

EFFECT OF NITRATE/AMMONIUM RATIO AND CONCENTRATION ON VEGETATIVE GROWTH, SEED YIELD AND YIELD COMPONENTS OF DRY BEAN UNDER GREENHOUSE CONDITIONS

5.1 INTRODUCTION

Ammonium (NH_4^+) and nitrate (NO_3^-) are the two forms of nitrogen available for plant growth. The use of NH_4^+ in nutrient solutions has been reported as early as 1860, and mixtures of NH_4^+ and NO_3^- have been adapted into many nutrient solution formulas (Hewitt, 1966). Barker & Mills, 1980 and Cao & Tibbitts (1993) state that most plant species grow better with NO_3^- than NH_4^+ nutrition. Other hydroponics studies have demonstrated advantages of mixed nitrogen forms with several different crops such as wheat (Cox & Reisenauer, 1973), triticale, wheat and rye (Gashaw & Mugwira, 1981), corn and sorghum (Clark, 1982), potato (Davis, Loescher, Hammond & Thornton, 1989) and tomato (Ikeda & Tan, 1998). For potatoes, applications of combined nitrogen forms are generally recommended for production in most growing areas (Hendrickson Keeney, Walsh, & Liegel, 1978). Studies with spring wheat showed increase in growth and yield when plants were fed on combined NH_4^+ -N and NO_3^- -N compared with those fed on predominantly NO_3^- -N (Wang & Below, 1992). In similar experiments, Cox & Reisenauer (1973) report that plants grown with NO_3^- as the sole source of nitrogen often develop Fe deficiency while those grown in NH_4^+ as the sole source of nitrogen may show NH_4^+ toxicity effects if the NH_4^+ levels are too high. The growth rates of both wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) were stimulated when NH_4^+ was added to growth media containing NO_3^- over the growth rates of plants grown with NO_3^- alone. More nitrogen is said to be absorbed and assimilated when both NH_4^+ and NO_3^- are provided in the growth solutions than when either NH_4^+ or NO_3^- alone is given (Jackson, Kwik & Volk, 1976).

The form of nitrogen in a nutrient solution influences the solution pH. The pH of nutrient solutions with NO_3^- as the sole source of N rises to near or above 7 for many plants (Hewitt, 1966). On the other hand the pH of nutrient solutions with NH_4^+ as the sole source of nitrogen decreases to near 4 when many plants are grown in them. This prompted Trelease &

Trelease (1935) to suggest a balance of NO_3^- and NH_4^+ as a buffer against large changes in pH.

According to Claassens (2000, personal communication³) the nutritional status of the Hoagland type nutrient solutions commonly used are relatively high, especially for environmental conditions like in South Africa where the rate of crop transpiration may be much higher than in Europe. Lower concentration of nutrient solutions would be less expensive and make production more cost efficient. The objective of this experiment was to determine the optimum $\text{NO}_3^-:\text{NH}_4^+$ ratio, and the concentration of nutrient solution that would optimise growth and yield of dry bean under greenhouse conditions.

5.2 MATERIALS AND METHODS

Experiment 1

Seed of cultivar Kranskop was planted in 10 litre Mitscherlich pots filled with sterilised sand media. Three seeds were planted in each pot and thinned to one plant per pot during the V2 growth stage. Four treatments were used each replicated three times. There were an adequate number of pots to allow five serial harvests at two weeks intervals and a final harvest at physiological maturity (figure 5.1a). Treatments included two $\text{NO}_3^-:\text{NH}_4^+$ ratios of 14:1 meq/l (93% NO_3^- -N: 7% NH_4^+ -N) and 7:7 meq/l (50% NO_3^- -N: 50% NH_4^+ -N), designated as 14:1 and 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratios respectively, each applied at full strength (FS) and half strength (HS) concentrations. The four nutrient solutions were prepared separately. The full strength (FS) concentration of the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio is equivalent to the standard Hoagland nutrient solution. Micronutrients as suggested by Nitsch (1972) (Table 1c) were added at the same concentration (20ml per 20 litre water) to all the four solutions. The pH of the nutrient solutions was not adjusted and ranged between 5.58 - 8.5 while electrical conductivity (EC) ranged between 1.25 - 2.25 $\text{mS}\cdot\text{cm}^{-1}$ (Table 5.1a). The recommended pH values are between 5.3 - 6.3 and electrical conductivity (EC) between 1.5 - 2.5 $\text{mS}\cdot\text{cm}^{-1}$ (Association for Intensive Plant Production, 1999). The nutrient solutions were applied three times a week, twice with fresh solution and a third with recycled solution after which the pots were flushed with water to avoid salt accumulation. The composition of the nutrient solutions is outlined in Table 1b.

