Effects of nitrogen, phosphorus and sulfur on the yield, growth and quality of canola (Brassica napus L.)

by Gregory B Popove

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy

Montana State University



Abstract:

The recent advent of canola production in the United States has underscored the need for current agronomic guidelines for optimal production. Although much research information from around the world pertaining to nutrient response of canola (spp. Brassica napus) has been disseminated, the soil and environmental conditions, as well as the production practices in Montana and the Intermountain Northwest area are sufficiently unique as to warrant further research.

Three varieties, Westar and two from the Intermountain Canola Company: IMC-01 and IMC-129 were tested at three locations in Montana. The Bozeman and Fairfield trials were irrigated and the Moccasin site was dryland. Eight treatments consisted of: a non-fertilized check, a standard application, and six experimental rates of nitrogen (N), phosphorus (P) or sulfur (S) respectively. Measured variables were: seedling vigor, date of first-flower, date of end-of-flower, maturity date (swath date), seed yield, 1000-seed weight, oil content and lodging score. Soil samples were taken to a depth of 1.2 m prior to fertilizer application.

Results suggest significant effects between applied nutrients and most measured variables. Curvilinear analysis of nitrogen-yield response curves indicates a linear response at the highest levels of fertilization at the flood-irrigated location (Fairfield). Some variety-treatment interactions were observed. Optimum nutrient application levels appeared to produce higher stand counts and plant vigor, as well as promoting wider plant-branching to compensate for low stand densities. Over-all response of canola to N was greater than to S and P. Higher moisture levels appeared to alter the variety-yield ranking at some locations. Yield was significantly affected by fertilizer treatments, particularly N, and by variety. Oil content was generally correlated negatively with N at higher fertility levels and positively correlated at lower soil nutrient levels.

This study supports previous studies which indicate a different fertilization profile is required for canola than for cereal grains. Canola generally has a higher requirement for N than cereal grains, while response to P is often less. Phosphorus response can differ due to the amount of less-labile P which may be present at significant levels resulting from several years of P fertilization. Sulfur response may be highly variable due to differences in the sulfate reserves in the subsoil profile.

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Gregory B. Popove

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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## ABSTRACT

The recent advent of canola production in the United States has underscored the need for current agronomic guidelines for optimal production. Although much research information from around the world pertaining to nutrient response of canola (spp. *Brassica napus*) has been disseminated, the soil and environmental conditions, as well as the production practices in Montana and the Intermountain Northwest area are sufficiently unique as to warrant further research.

Three varieties, Westar and two from the Intermountain Canola Company: IMC-01 and IMC-129 were tested at three locations in Montana. The Bozeman and Fairfield trials were irrigated and the Moccasin site was dryland. Eight treatments consisted of: a non-fertilized check, a standard application, and six experimental rates of nitrogen (N), phosphorus (P) or sulfur (S) respectively. Measured variables were: seedling vigor, date of first-flower, date of end-of-flower, maturity date (swath date), seed yield, 1000-seed weight, oil content and lodging score. Soil samples were taken to a depth of 1.2 m prior to fertilizer application.

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# CHAPTER 1

## INTRODUCTION

Oilseed Rape or Rapeseed, otherwise described scientifically as *Brassica napus* L., is a relatively recent addition to American agriculture as a source of edible oil. Newer varieties, described as double-low types (low glucosinolate and low erucic acid) are widely referred to as *Canola* in North America.

This study was conducted to document nutrient requirements and responses for canola in western Montana soils. Although considerable information exists from Canada and other countries, soil and climate conditions differ significantly between Montana and other regions. Canadian soils are almost exclusively glacial in origin, and much of Montana is non-glacial. Day length can vary by as much as two hours per day in June, between southern Montana and northern Alberta. The climate of Montana is generally warmer and drier than most of the major canola-growing areas of Canada, therefore the possibility for unique production problems may occur.

The hypothesis to be tested in this study is that conditions in Montana are sufficiently different from other canola-growing areas, and this will require a set of production recommendations for growing canola which is unique for the region.

Special attention was paid to identify possible problems which may have appeared, that could be reflected in future canola production. The canola test plots were managed with typical canola production practices. Pest control guidelines were followed, in that pesticides were not applied when pest counts were below economic threshold levels as defined by the Intermountain Canola Company's agronomic production guidelines. No fungicide and/or insecticide seed treatments were used as often happens in U.S. canola production at this time. Irrigation water was applied in quantities and timing in accordance with typical field practices. Herbicide application was restricted to grass control with POAST (sethyoxydim) and canada thistle eradication with STINGER (clopyralid) where infestations were substantial or severe. A particular focus for the study was for sulfur response, because it can often be more important in canola than in cereal grain production. Sites were chosen which were candidates for possible sulfur deficiency. Potassium was not included in this study. The test sites are generally not K-deficient, and few studies in the past have shown K-response on these types of soils.

