate that the various fatty acid constituents of L. alba seed oil can be influenced by N fertility management practices. Taking into consideration the wide range of variation of these constituents existing between selected accessions of the genus (Miller et al.), and the variation in fatty acids resulting from N management practices, it seems feasible that plant selection, breeding, and N application at optimum dates could result in an oil with a desirable blend of fatty acids for industrial use.

LITERATURE CITED

1. Bagby, M. O., C. R. Smith, T. K. Miwa, R. L. Lohmar, and I. A. Wolff. 1961. A Unique Fatty Acid from Limnanthes douglasii Seed Oil: The C22 Diene. Jour of Organic Chem. 26: 1261-1265.
2. Collins, F. L., and R. W. Howell. 1957. Variability of Linoleic and Linolenic Acid in Soybean Oil. Jour of Amer Oil Chem Soc. 34: 491-493.
3. Cook, A. A. 1971. Thar She Grows. The Farm Index. October. pp. 4-6. Economic Research Service, USDA.
4. Dybing, C. D. 1964. Influence of Nitrogen Level on Flax Growth and Oil Production in Varied Environments. Crop Sci. 4: 491-494.
5. Earle, F. R., E. H. Melvin, L. H. Mason, C. H. Van Etten, and I. A. Wolff. 1959. Search for New Industrial Oil I. Selected Oil from 24 Plant Families. Jour of Amer Oil Chem Soc. 36: 304-307.
6. Gentry, H. S., and R. W. Miller. 1965. The Search for New Industrial Crops IV. Prospectus of Limnanthes. Econ Bot. 19: 25-32.
7. Howell, R. W., and F. I. Collins. 1957. Factors Affecting Linolenic and Linoleic Acid Content in Soybean Oil. Agron J. 49: 593-597.
8. Jones, Q., and I. A. Wolff. 1961. Using Germ Plasm for New Products. Germ Plasm Resources. AAAS, Washington, D.C. pp. 265-277.
9. Kinman, M. L., and F. R. Earle. 1964. Agronomic Performance and Chemical Composition of the Seed of Sunflower Hybrids and Introduced Varieties. Crop Sci. 4: 417-420.
10. Miller, R. W., M. E. Daxenbichler, and F. R. Earle. 1964. Search for New Industrial Oils VIII. The Genus Limnanthes. Jour of Amer Oil Chem Soc. 41: 167-169.
11. Smith, C. R., M. O. Bagby, T. K. Miwa, R. L. Lohmar, and I. A. Wolff. 1960. Unique Fatty Acids from Limnanthes douglasii Seed Oil: The C20 and C22 Monoenes. Jour of Organic Chem. 25: 1770-1774.
12. Stansbury, M. F., C. L. Hoffpauir, and T. H. Hopper. 1953. Influence of Variety and Environment on the Iodine Value of Cottonseed Oil. Jour of Amer Oil Chem Soc. 30: 120-123.

INFLUENCE OF RATE AND FORM OF NITROGEN APPLICATION
ON SEED WEIGHT, GERMINATION, AND SEEDLING
GROWTH OF LIMNANTHES ALBA

ABSTRACT

The effect of various levels and forms of nitrogen (N) on seed characteristics in Limnanthes alba was studied under field conditions. Treatments used were a control (no N applied), and two rates of N (50 and 100 kg/ha) in two forms of N. The forms used were ammonium sulfate (AS) and ammonium nitrate (AN). From harvested plots, seed weight, germination, and seedling growth determinations were made. The relationships between oil, protein, and seed characteristics were evaluated.

N rate and form had no effect on seed weight. However, seed weight was found to be positively correlated to oil and negatively correlated to protein content.

Germination was higher in seed samples where 100N as AS was applied. Germination percent in other treatments was not different from the control. Percent germination was positively correlated to protein and negatively related to oil content of seed.

Root and shoot growth were not correlated to seed protein or oil content. Both rates of AN and 50N as AS increased shoot growth, whereas 100 N as AS did not. Root growth at 14 days was increased by both rates of AN, whereas AS treatments were not different from the control. At 16 days, the two rates and forms of N were not different from the control, but 100 N as AS had less root growth than either rate of AN.

INTRODUCTION

Limnanthes spp. seed oils are unique (8) and are a possible substitute for sperm whale oil (2). Since the genus is well adapted to the area (11), there has been recent interest in Limnanthes as a potential oilseed crop for Western Oregon. It was therefore desirable to examine management practices as they affect germination and seedling growth. Such information would be of benefit as we consider stand establishment.

Studies on germination of the genus Limnanthes have been limited. Seedling growth studies measuring the effect of management practices are non-existent.

In other crops, seed weight and chemical composition, factors often influenced by management practices, have been shown to affect germination and seedling growth (9).

Toy and Willingham (12,13) studying 10 accessions of the genus, representing 7 species, observed secondary seed dormancy as an effect of temperature during germination. They also measured speed of germination of the ten accessions at various temperature levels. Cole (1) studying two accessions of L. alba used a two-way thermogradient plate to determine optimum temperature and light conditions for germination.

If L. alba proves to be feasible for production in Western Oregon, there will be an effort to increase seed for distribution to growers. The objective of this study was to determine what N application rate and form will be most favorable for high quality seed production, and what influence these management practices will have on seed weight, germination, and seedling growth. In addition, relationships of these seed characteristics to seed chemical components (protein and oil) are reported.