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Serial harvesting started two weeks after emergence. Three pots were harvested fortnightly from each treatment over a period of eight weeks to monitor fresh biomass, dry matter accumulation and leaf area development. The data was plotted in a graph to observe the growth trend of each treatment. Furthermore, statistical analysis was applied to the vegetative growth data at 58 days after emergence (DAE). Three replicates from each treatment were left to reach maturity. These were used to collect data for evaluation of seed yield and yield components of the crop.

Table 5.1a. Composition of four nutrient solutions with two $\text{NO}_3^-:\text{NH}_4^+$ ratios and two concentrations (full strength (FS) and half strength (HS)) used in the first experiment.

$\text{NO}_3^-:\text{NH}_4^+$ RATIO (meq/l)				
SALT	14:1 (FS)	14:1 (HS)	1:1 (FS)	1:1(HS)
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	8	4	7	3.5
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	4	2	4	2
KNO_3	6	3	0	0
K_2SO_4	0	0	6	3
$\text{NH}_4\text{H}_2\text{PO}_4$	1	0.5	1	0.5
$(\text{NH}_4)_2\text{SO}_4$	0	0	6	3
TOTAL-N	15	7.5	14	7
Mean pH	8.45	8.23	5.58	6.70
Mean EC (mS cm^{-1})	1.65	1.25	2.25	1.35

Table 5.1b. Composition of micronutrients*

INGREDIENT	QUANTITY PER LITRE
KCl	2.7g
H_3BO_3	2.9g
MnSO_4	1.7g
ZnSO_4	0.27g
$(\text{NH}_4)_2\text{Mo}_7\text{O}_{24}$	0.27g
CuSO_4	0.14g
H_2SO_4	0.31ml

* Added at rate of 20ml per 20l nutrient solution.



Figure 5.1a General appearance of the bean crop in experiment 1 of the nitrate / ammonium ratio and concentration.



Figure 5.1b General appearance of the bean crop in experiment 2 of the nitrate / ammonium ratio and concentration.

Experiment 2

In the second experiment, cultivar Kranskop was planted in 8 litre capacity pots filled with sterilised sand media (Figure 5.1b). Three seeds were planted in each pot and thinned to one plant per pot during the V2 growth stage as in experiment 1. Nine treatments were compared each replicated three times. Treatments included three $\text{NO}_3^-:\text{NH}_4^+$ ratios of 14:1 meq/l (93% NO_3^- -N: 7% NH_4^+ -N), 7:7 meq/l (50% NO_3^- -N: 50% NH_4^+ -N) and 1:14 meq/l (7% NO_3^- -N: 93% NH_4^+ -N), designated as 14:1, 1:1 and 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratios respectively, each at, full strength (FS), half strength (HS) and quarter strength (QS) concentrations. The nine nutrient solutions were prepared separately and their composition is presented in Table 5.2. The full strength nutrient solution of the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio is equivalent to the standard Hoagland nutrient solution while the others are modifications of it. Micronutrients were added as suggested by Nitsch (1972) (Table 5.1b) at the same concentration (20ml per 20 litre water) to all the nine solutions. The pH of the fresh solution was monitored and adjusted to the recommended values of between 5.3 and 6.3 (Association for Intensive Plant Production, 1999). Electrical conductivity (EC) varied between 0.9 and 2mS.cm⁻¹ although the recommended conductivity is between 1.5 and 2.5mS.cm⁻¹ (Association for Intensive Plant Production, 1999). The drain to waste system with no recycling of nutrient solution was used. The nutrient solutions were prepared in 20 litre plastic containers and applied three times a week and then flushed with water to avoid salt accumulation. Three replicates were harvested from each treatment at 40 DAE for comparison of vegetative and reproductive growth. Three replicates from each treatment were left to reach maturity. These were used to collect data for evaluation of seed yield and yield components of the crop.

