EFFECT OF PLANT SPACING AND NITROGEN FERTILIZER LEVELS ON YIELD , LEAF CHLOROPHYLL CONTENT AND NITRATE REDUCTASE ACTIVITY OF BROCCOLI (*Brassica oleracea* L. var. *italica*, Plenck)

by

HARVEY ERNESTO ARJONA

B.S. Agronomy, Colombian National University

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Approved by:

J. K. Greig
Major Professor

To my mother

who constantly encouraged me and supported me

in continuing my studies

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INTRODUCTION

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) whose green shoots have been esteemed as an important vegetable by the Italians for centuries (3) has become a popular and widely grown crop in many countries. As a vegetable, broccoli is an important source of Ca, P, Fe, vitamins A, C, thiamine, riboflavin and niacin (22, 25, 31).

The commercial production of broccoli in the U.S. began around the 1930's when Italian immigrants started demanding it in considerable amounts (22). Since then, its importance as a vegetable crop either for fresh market or for the freezing industry has increased greatly. According to the U.S. Department of Commerce (30) there were 68,300 acres grown in 1978 in the U.S.A. which produced 287,600 short tons for a total value of $102,100,000. The main producing states are: California, Texas, and Oregon.

Within the last 50 years limited research has been done on this crop, and several aspects of the crop still need additional study. Among those are certain cultural practices including plant spacing, fertilization, harvesting etc. because of the great effects that they have on yields, quality, hand labor and in general cost and returns of the crop.

Studies involving the already mentioned aspects have been conducted in California, Arkansas and a few other states. However, no reports in the literature involving broccoli production in Kansas were found, although the crop thrives well under Kansas conditions and it has a potential as a commercial vegetable crop in the state.

For this reason, and given the importance of the cultural aspects on the crop, the present study was conducted in order to accomplish the following objectives:

1. To determine the effects of 4 nitrogen fertilizer levels and 4 plant spacings on yield of broccoli.
2. To determine the effects of nitrogen and plant spacing on size and weight of central heads of broccoli.
3. To determine the effects of nitrogen and plant spacing on the number and weight of lateral shoots per plant.
4. To determine the effects of 4 nitrogen fertilizer levels on chlorophyll content and nitrate reductase enzyme activity on leaves of broccoli plants, as an indication of nitrogen uptake by the plant.

LITERATURE REVIEW

Plant populations, spatial arrangement, nitrogen fertilizer rates, variety trials and other aspects of broccoli production have been reported in the literature by several authors. These studies have been conducted under different experimental conditions in Canada, the United Kingdom, Israel, Costa Rica, the United States, and others. A common purpose in these studies was to determine the most appropriate combination of cultural practices under each particular circumstance to obtain the best yield and quality of harvested product.

Zink and Akana (32) working on the effect of spacing on broccoli reported that the size of central heads and yield of shoots per plant decreased as the distance between plants in single rows decreased from 91 to 46 cm at 15 cm intervals. However, the total yield per hectare increased as the plant density increased at the closer spacings. The same authors reported that the number and size of lateral shoots depends to a certain extent on the length of the center stem left after cutting the central head, as well as on the plant density. They found an optimum plant spacing of 20 cm. At wider spacings the yield was reduced significantly and closer spacings produced small size heads and stems.

Aldrich _et_ _al_ (1) observed in a two-year experiment on plant spacing on broccoli that the total yield per unit area drops off abruptly if the space between plants is reduced only a few inches below the optimum, which depends on season and variety. They found an optimum between 20 and 28 cm apart. They also found that, as the plant spacing is reduced, the plant weight and the diameter of the heads and the shoots decreases while the average rate of maturity is delayed due probably to competition among plants. They observed that when the number of plants per area is reduced within certain limits, the increase in yield per plant offsets the reduction in number of plants.

Massey et al (20) reported significant differences in the yield of central heads and lateral shoots, edible leaves and total edible material when the distance between plants in rows 91 cm apart was reduced from 45 to 30 cm. In the same study they did not find significant differences in ascorbic acid and carotene content of leaves, central and lateral inflorescences between the two plant spacings.

Mack and Baggett (21) studying the effect of row spacing on yield of central heads of broccoli in Oregon, found significant differences when the spacing was reduced from 107 to 45 cm. They noted that the formation of poor heads and the weight of unacceptable central heads tended to be greater as row spacing was reduced.

In a study conducted in Arkansas from 1961 to 1964 Bradley et al (5) indicated that in broccoli for the freezing industry it is necessary to plant the crop reasonably close within and between rows in order to get the maximum yield of central heads. They suggest direct seeding rather than transplanting for the closer spacings to reduce costs of hand labor.

Campbell and Thomas (8) studying the effect of plant spacing and harvesting time found that increases in plant population consistently increased the yield per unit area if the plants were harvested when 10 to 15% of the inflorescences are overmature.