MATERIALS AND METHODS

A nitrogen (N) fertility trial was conducted at the Jackson Farm, Lebanon, Oregon in 1973-1974. The experiment was located on a Dayton series soil, previously cropped with soft white winter wheat (Triticum aestivum L.). A weather summary for the crop year is presented in Appendix Table 2.

After initial seedbed preparation, an entire range was seeded solid, using a standard grain drill set at 17 kg/ha, with row spacings of 17.8 cm, or approximately 4 seeds per dm of row. Planting took place October 15, 1973. During the first week of March, N treatments were applied using a hand-moved spreader.

The experimental design was a randomized complete block, with five treatments and four replications. Treatments included a control (no N applied), and two rates of N (50 and 100 kg/ha) applied in two forms. The forms used were ammonium sulfate (AS) and ammonium nitrate (AN). At the time of N application, plants were primarily in a vegetative stage of growth.

During the first week of July, 1974, entire plots (1.2 x 6.1 m) were harvested using a small plot combine. Harvested seed samples were dried, cleaned, and weighed. Samples for seed weight, germination, and seedling growth determinations were obtained from these lots.

Plot design for seed weight, germination, and seedling growth determinations was a randomized complete block with sub-samples. The number of sub-samples for the three experiments varied. The data were statistically analyzed using the analysis of variance technique. Data with significant F values were subjected to Duncan's multiple range test. Coef-

ficient of variation and standard error of the mean were also calculated.

A simple linear correlation analysis of seed weight, germination, and seedling growth to seed oil and protein content was calculated to determine if any significant relationships existed.

Seed Weight Determinations

Two sub-samples of 15 grams were taken from each treatment replicate after cleaning. From each sub-sample, 1000 seeds were counted using a Count-A-Pak electric eye counter. Each 1000 seed sample was weighed in grams, and the two sub-sample values were averaged.

Germination Studies

Samples for germination studies were sized. Seeds which would pass through a number eight but not through a number seven screen were used. Since the experiment was being conducted to determine the influence of treatments and chemical components (protein and oil) on germination, mechanically damaged seed was not selected, as damage would primarily reflect the quality of harvesting techniques.

Five samples of 50 seeds each were taken from each treatment replicate. This gave a total of 20 samples for each treatment. Eleven cm petri dishes were used with a 10 cm diameter by 5 mm thick foam sponge and 10 cm diameter blotter paper. Seed samples were moistened adequately with distilled water.

Petri dishes were placed in a growth chamber at 15° C for eight hours and 10° C for 16 hours in continuous dark. This diurnal temperature scheme was selected as optimum from the report by Cole (1).

Samples were allowed 14 days for completion of germination. Any seed in which the radicle had penetrated the seed coat was considered germinated (Cole 1973).

Seedling Growth Determinations

From each treatment replicate, a 15 gram seed sample was sized for uniformity. Those which would pass through a number eight but not through a number seven screen were used for the study. Once screened, five sub-samples of ten seeds each were placed in Seed Pack Growth Pouches ^{1/}. Each germination pouch received 20 ml of 25% Hoagland's nutrient solution.

The pouches were placed into a growth chamber in a wood-framed slotted box. A thermoperiod of 15° C for eight hours and 10° C for 16 hours was used. After ten days of continuous dark, chamber lights were activated. The photoperiod was eight hours of light and 16 dark, to correspond with the thermoperiod. Also at ten days, pouches received an additional 10 ml of distilled water.

After 14 days, pouches were removed and shoot and root measurements were recorded. Each sub-sample mean was composed of the sum of root or shoot lengths (in mm's) divided by the number of seedlings germinated.

At 16 days, measurements were repeated and the experiment was ended.

^{1/} Manufactured by Scientific Products. These pouches are made of clear plastic material and are 10 x 20 cm in size. Blotter paper with a V-shaped trough at the top of the pouch allows placement of seed and shoot growth measurements originated from this trough. The clear plastic design of the pouch allows non-destructive measurement of root growth.

RESULTS AND DISCUSSION

Seed Weight

Seed weight was not affected by rate and form of N application (Table 14). However, a trend towards reduced seed weight with increased N rates is evidenced (Figure 7).

Since seed weight was correlated to oil content (Table 15) and increased N levels reduced oil content (Chapter I), it would follow that seed weight could be reduced by N application. To the contrary, some researchers working with safflower have reported a negative correlation between kernal weight and oil content ($r = -.790^{**}$) (3).

Seed weight in this study was negatively correlated to protein content ($r = -.453^{*}$). This seems logical since there was negative relationship observed between oil and protein content ($r = -.962^{***}$), and seed weight was positively related to oil content.

Further investigations using a larger number of samples per replicate may allow the establishment of some significant trends.

Germination

Germination of L. alba was influenced by N application. The 100 N as AS treatment increased germination percent (Table 14 and Figure 8). Germination was not affected by rate or form of other N treatments. This difference could be attributed to protein levels of the seed lots. The high rate of AS was recorded in other studies (Chapter I) to result in the highest level of seed protein. Seed protein resulting from N application was shown to be positively correlated to germination ($r = .522^{*}$). These

Table 14. The Effect of Rate and Form of Nitrogen ^{1/} Application on Seed Weight, Germination, and Seedling Growth of L. alba, 1974.