Data analysis

In both experiments data was analysed using the General Linear Models (GLM) procedure of the Statistical System (SAS Institute, 1989) computer program. Differences at the $P < 0.05$ level of significance are reported. Means were separated using Tukey's studentised range test.

Table 5.2. Composition of nine nutrient solutions of three $\text{NH}_4^+ : \text{NO}_3^-$ ratios (meq/l) and three concentrations (full strength (FS), half strength (HS) and quarter strength (QS)) used in the second experiment.

SALTS	$\text{NO}_3^- : \text{NH}_4^+$ RATIO (meq/l)								
	14:1 (FS)	14:1 (HS)	14:1 (QS)	1:1 (FS)	1:1 (HS)	1:1 (QS)	1:14 (FS)	1:14 (HS)	1:14 (QS)
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	8	4	2	7	3.5	1.75	1	0.5	0.25
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	4	2	1	4	2	1	2	1	0.5
KNO_3	6	3	1.5	-	-	-	-	-	-
K_2SO_4	-	-	-	6	3	1.5	3	1.5	0.75
$\text{NH}_4\text{H}_2\text{PO}_4$	1	0.5	0.25	1	0.5	0.25	1	0.5	0.25
$(\text{NH}_4)_2\text{SO}_4$	-	-	-	6	3	1.5	13	6.5	3.25
TOTAL-N	15	7.5	3.75	14	7	3.5	15	7.5	3.75

5.3 RESULTS

Experiment 1

General observations

Plants receiving the nutrient solution containing 50% NO_3^- -N with 50% NH_4^+ -N (1:1 $\text{NO}_3^- : \text{NH}_4^+$ ratio) showed deep pigmentation. The leaves developed deep green colouration unlike those receiving the nutrient solution containing 93% NO_3^- -N with 7% NH_4^+ -N (14:1 $\text{NO}_3^- : \text{NH}_4^+$ ratio) at both the full strength and half strength concentrations. This gives an indication that NH_4^+ -N enhances chlorophyll development, the green pigmentation in leaves. According to Makus (1984) vegetable amaranth plants fertilized with more NH_4^+ -N were higher in leaf pigments than those receiving nitrogen in the other form. Cao & Tibbits (1993) attributed this to the concentration and accumulation of more nitrogen in the shoots and roots with a combination of NH_4^+ -N and NO_3^- -N nutrition. The physiological aspect of this is not yet established.

Effect of nitrate / ammonium ratio and concentration on vegetative growth

Fresh biomass (g)

Data from the five serial harvests is presented in Figure 5.2, and the results of the last of these sampling periods (58 DAE) are summarised in Table 5.3. The nitrate / ammonium ratio main effect significantly affected fresh biomass accumulation while the concentration main effect and the ratio x concentration interactions did not.

Fresh biomass accumulation of plants differed in the different treatments. Plants receiving the nutrient solutions containing 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio at the full strength and half strength concentrations showed better growth rates than those receiving the nutrient solutions containing 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio at the two concentrations (Figure 5.2). Within the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio, plants receiving the half strength nutrient solution grew better than those receiving the full strength nutrient solution.

Significant ratio main effect indicates that the different ratio treatments affected the fresh biomass differently. The 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio accumulated significantly more fresh biomass (89.3g) than the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (66.1g). Differences between plants receiving the full strength and half strength nutrient solution were not significant, although there was a tendency by plants receiving the full strength nutrient solution developing a somewhat larger (80.5g) fresh biomass than those receiving the half strength nutrient solution (74.9g)(Table 5.3).

Leaf area (cm^2)

Data from the five serial harvests is presented in Figure 5.3, and the results of the last of these sampling periods (58 DAE) are summarised in Table 5.3. The nitrate / ammonium ratio main effect significantly affected the leaf area development while the concentration main effect and the ratio x concentration interactions did not.