The use of nitrogen, phosphorus and sulfur at 0x, 1x and 2x rates was seen as an effective way to analyze linear and curvilinear response. This type of information is useful in establishing fertilization guidelines for specific cropping situations, and identifying nutrient deficiencies.

It is hoped that all the methods employed in this study cannot only test the hypothesis, but can add to the knowledge-base for canola production. Effects observed in this study may also lend support to further research in canola production.

# CHAPTER 2

## LITERATURE REVIEW

### Nitrogen

Canola requires higher amounts of nitrogen (N) than most other crops (Soper, 1971), and canola continues to respond to increasing levels of applied nitrogen until soil nitrate levels are above 100 kg $ha^{-1}$. In some soils, canola has responded to N levels of up to 200 kg $ha^{-1}$ (Nuttall et al., 1987; Lewis and Knight, 1987; Bailey, 1990). Increased N rates have been shown to promote lodging also (Yusuf and Bullock, 1993; Sheppard and Bates, 1980).

The effects of N-deficit in the canola plant have also been explored. Leaf senescence in canola was related to very low soil nitrogen levels (Rood et al., 1984). In the first year of a two-year study, nearly all N was depleted from the upper soil layers, and rapid leaf-loss was observed. Slightly higher N levels in the second year resulted in much-reduced leaf-loss at maturity. This is a possible field indicator of low N status in the soil.

The influence of N on canola's quality factors was shown to be substantial by several researchers. Protein content in canola generally increases with N availability (Henry and MacDonald, 1978; Sheppard and Bates, 1980; Nuttall et al., 1987). Since N is a major component of all proteins, protein synthesis may be inhibited without adequate N and other nutrients (Finlayson et al., 1970).

Although the inverse relationship between N levels and oil content has been well described (Bhatty, 1964; Allen and Morgan, 1972; Sheppard and Bates, 1980), a mechanism for this phenomenon was better-elucidated by Diepenbrock and Geisler in 1981. Chlorophyll levels and chloroplast characteristics in canola leaves were seen to be influenced by available N. Chlorophyll levels were reduced only under extreme N deficiency, but galactolipid levels were highly correlated with N levels. The production of linolenic acid (C18:3) rose in proportion to available N until levels of latent, moderate deficiency were reached, where it plateaued. As levels of N rose to what was optimal for growth and above, levels of galactolipids and linolenic acids fell sharply. This was partially explained by changes in the fine structure of the thylakoid membranes in the chloroplasts under high N levels.

Water-use efficiency (WUE) was observed to increase with moderate (100 kg $ha^{-1}$) and high-rate (200 kg $ha^{-1}$) additions of N on canola (Taylor et al., 1991). The high N rates reduced oil concentration in seed, but increased overall oil yield.

Response of canola to other nutrients was slight in a study by Sheppard and Bates (1980), but N elicited the greatest yield response. Leaf concentrations of N varied considerably with the level of treatment. Band-placement fertilizer applications increased yields significantly over other types of placement.

## Phosphorus

Canola requires phosphorus (P) in levels comparable to cereal grains when grown on P deficient soils (Soper and Racz, 1963). It has often shown a lesser P response than many other crops (Soper, 1971) in soils with higher levels of reserve-P (less-extractable phosphate). Soils that require P amendment for cereal grains often have adequate P for canola. There appears to be a better P extraction mechanism in canola roots than in other plants.

Grinsted, et al. (1982) found that *Brassica napus* caused an acidification of its rhizosphere by as much as 2 to 4 pH units in P deficient soils. This decrease in pH facilitated a tenfold increase in P uptake by the plant. Phosphatase activity appeared to correlate to root density (Hedley et al., 1983). In soils sensitive to pH change, preferential uptake of anions contributed to the acidification of the rhizosphere. Acid levels below 5.5 can have a detrimental effect on yield (Edwards, 1991) however, and annual applications of P are needed to alleviate this condition.

The role of plant metabolism on P influx into plant roots was tested with a protein synthesis inhibitor and a metabolic uncoupler (Schjørring and Jensén, 1984b). The concentration of P in roots appeared to vary 60 to 70% due to the influence of disabled plant metabolic factors in this experiment. Influx at low P concentrations and efflux at high levels were significantly reduced in canola with the addition of these inhibitors. From these data it appears that *Brassica napus* utilizes active-transport processes for P uptake in its roots.