Treatment	Seed Weight g/1000	Percent Germination	Seedling Growth (mm)			
			Shoots 14 Days	Shoots 16 Days	Roots 14 Days	Roots 16 Days
CONTROL	7.61 ^{2/}	88.3 b ^{3/}	16.0 c	22.8 c	34.7 bc	56.6 ab
50N AS	7.36	89.7 b	19.5 b	25.9 b	37.6 b	54.8 ab
100N AS	7.28	93.9 a	16.6 c	23.6 c	34.2 c	52.3 b
50N AN	7.47	90.2 b	20.2 b	26.8 b	48.8 a	59.1 a
100N AN	7.40	90.9 b	23.2 a	30.4 a	50.6 a	58.7 a
Coefficient of Variation	2.5%	5.1%	10.2%	7.8%	12.5%	12.6%
Standard Error of the Mean	0.06	0.52	0.44	0.45	1.15	1.58

^{1/} kg/ha of N, as AS = ammonium sulfate, and AN = ammonium nitrate.

^{2/} Means of four replications.

^{3/} Means within columns followed by the same letter(s) are not significantly different from each other at the .05 level of P. according to Duncan's Multiple range test.

Figure 7. The Effect of Rate and Form of Nitrogen Application on Seed Weight of *L. alba*, 1974.

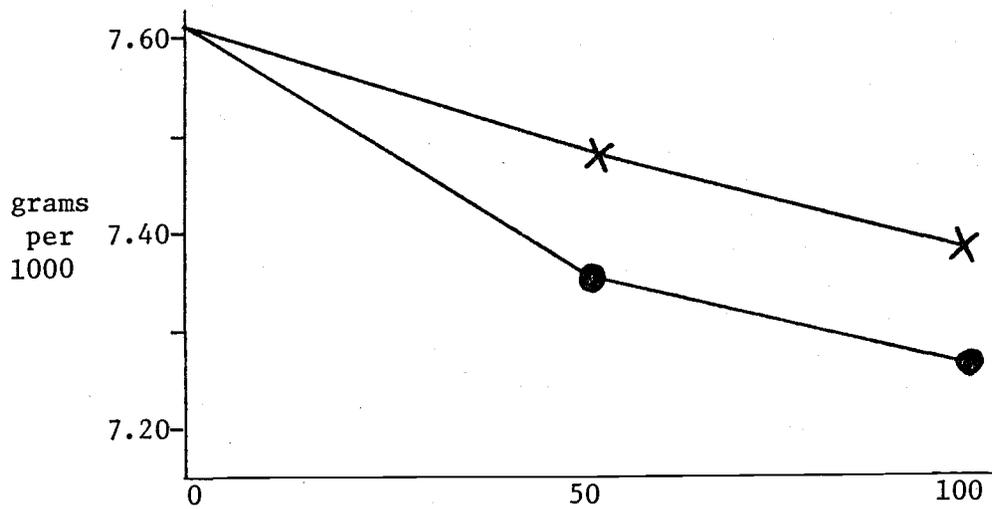


Figure 8. The Effect of Rate and Form of Nitrogen Application on the Percent Germination of *L. alba* Seed, 1974.

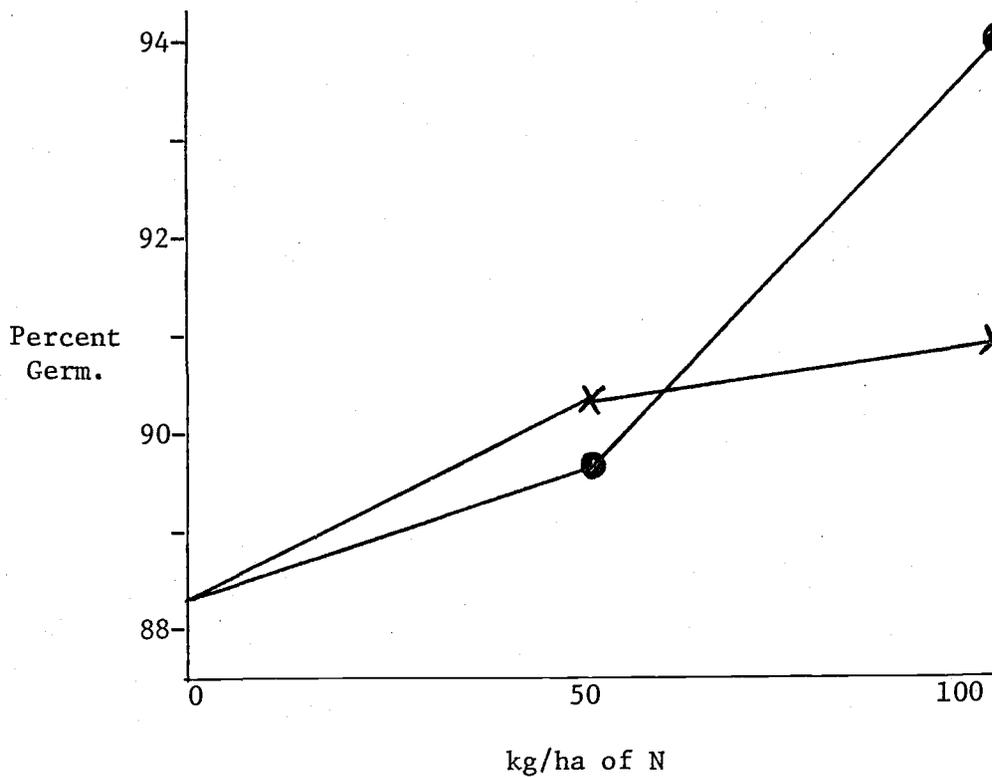


Table 15. Simple Correlation Coefficients of Some Seed Quality and Seedling Growth Characters of L. alba, 1974. 1.2/