The leaf area development followed a similar trend as fresh biomass accumulation. Poor leaf area development was observed in some plants receiving the full strength nutrient solution treatment containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio. This affected the treatment's overall performance. On the other hand, plants receiving the half strength nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio maintained a steady increase in leaf area similar to plants

receiving the full strength and half strength nutrient solutions containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (Figure 5.3). Differences emerged 29 DAE where plants receiving the full strength and half strength nutrient solutions containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio developed a somewhat larger leaf area than those receiving the nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (Figure 5.3).

Plants receiving the nutrient solution containing 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio developed a somewhat larger leaf area (1365.7cm^2) than those receiving the nutrient solution containing 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (1159.3cm^2). No differences were observed between plants receiving the full strength and half strength nutrient solution concentration treatments (Table 5.3). The non significant ratio x concentration interaction shows that there were no difference in leaf area development among plants receiving the different treatment combinations.

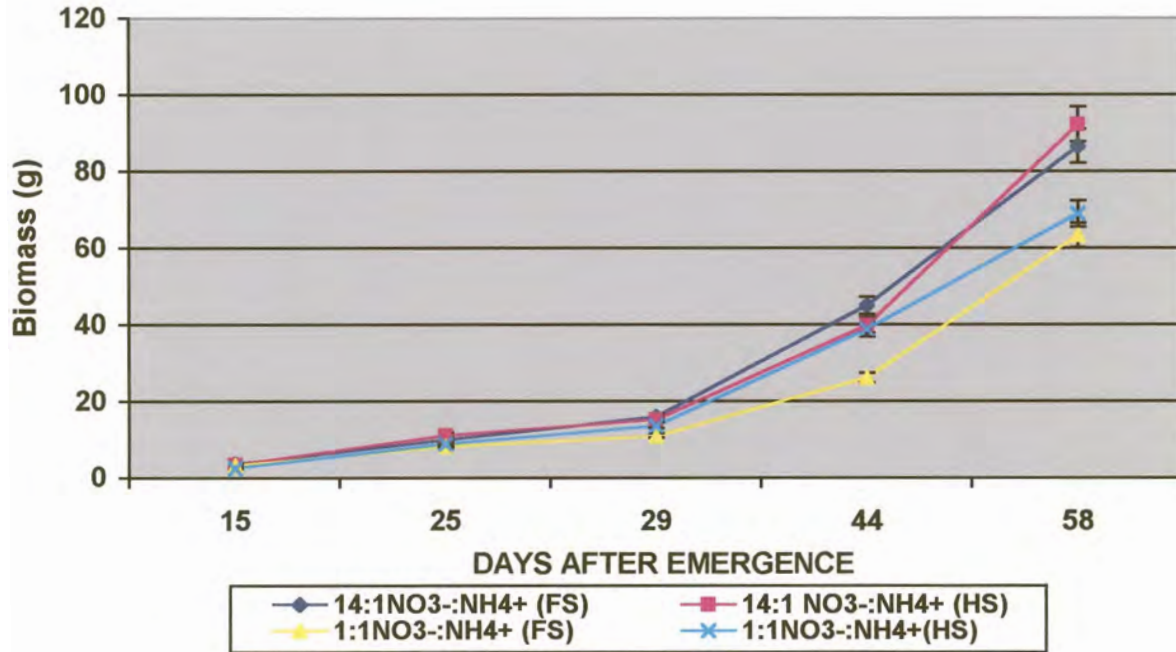


Figure 5.2 Effect of nitrate / ammonium ratio and concentration on fresh biomass accumulation