Tereshkovich (27) observed that plants spaced 20 and 30 cm apart produced terminal heads smaller in size and weighed less than plants spaced further apart. He attributed this diminution in size and weight of heads to a shading effect between plants when they are grown at the closer spacings. He found optimum yields when plants were spaced 20 cm in rows 1.0 m apart.

Palevitch (23) combining two different distances between rows (50 and 70 cm) with three distances between plants (20, 30, and 40 cm) showed that a square arrangement approaching equidistant spaces between rows and between plants increased the yield of central heads of proper size more than a rectangular plant pattern. He noted that high yields of central heads of adequate size were obtained by reducing the space between plants rather than the space between rows.

Several studies involving nitrogen, plant spacing and cultivars and their effects on yield and quality on single-harvested broccoli have been conducted by Cutcliffe in Canada (11, 12, 13, 14). Although the spacing effect varies among the different cultivars, he found that in general single harvest yield increased and central head weight decreased as the distance between plants decreased. He observed that the increase in yield due to higher nitrogen application rates was greater when broccoli was single-harvested than when harvested sequentially. He also found that nitrogen had more effect on yield than plant spacing and that plant spacing has only a slight effect on marketable yield. He did not find a nitrogen-space interaction.

Maturity is reported by Cutcliffe (12) to be influenced by nitrogen and spacing. He found that the percentage of overmature heads generally decreased as spacing decreased and that maturity was delayed by high nitrogen rates. Aldrich _et al_ (1) also found a delay in maturity at the closer spacings.

Ramos and Martinez (25) found in an experiment involving 8 different cultivars and 3 different spatial arrangements that the yield per unit area decreased in a linear relationship as the distance between plants and rows increased. They obtained high yields at a distance of 20 cm between plants and 40 cm between rows. However, for practical purposes they recommended a distance of 20 x 60 cm.

Thompson and Taylor (29) observed that yield and inflorescence size may differ between single harvest and successive harvested crops. They noted that the maturity of secondary inflorescences was delayed as population increased and that the number of these per plant declined with increasing populations. They did not find significant differences in yield between 3.5 and 19 plants per square meter. Contrary to Aldrich (1) and Cutcliffe (12), they observed that plants from low population densities reached maturity later than plants from higher populations. Their results agreed with those of Ramos and Martinez (25) in that a decrease in population below the critical level result in a linear decrease in yield.

From the results obtained by Thompson and Taylor (29), and those obtained by other researchers [Zink and Akana (32), Palevitch (23)], Thompson and Taylor indicated that little increase in yield of broccoli would be expected from populations greater than 10 plants per square meter. They also stated that the major determining factor in choice of a plant population for a commercial crop is the inflorescence size required for the market. They observed that increases in plant population densities reduce "bractiness," as well as axillary bud development and that the visual quality of the inflorescence improved as density increased. Contrary to Palevitch (23), they found similar yields for square and rectangular patterns of plant arrangements.

Chlorophyll

Nitrogen is a component of the chlorophyll molecule. Therefore, a deficiency of nitrogen results in lighter colored leaves (Greig et al, 16). It may also result in lighter colored fruits like apples, as it was reported by Boynton et al (4).

In an experiment with nitrogen levels and micronutrients on chlorophyll content of spinach Greig et al (16) found that although it was difficult to see the effect of nitrogen levels on color of spinach, nitrogen significantly increased the chlorophyll content of the leaves as well as the content of Ca, Mg, and K at the high nitrogen levels, increasing consequently the food value. They found a significant linear increase of chlorophyll content in leaves with increased nitrogen levels.

The quantitative method to determine chlorophyll content by light absorbance is based on the strong absorption band in the red end of the spectrum for chlorophyll (640 nm). At this wave length there is no carotenoid pigments absorption and therefore there is no cumulative absorbance effect due to these pigments (Compton and Boynton, 4).

Nitrate Reductase Enzyme

Nitrate is the most common form of nitrogen available to plants since the reduced forms of nitrogen commonly applied rapidly undergo nitrification in well aerated soils above 5°C (Thompson, 28). However, after being absorbed by the plant, the nitrate has to be reduced to the ammoniacal form before nitrogen is fixed in organic compounds (Elrich and Hageman, 15). The enzyme that catalyzes the first step in this assimilatory process is called nitrate reductase.

This enzyme is substrate-inducible and its concentration and activity in plants is a limiting step in the reduction of nitrate to ammonia (26). Being a substrate-inducible enzyme, it is expected that nitrogen treatments enhance the nitrate concentration of the plant tissues, causing induction of the enzyme and increasing its level of activity. Higher levels of the enzyme in turn would increase the amount of ammoniacal nitrogen which would increase the synthesis of nitrogen compounds in the plant.