Character	Oil	Germination	Shoots 14 Days	Shoots 16 Days	Roots 14 Days	Roots 16 Days	Seed Weight
Protein	---- -.962***	.522*	.011	.042	-.112	-.130	-.453*
Oil	-----	-.588**	.035	-.011	.136	.181	.536*
Germination	-----	-----	-.109	-.079	-.103	-.216	-.240
Shoots 14 Days	-----	-----	-----	.970***	.872***	.609**	-.221
Shoots 16 Days	-----	-----	-----	-----	.906***	.661**	-.183
Roots 14 Days	-----	-----	-----	-----	-----	.789**	-.062
Roots 16 Days	-----	-----	-----	-----	-----	-----	.031

1/ Number of observations = 20.

2/ A data description of the above correlation analysis characters is presented in Appendix Table 5.

3/ The following codes indicate levels of significance: *p = .05, **p = .01, and ***p = .001.

results are in agreement with many of the research findings summarized by Pollock and Roos (9) in their review of seeds and seedling vigor.

Oil content was negatively correlated with germination ($r = -.588^*$). This would follow since there was a negative relationship between protein and oil ($r = -.962^{***}$), and germination was positively related to protein.

Seedling Growth

Shoot growth at 14 days was increased by 50N as AS and AN, and by the 100N as AN treatments (Table 14 and Figure 9). Shoot growth was increased to a greater degree by 100N as AN than by other treatments.

The same responses were recorded for the 16 day measurements. Here again, 100N as AN resulted in a greater shoot growth response than other treatments, whereas the high rate as AS was not different from the control.

Root growth at 14 days was increased by the 50 and 100N as AN treatments (Table 14 and Figure 10). Although root growth of the control was not different from that of both rates of AS, the 100N rate of AS was less than the 50N rate of the same form.

The 16 day root growth measurement exhibited similar trends. Root growth in the control and 50N as AS treatments did not differ from the other treatments. But, 100N as AS had less root growth than both rates of the AN treatments.

Interestingly, the roots exhibited either negative or no growth responses to both rates of AS, whereas shoot growth was increased by the 50N as AS treatment.

The results observed in this study show that high protein level seed lots do not necessarily result in increased seedling growth. This is in

Figure 9. The Effect of Rate and Form of Nitrogen on Seedling Growth of L. alba at 14 and 16 Days.