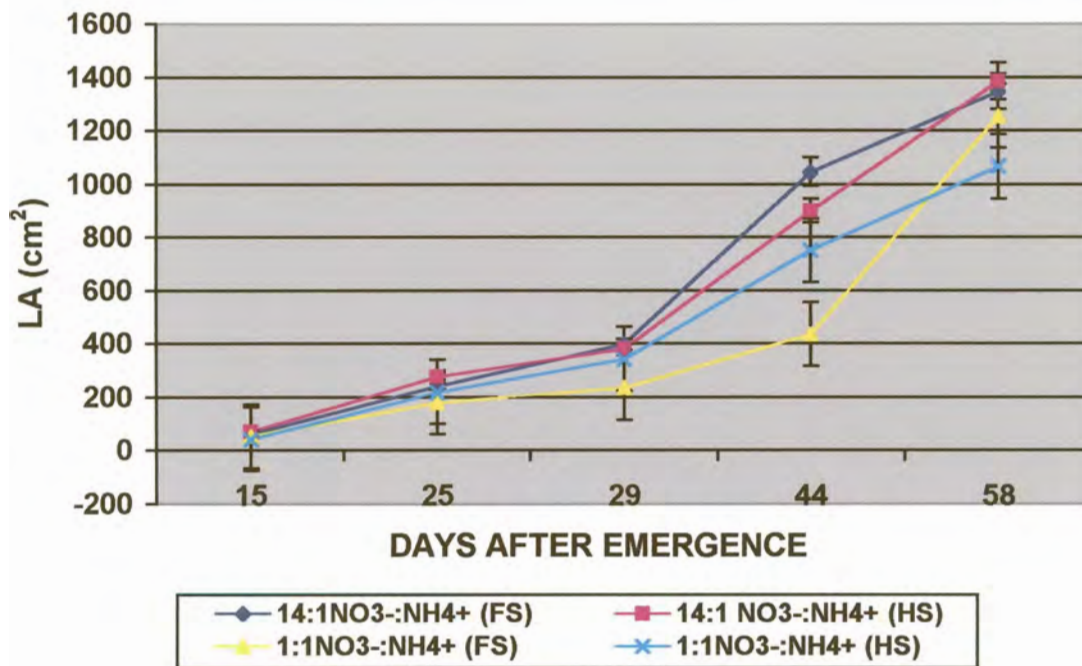


Figure 5.3 Effect of nitrate / ammonium ratio and concentration on leaf area (cm²)

Table 5.3 Effect of NO₃⁻:NH₄⁺ ratio and concentration on vegetative growth of dry bean 58 DAE (ANOVA: Appendix Tables 8.5A – 8.5E).

	FRESH BIOMASS (g)	LEAF AREA (cm ²)	DRY BIOMASS (g)	SHOOT DRY WEIGHT (g)	ROOT DRY WEIGHT (g)
NO₃⁻:NH₄⁺ RATIO					
14:1	89.3a	1365.7a	12.4a	11.5a	0.85a
1:1	66.1b	1159.3b	9.5b	8.77b	0.71a
CONCENTRATION					
Full strength (FS)	80.5a	1300.2a	11.4a	10.6a	0.80a
Half strength (HS)	74.9a	1224.8a	10.5a	9.7a	0.76a
SE	4.7	39.3	0.58	0.61	0.07
LSD (P≤0.05)	15.4	128.2	1.88	1.98	0.24
R ²	0.61	0.71	0.63	0.58	0.21
CV (%)	14.9	7.62	12.9	14.7	23.2

Means within the columns followed by the same letter are not significantly different (P≤0.05) according to Tukey's studentized range test.

Dry biomass (g)

Table 5.3 shows the effect of $\text{NO}_3^-:\text{NH}_4^+$ ratio and concentration on dry biomass accumulation of dry bean. The ratio main effect was significantly different while the concentration main effect and the ratio x concentration interactions were not.

Plants receiving the nutrient solution containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio accumulated larger dry biomass (12.4g) than those receiving the nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (9.5g). No differences in dry biomass were observed among plants receiving the full strength and half strength nutrient solutions. A somewhat larger dry biomass (11.4g) was produced by plants receiving the full strength nutrient solution than 10.5g produced by plants receiving the half strength nutrient solution.

Shoot dry weight (g)

The effect of nitrate / ammonium ratio and concentration on shoot dry weight is shown in Table 5.3. Only the ratio main effect was significant while the concentration main effect and the ratio x concentration interactions were not.

Plants receiving the nutrient solution containing 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio produced significantly larger shoot dry weight (11.5g) than those receiving the nutrient solution containing 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (8.77g). No differences were observed between plants receiving the different nitrogen concentration treatments. However, plants receiving the full strength nutrient solution produced a somewhat larger (10.6g) shoot dry weight than those fed on the half strength nutrient solution (9.7g).

The non significant ratio x concentration interactions effects show that different treatment combinations produced similar shoot dry weights.

Root dry weight (g)

The data for the effect of nitrate / ammonium ratio and concentration on the root dry weight of dry bean cultivar Kranskop at 58 DAE is presented in Table 5.3. Both the ratio and concentration main effect and the ratio x concentration interactions were not significant.