Croy and Hageman (10) showed that spring applications of nitrogen increased N.R. activity, soluble protein concentration of leaf tissue and yield of grain protein per hectare in wheat.

Nitrate reductase has been extracted from diverse plant tissues such as leaves, petioles, stems, shoots, roots, barley aleurone layer, corn scutella, cotyledons, glumes from seeds of pod corn, corn husks, and cultured cells (17). However, higher activities are obtained from chlorophyllous than from non-chlorophyllous tissues. The amount of extractable enzyme varies drastically with: a. plant species, b. varieties within a species, c. plant age, d. cultural techniques, and e. nitrate supply and environment (17).

The enzyme exhibits diurnal variation and therefore Hageman and Hucklesby (17) suggest allowing 3 to 4 hours of illumination before taking samples.

Broccoli leaves are reported by Hageman and Hucklesby (17) as good sources of nitrate reductase (22). Jaworski (19) reported that nitrate reductase could be measured in green leaf disks vacuum infiltrated with a nitrate containing phosphate buffer. These disks incubated in the dark rapidly accumulated nitrate. The measurement of enzyme activity in intact plant tissue is based on reduction of NO_3^- to NO_2^-

(Jaworski, 19). The *in vivo* assay has proven to be an acceptable method to determine N.R. activity, since it estimates closer the actual amount of nitrogen accumulated, compared to the *in vitro* assay (Brunetti and Hageman, 7).

Al-Shaibani (2) studying the nitrate reductase activity in honeydew melon leaves did not find any significant change in enzyme activity with increased application rates of nitrogen fertilizer. He found an increase in the enzyme activity as fruits started ripening and then a decrease in plants that had produced overripe fruits.

MATERIALS AND METHODS

Seeds of broccoli (Brassica oleracea L. var. italica Plenck) of the hybrid 'Green Comet' were seeded during the Spring of 1980 in plastic trays containing a mixture of 1 part sand and 1 part vermiculite. Two weeks after germination, the seedlings were transplanted to jiffy pots (5.76 x 5.76 cm square) containing a mixture of 1 part perlite, 1 part peat, 1 part sand and 2 parts soil. The pots were kept in a greenhouse and the seedlings were watered when necessary.

Six weeks from seeding they were transplanted into the field at the Ashland Horticultural Farm, Kansas State University.

The field was prepared for transplanting, but no fertilizer was supplied since a soil analysis showed an adequate fertility level except for a low nitrogen level (See Appendix Table 11).

Nitrogen as ammonium nitrate was supplied at 4 different rates (0, 56, 112, and 168 kg/ha). Plants were spaced 22, 30, 38, and 46 cm apart. A 91 cm between row spacing was used for all four plant spacings. Nitrogen and plant spacing treatments were combined in a split-plot design. The nitrogen levels were assigned at random for each plot and each plot was sudbivided in four subplots in which plants were spaced at different distances. The experiment was replicated four times.

Ammonium nitrate was applied in 56 kg/ha increments one week, three weeks and six weeks after transplanting, as side dressing applications.

The crop was cultivated at the third and fifth weeks after transplanting and three applications of insecticide were necessary to control aphids and cabbage loopers. The plants were overhead irrigated when necessary, at intervals of about two weeks.

Harvesting was initiated six weeks after transplanting and the plots were hand harvested every two days. The central heads were cut close to the first developing axillary shoot and classified in two groups: 1. greater than 5.0 cm in diameter and 2. smaller than 5.0 cm in diameter. Number of heads and total weight of heads were recorded for each subplot.

Lateral shoots 2.5 cm in diameter or greater were harvested by cutting them at the base and, number and weight recorded for each subplot. Shoots smaller than 2.5 cm in diameter were not considered of commercial size so harvesting was stopped when the laterals produced were smaller than this diameter.

A week after the last side dressing, leaf samples were taken from the different subplots for chlorophyll content and nitrate reductase analysis.

Chlorophyll Analysis

Twenty 0.5 square centimeter disks were taken from each subplot from plants at random for chlorophyll content analysis. The samples were taken from the closest fully developed leaf to the central head. The disks were put in bottles containing from 40 to 50 cc of methanol and the bottles were kept in an ice chest for further analysis in the laboratory.

The next day, the disks were blended for 1 minute in a Sorvall Omnimixer, filtered and brought to 100 ml volume. Absorbance was read in a spectrophotometer Beckman model 25 at a wave length of 650 nm. Chlorophyll content obtained from a standard curve was expressed as mg of chlorophyll per square centimeter of leaf tissue.

Nitrate Reductase Activity Analysis

Young leaves close to the central head were sampled from plants at random in each subplot for nitrate reductase activity analysis. The leaves were put in plastic bags, put immediately in an ice chest and taken to the laboratory for nitrate reductase activity analysis.