Shoot/root fresh weight ratios were strongly influenced by the external P supply in a study by Schjørring and Jensén (1984a). The shoot/root ratio was highest at low P levels. There was a near-linear relationship between P concentration in the root zone and an increased shoot-to-root ratio existed at low-to-intermediate levels. As higher P levels were reached, the increase in shoot/root ratio was less pronounced.

From past research, it seems useful when growing canola, to allow for the "soil phosphorus reserve" of less extractible P when gauging P requirements for a crop. This might be accomplished with soil tests that measure the less-labile forms of phosphorus such as "weak-Bray and strong-Bray" tests, or calibration of current tests. Continued P fertilization under cereal grain production may lead to a buildup of usable P for a subsequent canola crop.

## **Sulfur**

S requirements are higher for canola than for most other crops and have been established about 25-35 kg $ha^{-1}$ (Nyborg, 1974). Sulfate ($SO_4^{=}$) is the chief form taken up by the plant (Thompson et al., 1986), and is required for chlorophyll, protein synthesis and for production of volatile oils that constitute glucosinolates (Marschner, 1986).

Nitrogen-to-sulfur ratios in canola have been optimal at 7:1 (Janzen and Bettany, 1984). Seed yields were significantly reduced when the ratio exceeded 7, and excess S accumulated in stems and leaves when the ratio was below 7.

Subsoil reserves of S appeared to serve as both a "reserve" and a "sink" for sulfate in soils in a study by Castellano and Dick (1991). Sulfate and residual S levels in soil appeared to decrease in spring with downward leaching, and increase later in the season with upward soil water movement driven by evapo-transpiration. Another S-reserve, Carbon-bonded S in organic matter did not appear to fluctuate seasonally, or in response to cropping. These phenomena qualify soil testing of available soil S to allow for subsoil S reserves and net seasonal water movements in the soil profile.

The negative effects of excessive S accumulations have been studied recently. High rates of S fertilization that exceed 100-200 $\mu g\ g^{-1}$ (ppm) can increase oil content in canola seed and raise glucosinolate concentrations (Booth et al., 1991), while decreasing yield (Mailer, 1989). A depression in oil content was seen in a study by Wetter et al. (1970). Inorganic sulfate accumulations were seen to be higher in pods of double-low (low glucosinolate and low erucic acid) type canola than what was observed in single-low (low erucic acid) varieties (Zhao et al., 1993). This was explained by the hypothesis that the pod walls are the site of biosynthesis of seed glucosinolate, and blocking of this pathway in low glucosinolate varieties (originally Bronowski var.) results in the accumulation of inorganic sulfates (Josefsson, 1971).

Sulfate uptake into canola plasma-membrane vesicles was strongly influenced by an imposed pH gradient (Hawkesford et al., 1993). Acidifying the solution outside the membrane to pH 5.0 caused a twofold increase in sulfate uptake over solutions of pH 6.5.

Sulfur appears to be a critical nutrient for canola, required in higher amounts than for cereals. Nitrogen and sulfur are required by each other for optimal utilization, and excessive levels can lead to unfavorable growth and seed quality characteristics.

## Soil Test Correlation

The effects of nitrogen and P treatments had no significant interaction in a sixteen-year study of canola seed and straw yield correlated with soil tests (Nuttall et al., 1992). The greatest variability in yield was due to yearly fluctuations of total seasonal precipitation and mean daily temperature. Yield was negatively correlated to mean daily temperatures, and positively correlated to total seasonal precipitation. This effect was thought to contribute significantly to the soil test-to-yield correlations of $r^2=0.58$ for N and $r^2=0.46$ for P.

# CHAPTER 3

## MATERIALS AND METHODS

### Experimental Design

This study was conducted as a split-plot design, with three varieties as main plots, and eight fertilizer treatments as subplots. The experiment was conducted at three locations for two years. The three varieties were IMC-01, IMC-129 and Westar. The fertilizer treatments consisted of a no-fertilizer check, a normal-rate application, and 0x, 1x, and 2x rates of nitrogen, phosphorus and sulfur respectively. Each location was divided into four blocks.

### Fertilizer

Fertilizer types were the same at all sites and in both seasons. Nitrogen was supplied by ammonium nitrate (34-0-0) in pelleted form, phosphorus was provided by monocalcium phosphate (0-45-0), and ammonium sulfate (21-0-0-24) was the source of sulfur. Some additional nitrogen application was effected with the sulfur applications (21%N), and amounted to about 19 kg $ha^{-1}$ (Table 1).