A somewhat larger (0.85g) root dry weight was produced by plants receiving the nutrient solution containing 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio than those receiving the nutrient solution containing 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (0.71g). No differences were observed among plants receiving the two different nutrient solution concentrations. However, plants receiving the full strength nutrient solution developed a somewhat larger (0.80g) root dry weight than 0.76g produced by plants receiving the half strength nutrient solution.

Effect of nitrate / ammonium ratio and concentration on seed yield and yield components

Table 5.4 shows the effect of nitrate / ammonium ratio and concentration on seed yield and yield components of dry bean cultivar Kranskop. Both the ratio and concentration main effects and the ratio x concentration interactions effects were not significant.

Despite the differences observed in vegetative growth of dry bean plants receiving the two different nitrate / ammonium ratios, no significant differences were observed in seed yield and yield components. The number of pods per plant, seeds per pod, 100 seed mass, seed yield per plant and harvest index were similar among all plants receiving both the nutrient solutions containing 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio and 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (Table 5.4). Similarly no differences were observed in seed yields and yield components among plants receiving the full strength and half strength nutrient solutions (Table 5.4). Both the full strength and half strength nutrient solutions affected seed yields and yield components in a similar way, indicating that either of the concentrations can be used without affecting seed yields of the crop.

Table 5.4 Effect of $\text{NH}_4^+:\text{NO}_3^-$ ratio and concentration on yield and yield components of dry bean (ANOVA: Appendix Table 8.5 F – 8.5J).

	PODS/PLANT	SEEDS/POD	100 SEED MASS (g)	YIELD/PLANT (g)	HI (%)
$\text{NO}_3^-:\text{NH}_4^+$ RATIO					
14:1	10.17a	3.7a	54.4a	20.4a	47.2a
1:1	10.67a	3.7a	49.0a	17.2a	48.4a
CONCENTRATION					
Full strength (FS)	11.0a	3.7a	52.2a	21.0a	47.7a
Half strength (HS)	9.83a	3.7a	51.2	16.6a	48.0a
SE	0.75	0.17	2.61	2.82	0.49
LSD ($P \leq 0.05$)	2.46	0.49	8.53	4.60	1.61
R^2	0.30	0.08	0.23	0.58	0.30
CV (%)	17.7	9.92	12.4	18.4	2.53

Means within the columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Tukey's studentized range test.

Experiment 2

General observations

Initially, plant growth was normal for all the treatment combinations as observed in figure 5.1b. However visual differences were observed among the different treatments with time, especially between plants receiving the nutrient solution containing 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio and those receiving the 14:1 and 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratios. Plants receiving the nutrient solution containing 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio were poorly developed, stunted and weak. The leaves were rather small, curled, thick and initially with a deep green pigmentation that faded with time becoming chlorotic at both the full strength and half strength nutrient solution treatments (see Figure 5.4). When grown to maturity the leaves of the plants in this treatment were completely chlorotic resulting in early harvest of the treatment (see Figure 5.5). On the other hand plants receiving the full strength and half strength nutrient solutions containing the 14:1 and 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratios were healthy.



Figure 5.4 Comparison of plants receiving different $\text{NO}_3^-:\text{NH}_4^+$ ratios and concentrations: A. 14:1 (FS); B. 14:1 (HS); C. 14:1 (QS); D. 1:1 (FS); E. 1:1 (HS); F. 1:1 (QS); G. 1:14 (FS); H. 1:14 (HS); I. 1:14 (QS).



Figure 5.5 Plants receiving 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratios showing chlorosis on the far left. From left: 1:14 (QS); 1:14 (HS); 1:14 (FS); 1:1 (QS)

Plants receiving the quarter strength nutrient solution initially grew better in all the three $\text{NO}_3^-:\text{NH}_4^+$ ratios but changed with age. The plants receiving the quarter strength nutrient solutions containing the 14:1 and 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratios were healthy and good looking throughout the growing period, becoming slightly yellowish with slight chlorosis especially on the edges (see Figure 5.4 & 5.5). On the other hand the plants receiving the quarter strength nutrient solution containing the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio became more yellowish and rather weak as they grew older. Slight curling of leaves and chlorosis were also observed (Figure 5.5)

Effect of nitrate / ammonium ratio and concentration on vegetative growth 40 DAE

Table 5.5 shows the effect of nitrate / ammonium ratio on the vegetative growth of dry bean cultivar Kranskop at 40 DAE.