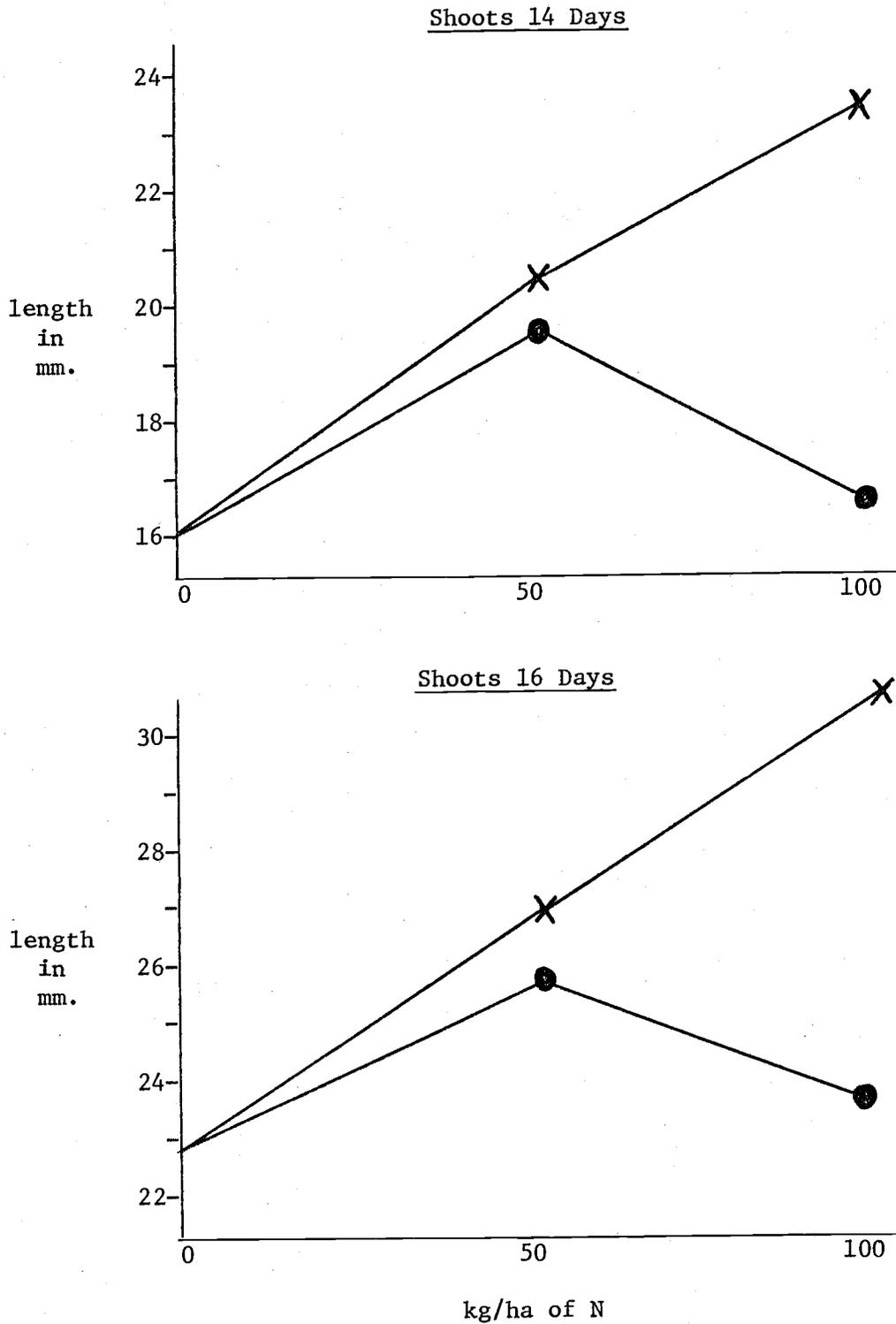
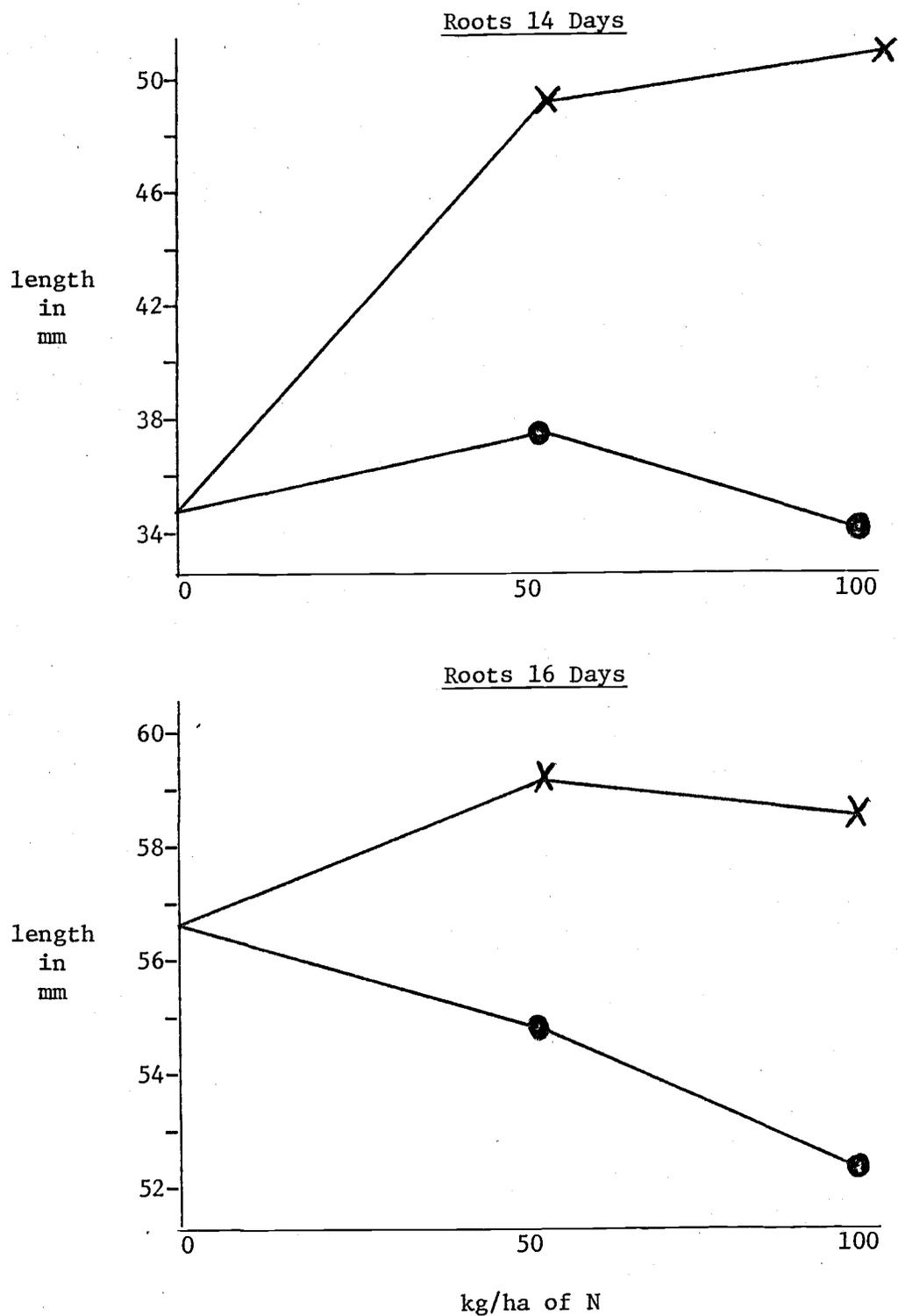


Figure 10. The Effect of Rate and Form of Nitrogen on Seedling Growth of *L. alba* at 14 and 16 Days.



contradiction to reports of other researchers who found seedling growth to be directly and positively related to seed protein (5,6,7,9,10).