**Table 1. Fertilizer Treatments: N-P-K-S (kg $ha^{-1}$)**

| | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 check | 2 normal | 3 high N | 4 no S | 5 high S | 6 no P | 7 high P | 8 no N |
| **Dryland** | 0-0-0-0 | 56-22-0-22 | 112-22-0-22 | 56-22-0-0 | 56-22-0-45 | 56-0-0-22 | 56-45-0-22 | 19-22-0-22 |
| **Irrigated** | 0-0-0-0 | 112-22-0-22 | 168-34-0-22 | 112-34-0-0 | 112-34-0-45 | 112-0-0-22 | 112-67-0-22 | 19-34-0-22 |

## Locations

The three Montana test locations consisted of a dryland site (Moccasin) and two irrigated sites (Bozeman and Fairfield). Site selection criteria were for a diversity of environmental and management factors in the major canola-growing regions of Montana, as well as for soils that were expected to have lower sulfate levels. Two of the three sites were characterized by soils with a gravely, glacial outwash parent material (Fairfield and Moccasin), which differ significantly from glacial-till type parent materials that have often been shown to contain higher sulfate levels. The soil at the Bozeman site developed from loess parent material and is similar to other soils in the area, which have shown response to S by cereals and forages.

## Soil Sampling and Testing

Soil was sampled at: 0 to 15 cm, 15 to 30.5 cm, 30.5 to 61 cm and 61 to 122 cm. All samples were collected before spring planting and analyzed at the Western Testing Laboratory at Great Falls Montana. Phosphorus was determined by Olsen bicarbonate test, $NO_3$ -N by CaOH extraction (.2%) with chromotropic acid for colorimetry and S by ammonium acetate (.5N) with acetic acid (.25N) extraction. The pH at all locations were in the 7.4 to 7.5 range and are known to have moderate to high potassium content.

Equipment consisted of truck-mounted hydraulic soil probe that was used at all locations and for all soil depths.

## Varietal Descriptions

### Westar

Westar was developed at the Agriculture Canada Research Station at Saskatoon, Saskatchewan, Canada and first licensed for use in 1982. The variety was obtained from a single plant selection in the $F_4$ generation of the cross: ((SD x 568-2895) $F_3$ x Midas) $F_2$ x Tower. It was selected for oil and meal quality characteristics, early maturity, high seed yield and standability (lodging-resistance).

The plant height, lodging resistance, protein content and disease reaction were comparable to most previously-established varieties. Yield, oil content, glucosinolate levels and maturity were slightly better than most other varieties licensed at the time of its introduction (*Source: Agriculture Canada description of variety dated 8-6-1982, issued by Secan Association 3-15-1994*).

### IMC-01

IMC-01 variety (Sunola 001) was developed by the DNA Plant Technology Corporation in 1988. The line was selected from an uncharacterized, private germplasm collection for its low-linolenic acid (C18:3) content. These oils have desirable characteristics such as higher oxidative stability and reduced odor development when heated. The yield of this variety is described as 80% of Westar under dryland conditions and 90% under irrigation. Maturity is about 8 days later than Westar, and lodging resistance is better than Westar under irrigated conditions. Plant height is about 1 - 1.3 m, and it has moderate resistance to blackleg (*Phloma lingam*). Leaves are narrower than Westar and have a distinctive inward curling (*Source: undated variety description bulletin, Intermountain Canola Company*).

**IMC-129**

This variety was developed by chemical mutagenesis from the Westar variety by the DNA Plant Technology Corporation in 1989. The line was self-pollinated and selected for high oleic acid (C18:1) for seven generations before breeder seed was distributed. IMC-129 appeared to be homogeneous and without morphological variation during the production of foundation seed. Maturity is about two days later, and the plant is about 5 cm. taller than Westar. The fatty acid profile differs in that it has about 15% more oleic acid than Westar, for a total of about 77% in the seed oil (*Source: excerpt from application for PVPA certificate, issued from IMC 2-16-93*).

## Planting

Seeding was carried out at all three locations with standard plot-seeding equipment. Canola seeds were weighed to obtain a seeding rate equivalent to 4 pounds per acre. This amount was calculated to be 2.2 grams per 100 $ft^2$ (9.29 $m^2$) plot, and was placed in paper envelopes for transport to the field and planting.