Fresh biomass (g)

The data for the effect of nitrate / ammonium ratio and concentration on fresh biomass of dry bean cultivar Kranskop at 40 DAE are presented in Table 5.5. The concentration main effect was significant while both the ratio main effect and the ratio x concentration interactions effects were not.

No difference was observed among plants receiving the different nitrate / ammonium ratios. Plants receiving the nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio accumulated somewhat more (45.2g) fresh biomass than those receiving the nutrient solutions containing 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (42.7g) and 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio (38.0g).

Significant differences in fresh biomass accumulation were observed among plants receiving the different nutrient solution concentrations (Table 5.5). Plants receiving the full strength and half strength nutrient solution concentrations accumulated more fresh biomass (48.5g and 44.6g respectively) than those receiving the quarter strength nutrient solution (32.8g).

Leaf area (cm^2)

The data for the effect of nitrate / ammonium ratio and concentration on leaf area of dry bean cultivar Kranskop at 40 DAE are presented in Table 5.5. The ratio and concentration main effects were significant while the ratio x concentration interaction effects were not.

Plants receiving the nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio developed a significantly larger (1216.3 cm^2) leaf area than those receiving the nutrient solution containing the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio (924.8 cm^2). The leaf area (1077.2 cm^2) for plants receiving the nutrient solution containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio was intermediate and not different from that of plants in the other two treatments.

Significant differences were also observed in leaf area among plants receiving the different nutrient solution concentrations. Plants receiving the full strength nutrient solution developed a significantly larger leaf area (1268.8 cm^2) than those receiving the quarter strength nutrient solution (804.4 cm^2). The leaf area of plants receiving the half strength nutrient solution was intermediate (1145.1) and significantly larger than that of plants receiving the quarter strength nutrient solution but not different from the leaf area of plants receiving the full strength nutrient solution.

Dry biomass (g)

Table 5.5 shows the data for the effect of nitrate / ammonium ratio and concentration on dry biomass of dry bean cultivar Kranskop 40 DAE. Significant concentration main effect was observed while both the ratio and ratio x concentration interaction effects were not.

Although the ratio main effect was not significant, plants receiving the nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio accumulated somewhat more (14.0 g) dry biomass than those receiving the nutrient solutions containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (13.0 g) and the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio (11.8 g). The significant concentration main effect shows that differences existed in dry biomass accumulation among plants receiving the different nutrient solution concentrations. Plants receiving the full strength and half strength nutrient solutions accumulated significantly more (14.5 g and 14.0 g respectively) dry mass than those receiving the quarter strength nutrient solution (10.3 g).

Shoot dry weight (g)

Table 5.5 also shows the data for the effect of nitrate / ammonium ratio and concentration on shoot dry weight of dry bean cultivar Kranskop 40 DAE. Significant concentration main effect was observed while both the ratio and the ratio x concentration interaction effects were not.

Plants receiving the nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio developed somewhat more (6.4g) shoot dry weight than those receiving the nutrient solutions containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (6.1g) and the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio (5.3g). The significant concentration main effect indicates that plants receiving the different nutrient solution concentrations differed in their shoot dry weight accumulation. Plants receiving the full strength and half strength nutrient solutions accumulated significantly more shoot dry weight (6.8g and 6.4g respectively) than plants receiving the quarter strength nutrient solution (4.8g).

Root dry weight (g)

Table 5.5 shows the data for the effect of nitrate / ammonium ratio and concentration on root dry weight of dry bean cultivar Kranskop 40 DAE. Both the ratio and concentration main effects and the ratio x concentration interaction effects were not significant.

Despite the non significant ratio main effect, plants receiving the nutrient solution containing the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio developed somewhat larger (0.99g) root dry weight than that of plants receiving the nutrient solutions containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (0.85g) and the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (0.71g). Plants receiving the full strength nutrient solution developed somewhat more (0.95g) root dry weight than those receiving the half strength (0.92g) and quarter strength (0.68g) nutrient solutions.