The leaves were immersed in 75% ethanol for 30 seconds to destroy micro-organisms on the leaf surface which may interfere with the determination. The leaves were dried and cut into approximately 0.5 cm long sections and approximately 0.3 grams of leaf tissue were weighed for each sample and put into 50 ml Erlenmeyer flasks. The samples were refrigerated until all samples were weighed. Five ml of incubation medium was added to each flask and they were vaccuum-infiltrated for a period of 60 second.

The samples were incubated at 25°C in darkness for one hour. Then a 1.0 ml aliquot was transfered to a 13 x 100 mm tube and 2.0 ml sulfanilamide reagent and 2.0 ml N-1-naphtyl-ethylenediamine di HCl reagent were added. After 10 minutes absorbance of solutions was read at 540 nm. A standard curve was prepared and used to establish nitrate reductase enzyme activity as micromoles NO_2^- per gram of fresh weight per hour. The procedure followed was described by Paulsen (24) and reagent compositions are shown in Appendix Table 12.

All variables studied were subjected to the appropriate statistical analysis for a split-plot design. An analysis of variance was obtained for each variable with the factors being nitrogen fertilizer rate and plant spacing.

EXPERIMENTAL RESULTS

Analysis of variance tables of the variables studied are shown in the Appendix (Tables 4-10). Of the variables studied, only the weight of central heads per unit area and the nitrate reductase content per gram of leaf tissue showed a significant nitrogen-space interaction.

A linear relationship was found between the nitrogen fertilizer rate and yield, number and weight of shoots per plant, leaf chlorophyll content, and nitrate reductase activity. A quadratic relationship was found between N fertilizer rate and the variables weight of central heads per plant and number of central heads greater than 5.0 cm in diameter per unit area. All the variables studied were related linearly to the distance between plants. The results of the statistical analysis are shown on Tables 1-3. Figures 1-7 show relationships between the two independent variables (nitrogen fertilizer rate and plant spacing) and the dependent variables studied.

1. Yield

Total yield per unit area (central heads plus lateral shoots) increased significantly as the rate of nitrogen fertilizer increased from 0 to 112 kg/ha at any given plant spacing but no difference in total yield was obtained between 112 and 168 kg/ha of nitrogen fertilizer (Table 1).

Yield increased dramatically between 0 and 56 kg/ha of N fertilizer and the increase between 56 and 112 kg/ha although significant, was not as pronounced as between 0 and 56 kg/ha (Fig. 1).

No significant difference was found in total yield when plants were spaced 30, 38, and 46 cm but total yield per plot increased significantly when plants were spaced 22 cm apart. No difference in total yield was found between the 22 and 30 cm spacing (Table 2).

2. Central Heads

The number of central heads greater than 5.0 cm in diameter increased significantly per plot as N fertilizer rate increased from 0 to 56 kg/ha at any given plant spacing. Further increases in fertilizer did not affect the number of heads greater than 5.0 cm in diameter at the different plant spacings (Table 1). At 56, 112, and 168 kg/ha of nitrogen fertilizer more than 75% of the heads in each plot were greater than 5.0 cm for any given plant spacing. At 0 kg/ha of fertilizer the percentage of heads greater than 5.0 cm in diameter varied at the different plant spacings, and although no significant differences in the ratio of heads greater than 5.0 cm were found among the different plant spacings for any given N fertilizer rate, A trend to produce larger heads at the widest spacings (38 and 46 cm) was observed.

The average weight of central head per plant increased significantly as N fertilizer rate increased from 0 to 56 kg/ha but no change in weight was noticed as the fertilizer rate increased further from 56 to 168 kg/ha for any given plant spacing (Table 1).

Average weight of central heads did not vary significantly in plants spaced 22, 30, and 38 cm apart but it increased significantly between plants spaced 46 cm apart and those spaced 22 and 30 cm. No significant increase in central head weight was found when the distance between plants was increased from 38 to 46 cm (Table 2). The number of central heads greater than 5.0 cm diameter per unit area decreased significantly as plant spacing increased from 22 up to 46 cm (Table 2), but it is difficult to determine if the decrease is due to the spacing effect in itself or to the lower number of plants at the wider spacings.

3. Lateral Shoots

The average number of lateral shoots per plant did not change significantly between plants receiving 0 and 56 kg/ha of N fertilizer or between those receiving 112 and 168 kg/ha at any plant spacing (Table 1). However, as the N fertilizer rate increased from 112 to 168 kg/ha, the average number of shoots per plant decreased causing no significant difference between 56 and 168 kg/ha (Table 1 and Fig. 4).

The average number of lateral shoots per plant increased as the distance between plants increased but significant differences were not found between a given distance and the distance immediately below or above. Further increases or decreases in