Fertilizer was applied in the field by several methods. At Moccasin, measuring scoops were prepared for N, P and S fertilizers that were dipped from a bulk supply. These measured amounts were poured into the planter cone-bins in the required number at each respective plot. At Fairfield, fertilizer was applied through drill-mounted hoppers that could be adjusted quickly at each plot to deliver the desired amount of each fertilizer treatment. Water was applied after seeding at this location in 1992 due to excessively dry soil conditions. At Bozeman, the required amount of each fertilizer was measured for each plot, and spread over the surface by hand as uniformly as possible. It was then worked into the soil to a depth of 5 cm.

## Harvesting

All plots were swathed prior to drying and threshing. At Moccasin, a plot forage harvester delivered acceptable swath-rows. Only the four central rows of the six-row plots were harvested to minimize edge-competition effects. The chosen method of swathing at the Bozeman location was hand-cutting and windrowing of the four central rows. Because of the inaccessibility of the Fairfield location by harvesting equipment, a smaller sample, 1.5 $m^2$ was taken for harvest. This sample was left in the field for several days drying, and then transported to a stationary threshing machine. At Bozeman and Moccasin, threshing was conducted with standard small-plot combines.

The grain samples were then cleaned with a stationary air separation cleaner. The air-choke setting and screen size were selected for the optimum balance of chaff removal and seed loss. This setting was not altered during the time when the entire lot of seed from a particular location was being cleaned. This procedure was used to minimize variability among individual seed samples within each location.

## Statistical Analysis

Variables were analyzed using analysis of variance, correlation and regression. These were completed with the MSUSTAT statistical analysis package developed at Montana State University, and with the Microsoft EXCEL application's analysis tools. Response to N, P and S were investigated using an orthogonal polynomial coefficient for equally spaced treatments.

## Description of Measured Variables

The variables measured in this study were: seedling vigor, date of flower initiation, date of flower cessation, date of seed color change (swathing date), grain yield, seed oil content, 1000-seed weight, and lodging scores. Measurement techniques did not differ appreciably between the two seasons, with the exception of seedling vigor measurements. A dry sample weight of seedlings was used the first year, and showed little significant variation, so an alternate method of scoring based on seedling height and density was employed in the second season.

These data were collected in the field or lab, and transcribed directly to a dBASE IV data file. All data transformations were calculated within the database.

### Seedling Vigor

In the 1992 season an attempt was made to correlate seedling dry weight to final yield. At the four leaf growth stage, a 30 cm sample from two rows of each plot was cut at ground level. The method employed for inter-plot sample selection was to visually select a location which was representative of the entire plot, and to cut the sample with clipping shears at ground level. Plants were sampled at the 4 to 5-leaf stage of development. This method was used due to high variability in plot density at some sites with differing emergence times. The samples were oven-dried at 100 $^{o}$C for 48 hours. The dry weights were recorded and later correlated with final yield seed weights, to determine yield potential estimates at the seedling stage.

In the second year of the study (1993), a different method was attempted due to the absence of a consistent correlation between seedling weights and final yield in the previous season. A visual vigor score was established from a composite of stand density rating and average plant height rating. Each factor was assigned a 0 to 5 score. The plots within the first quintile of plant density rankings at the site (thinnest stand or smallest plant) were assigned a 0-value. The plots within the fifth quintile (highest at the location) received a 5. The two factors were combined for a maximum possible vigor score of 10. Plants were examined at the 4 to 5-leaf stage of development. This method was also chosen for its compatibility with agronomic field-evaluation situations.

**Flowering Dates**

The date at which the first flowers had appeared in approximately 5% of the visible canola racemes in each plot was recorded as date of flower initiation. It was then converted to a numeric variable expressed as days after seeding. All sites appeared to make the transition from bolting to bloom quite rapidly, so it is estimated that the visual observations of date of flower initiation are accurate to within 2 days.

The flower cessation measurement was determined by the time when approximately 95% of the canola racemes had ceased bloom of the terminal flower. The end of blooming did not occur as succinctly as did first-bloom, and required the determination of a mean date by estimation. These dates were also converted to numeric values expressed as days after seeding.

**Color Change**

This variable corresponds approximately to the date when canola is normally swathed, according to field guidelines established in Canada and the US. This is the approximate date when one-half of the canola seeds have turned from green to a brown-black color. Pods from the central racemes of several plants were collected at approximately one-third height from the base. If seeds from these samples were approximately one-half brown or black, the color-change date was recorded and converted to days after seeding. This measurement was the most time-critical, as the seed-ripening process occurs quite rapidly in canola and pod shattering often becomes a problem soon after ripening.