In all measurements, a trend towards a reduction of seedling growth from high protein level seed and of a difference in growth response to form of N was noted. In fact, the treatment with the highest seed protein content (100 N as AS) repeatedly had less growth than 100 N as AN.

When protein was correlated to seedling growth, no significant relationships were established for roots and shoots at either date of measurement ($r = .011, .042, -.112, \text{ and } -.130$ respectively). This would be expected since one treatment with high seed protein had the least seedling growth, and another treatment (100N AN) also having high seed protein had the greatest seedling growth.

Ries and Everson (10) and others (7,6) studying seed protein and seedling growth relationships of wheat have reported significant correlations between these parameters ($r = 0.92, 0.93, \text{ and } 0.926$ respectively). One possible explanation for the contradiction between the reports of these researchers and those of this study is that as N rates increase, especially with the AS form (Figure 7), seed weight decreases (not significant though). Associated with increasing N rates in the AS form is increased protein content. As explained in the materials and methods section, seeds from treatments were sized. With the smaller seed removed, then quite possibly those seeds with higher concentrations of protein were removed.

Lopez and Grabe (5), studying seed quality of barley and wheat, observed that when high N application rates were made to increase seed protein, seed size decreased. High protein seeds were obtained at the ex-

pense of seed size and yield.

Another explanation is that in the field experiments, plots receiving the 100 N as AS treatment were observed as having a delayed and prolonged flowering period. Since this treatment delayed flowering, results of germination and seedling growth studies may well be expected to differ from those of other treatments, as the seeds were exposed to and developed under entirely different environmental and physiological conditions. Pollock and Roos (9) concluded similarly that during curing, seeds of different stages of maturity are exposed to different sets of environmental conditions, and subsequent seed quality is affected.

Additionally, from studies of safflower seed development, Leininger and Urie (4) reported that maximum germination of safflower varieties occurred at 14-16 days after flowering, but maximum seedling vigor did not occur until approximately seven days later. Pollock and Roos (9) also reported that the more mature a seed when harvested, the greater its vigor. Results of this study suggest that a similar type of maturity pattern could have occurred in seed from the 100N as AS treatment observed as having delayed maturity. Consequently, even with a high level of seed protein, possibly responsible for the high germination recorded, seeds from the 100N as AS treatment may not have been physiologically mature enough to have a greater rate of seedling growth.

Roots and shoots were not similar in their growth response to rate and form of N treatments. Root growth from the AS treatment showed either a negative or no growth response when compared to the control. Since roots and shoots were not similar in growth responses, it is suggested that the differences were due to variation in biochemical components available to

these organs resulting from N application.

Results reported here suggest that AN at 50 or 100 N would be the most favorable N fertilization practice for producing high quality seed. Even though the highest germination percent was observed with 100 N as AS, this treatment resulted in either a negative or no effect on seedling growth. Therefore, this treatment would not be desirable when stand establishment qualities are of importance.

It must be mentioned though, that the N rate and form which appears most adventitious from the standpoint of germination and seedling growth qualities may not be consistent with the rate and form appearing most desirable for yield, or oil and protein content of seed. Since AN has not been observed to increase seed yield (Chapter I), further studies should be attempted to establish if there is an advantage in using AN as a N fertility practice.

In future experiments, it is recommended that speed of germination or germination energy be included in efforts to determine what influence seed oil and protein content, resulting from N application rates, have on emergence. This would be particularly helpful in determining true stand establishment potential. Methods employed in this study evaluated germination at only one date- 14 days, and did not allow for establishing differences on germination vigor during the first two weeks of growth: a period of critical importance under field conditions.

LITERATURE CITED

1. Cole, D. F. 1973. Effect of Light and Temperature on Germination of Two Accessions of Limnanthes alba Seed. Unpublished Data. Seed Quality Laboratory, AMPI, ARS, USDA, Beltsville, Md.
2. Cook, A. A. 1971. Thar She Grows. The Farm Index. ERS, USDA. Oct. pp. 4-6.
3. Hoag, B. K., J. C. Zubriski, and G. N. Geiszler. 1968. Effect of Fertilizer Treatments and Row Spacing on Yield, Quality, and Physiological Response of Safflower. Agron. J. 60: 198-200.
4. Leininger, L. N., and A. L. Urie. 1964. Development of Safflower Seed From Flowering to Maturity. Crop Sci. 4: 83-87.
5. Lopez, A., and D. F. Grabe. 1971. Effect of Seed Protein Content on Plant Growth of Barley and Wheat. Agron Abst. pp. 44.
6. Lowe, L. B., G. S. Ayers, and S. K. Ries. 1972. Relationships of Seed Protein and Amino Acid Composition to Seedling Vigor and Yield of Wheat. Agron. J. 64: 608-611.
7. Lowe, L. B., and S. K. Ries. 1973. Endosperm Protein of Wheat Seed as a Determinant of Seedling Growth. Plant Physiol. 51: 57-60.
8. Miller, R. W., M. E. Daxenbichler, and F. R. Earle. 1964. Search for New Industrial Oil VIII. The Genus Limnanthes. Jour. of Amer. Oil Chem. Soc. 41: 167-169.
9. Pollock, B. M., and E. E. Roos. 1972. Seed and Seedling Vigor. IN T. T. Kozlowski (Ed.). Seed Biology Series. Vol I, pp. 313-387. Academic Press, New York.
10. Ries, S. K., and E. H. Everson. 1973. Protein Content and Seed Size Relationships with Seedling Growth of Wheat Cultivars. Agron J. 65: 884-886.
11. Stamp, D. L. 1973. The Culture of Limnanthes spp.: A Substitute for Sperm Whale Oil. Agron Abst. pp. 69.
12. Toy, S. J., and B. C. Willingham. 1966. Effect of Temperature on Seed Germination of Ten Species and Varieties of Limnanthes. Econ Bot. 20: 71-75.
13. Toy, S. J., and B. C. Willingham. 1967. Some Studies on Secondary Seed Dormancy in Limnanthes Seed. Econ Bot. 21: 363-366.

SUMMARY AND CONCLUSIONS

Field investigations were conducted to determine what effect N application rates had on seed yield, oil, protein, and fatty acid composition of seed oil of L. alba. Seed from the field plots was used for laboratory experimentation to determine if seed weight, percent germination, and seedling growth differences existed as an effect of N fertilization practices.

N application greatly influenced seed yield one out of two years. Seed oil and protein content were also affected by N. The fatty acid composition of seed oil was affected by rates and forms of N at both dates of application. Seed weight was not influenced, but germination and seedling growth were affected by rates and forms of N application.

Overall, the 50 kg N rate as AS applied in March when L. alba followed wheat had the most beneficial influence on increasing seed and protein yield. Applications of N when L. alba followed alfalfa had no desirable effects on either oil or protein yield, and seed yield was decreased.

None of the treatments used during the two years of study increased oil yield, but some did increase protein yield. Of course, oil yield is a main concern with an oilseed crop, but protein yield has come under attention recently due to the value of protein meals for livestock and poultry consumption. With this in mind, if N fertilization can increase protein yield without sacrificing oil yield, there may be an advantage in the use of N if industry offer a premium for higher protein seed.

At the present time, with the seed yield data recorded to date, the

economic feasibility of N application to stimulate yields of this potential oilseed crop is questionable. An indirect advantage of N use would be the reduction of shattering during harvest due to a more dense vegetative canopy. Although, in the future, plant breeding and machine technology could reduce or eliminate shattering as a problem during harvest.

It must be pointed out that interpretation of the results of these experiments may be difficult due to a lack of soil sampling before and after the N trials in both years.

In many agronomic field studies of N fertilization, soil samples are not taken, but a range of yield response to N is usually recorded. Over a period of time, with yield responses observed within a certain range of N fertilization, recommendations may be made as to what N practice should be followed for a specific crop and soil condition. In this study, an optimum range of N application rates has yet to be identified. When following alfalfa, the highest yield was the control, where no N was applied. Soil samples before and after the experiment may have enabled us to quantify N use under these conditions. When following wheat, both March application rates of 50 and 100 kg N increased yield, but this yield level was significantly below the level of the control when L. alba followed alfalfa. The reason for this difference could not be identified without proper soil sampling.

In the first year, at Hyslop Farm, the two N fertility experiments were conducted on entirely separate areas, and soil samples were not taken. Since the two trials were set out on different areas (one following alfalfa and the other wheat), any significant differences between the results for the two experiments cannot be attributed solely to effect of

rotation, but rather partly to soil differences resulting from past management and cultural practices of the two locations. Ideally, it would have been hard to measure the true effect of rotation in terms of available N from a previous legume crop on the following crop. The differences between management practices and in soil characteristics resulting from an alfalfa or wheat rotation would represent variables which could not be controlled experimentally or quantified for purpose of identification.

A second year of data from Hyslop Farm with adequate soil sampling could have answered some of these questions. Unfortunately, in the second year the N fertility plot areas planted at Hyslop drowned out due to a prolonged freezing period followed by sudden heavy rains which could not penetrate the frozen soil profile. Since the stands were lost, an area at the Jackson Farm, near Lebanon, Oregon, which had been planted for seed increase, was used. Here again, no soil samples had been taken. Secondly, no weed control measures were employed, which may have been responsible for a lack of differences between treatments. Consequently, N use was again not quantified.

In an effort to establish in an analytical manner what the N requirements for L. alba are, a greenhouse study under controlled conditions is in order. With data from a greenhouse study, field experimental results may be more easiler understood.

APPENDIX TABLES

Table 1. Weather Summary for Crop Year 1972-73, Corvallis, Oregon.

Temperature							
Year	Month	Avg. Max.	Avg. Min.	Mean	Dev. Norm.	High	Low
1972	Sept.	72.7	46.2	59.5	-2.6	96	32
	Oct.	65.2	40.6	52.9	-0.7	81	27
	Nov.	53.6	40.8	47.2	+2.5	62	29
	Dec.	43.0	27.2	35.1	-5.9	60	-07
1973	Jan.	44.8	31.3	38.1	-0.2	56	20
	Feb.	52.6	36.8	44.7	+2.6	63	27
	March	53.3	36.3	44.8	-0.6	62	31
	April	60.9	39.1	50.0	-0.8	73	29
	May	70.1	43.5	56.8	+0.2	88	34
	June	73.4	49.6	61.5	+0.4	92	33
	July	82.9	51.0	67.0	+0.6	99	43
	Aug.	78.9	48.6	63.7	-2.4	88	37

High: 99 July 15

Low: -07 December 8

Precipitation								
Year	Month	Precip.	Dev. Norm.	Snow	Clear	Partly Cldy	Cldy	Rainy
1972	Sept.	2.28	+0.94	0	14	4	12	11
	Oct.	0.88	-2.90	0	14	1	16	6
	Nov.	4.92	-0.81	0	3	2	25	27
	Dec.	9.33	+2.28	9.3	4	0	27	22
1973	Jan.	5.56	-0.96	0.2	8	0	23	21
	Feb.	1.65	-3.39	T	8	1	19	12
	March	3.63	-0.75	T	10	6	15	19
	April	1.75	-0.45	0	17	3	10	9
	May	0.85	-1.08	0	20	3	8	11
	June	1.38	+0.07	0	13	5	12	8
	July	0.02	-0.32	0	22	1	8	1
	Aug.	0.70	+0.29	0	23	1	7	3
Totals		32.97	-7.08	9.5	156	27	182	150

Table 2. Weather Summary for Crop Year 1973-74, Corvallis, Oregon.

Temperature							
Year	Month	Avg. Max.	Avg. Min.	Mean	Dev. Norm	High	Low
1973	Sept.	75.1	50.5	62.8	+0.7	97	41
	Oct.	62.0	43.6	52.8	-0.8	72	34
	Nov.	49.3	38.3	43.8	-0.9	62	27
	Dec.	48.9	46.8	43.7	+2.7	56	30
1974	Jan.	43.5	29.9	36.7	-1.6	58	09
	Feb.	47.3	35.0	41.2	-1.9	54	28
	March	54.0	37.2	45.6	+0.1	68	26
	April	57.5	40.7	49.1	-1.7	70	30
	May	63.6	42.4	53.0	-2.7	77	34
	June	74.6	48.4	61.5	+0.5	92	40
	July	77.5	49.5	63.5	-2.9	95	44
	Aug.	82.2	51.8	67.0	+1.2	96	43

High: 97 Sept 9

Low: 09 Jan. 8 and 10

Precipitation							
Year	Month	Precip.	Dev. Norm.	Snow	Clear	Pt. Cldy	Cloudy
1973	Sept.	2.52	+1.18	0	18	3	9
	Oct.	2.70	-1.08	0	4	6	21
	Nov.	18.28	+12.55	T	5	1	24
	Dec.	12.40	+5.35	0	1	3	27
1974	Jan.	11.59	+4.53	0.4	15	0	16
	Feb.	7.52	+2.89	0.3	3	1	24
	March	8.87	+4.67	T	9	0	22
	April	2.39	+0.34	0	9	3	18
	May	1.46	-0.31	0	11	4	16
	June	0.61	-0.54	0	16	1	13
	July	1.81	+1.48	0	14	8	5
	Aug.	0	-0.55	0	17	5	9
Totals		70.15	+31.81	0.7	122	35	208

Table 3. Maximum, Minimum, Average, and Standard Error of the Mean Values for Correlation Analysis Factors of L. alba Seed Characters for Two Years.

Characters	Range		Average	Standard Error
	Minimum	Maximum		
Protein Percent	17.20 ^{1/}	23.20	20.34	0.22
	17.10 ^{2/}	24.60	20.40	0.33
Oil Percent	20.90	29.30	25.27	0.25
	21.70	27.20	24.94	0.19
			<u>kg/ha</u>	
Seed Yield	673	1794	1185	52.1
	422	995	839	58.7
Protein Yield	143	352	239	10.5
	104	204	170	13.9
Oil Yield	147	524	302	14.1
	92	265	210	13.9

^{1/} Data in this row represent 1973 observations (n= 40).

^{2/} Data in this row represent 1974 observations (n=20).

Table 4. Minimum, Maximum, Average, and Standard Error of the Mean Values for Correlation Analysis Factors of *L. alba* Seed Oil and Fatty Acid Constituents of Seed Oil, 1973 and 1974.

Characters	Range		Average	Standard Error
	Minimum	Maximum		
Oil	20.20 ^{1/}	29.30	25.27	0.25
	21.70 ^{2/}	27.20	24.94	0.19
C20:1 Monoene	57.90	63.10	61.15	0.20
	57.80	62.20	60.06	0.38
C22:1 Monoene	18.50	22.70	20.48	0.17
	16.30	17.70	17.18	0.19
C22:2 Diene	15.00	18.10	16.53	0.13
	18.60	22.80	20.50	0.27
Non C20-22	0.90	7.70	1.84	0.23
	1.50	3.40	2.35	0.21

^{1/} Data in this row represent the 1973 observations (n = 40).
^{2/} Data in this row represent the 1974 observations (n = 20).

Table 5. Maximum, Minimum, Average, and Standard Error of the Mean Values for Correlation Analysis Factors of L. alba Seed Characters, 1974.

Character	Range		Average	Standard Error
	Minimum	Maximum		
Protein Percent	17.1	24.6	20.40	0.33
Oil Percent	21.7	27.2	24.94	0.19
Seed Weight (g/1000)	6.93	7.97	7.42	0.06
Percent Germination	83.6	96.0	90.6	1.04
	<u>Millimeters</u>			
Shoots 14 Days	13.6	29.6	19.06	0.44
Shoots 16 Days	21.3	35.0	25.88	0.45
Roots 14 Days	29.0	64.4	41.19	1.15
Roots 16 Days	43.6	71.9	56.29	1.58