Table 5.5 Effect of $\text{NO}_3^-:\text{NH}_4^+$ ratio and concentration on vegetative growth of dry bean 40 DAE (ANOVA: Appendix Tables 8.6A - 8.6E).

	FRESH BIOMAS (g)	LEAF AREA (cm^2)	DRY BIOMASS (g)	SHOOT DRY WEIGHT (g)	ROOT DRY WEIGHT (g)
$\text{NO}_3^-:\text{NH}_4^+$ RATIO					
14:1	42.7a	1077.2ab	13.0a	6.1a	0.71a
1:1	45.2a	1216.3a	14.0a	6.4a	0.85a
1:14	38.0a	924.8b	11.8a	5.3a	0.99a
CONCENTRATION					
Full strength (FS)	48.5a	1268.8a	14.5a	6.8a	0.95a
Half strength (HS)	44.6a	1145.1a	14.0a	6.4a	0.92a
Quarter strength (QS)	32.8b	804.4b	10.3b	4.8b	0.68a
SE	3.02	66.14	0.91	0.39	0.17
LSD ($P \leq 0.05$)	10.9	238.7	3.28	1.40	0.62
R^2	0.53	0.70	0.52	0.58	0.19
CV (%)	21.6	18.5	21.1	19.6	60.6

Means within the columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Tukey's studentized range test.

Effect of nitrate / ammonium ratio and concentration on seed yield and seed yield components at harvest

After physiological maturity, seed yield and yield components data from all treatments were analysed.

Seed mass per plant (g)

The effect of nitrate / ammonium ratio and concentration on seed mass per plant (g) of dry bean cultivar Kranskop is presented in Table 5.6. Only the ratio main effect and the ratio x concentration interaction effects were highly significant while concentration main effect was not.

Table 5.6 Effect of nitrate / ammonium ratio and concentration on seed mass (g) per plant of dry bean cultivar Kranskop at maturity (ANOVA: Appendix Table 8.6F)

Concentration (C)	Nitrate / ammonium ratio (R)			Mean
	14:1	1:1	1:14	
Full strength	24.9	20.6	3.0	16.2
Half strength	18.8	22.7	6.4	16.0
Quarter strength	13.5	20.5	12.6	15.5
Mean	19.1	21.2	7.3	15.9
LSD (R) = 3.4	LSD (C) = ns		LSD (R x C) = 10.7	
CV (%) = 14.6	SE = 1.9		R ² = 0.5	

Main effects. Different $\text{NO}_3^-:\text{NH}_4^+$ ratio treatments affected the seed mass of dry bean differently. The highest seed mass per plant (21.2g) was produced by plants receiving the nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio even though this was not significantly different from the seed mass per plant (19.1g) produced by plants receiving the nutrient solution containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (Table 5.6). Plants receiving the nutrient solution containing the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio produced the lowest seed mass per plant (7.3g). No difference was observed among plants receiving the different nutrient solution concentrations. A somewhat larger seed mass per plant (16.2g) was produced by plants receiving the full strength nutrient solution. This decreased as the concentration treatment decreased. On average plants receiving the nutrient solution containing the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio produced the lowest seed mass per plant at all nutrient solution concentrations and over all concentration treatments.

Interaction. The significant ratio x concentration interaction shows that different ratio x concentration treatment combinations affected the seed mass per plant differently. The largest (24.9g) seed mass per plant was produced by plants receiving the full strength nutrient solution containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio followed by 22.7g produced by plants receiving the half strength nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio.

Table 5.7 shows the effect of nitrate / ammonium ratio and concentration on number of pods per plant. The ratio main effect and ratio x concentration interaction were significant ($P \leq 0.05$) while the concentration main effect was not.

Table 5.7 Effect of ammonium/nitrate ratio and concentration on number of pods per plant of dry bean cultivar Kranskop at maturity (ANOVA: Appendix Table 8.6G)

Concentration (C)	Nitrate / Ammonium ratio (R)			Mean
	14:4	1:1	1:14	
Full strength	12.0	8.8	1.2	7.3
Half strength	8.5	9.5	5.5	7.8
Quarter strength	6.5	8.0	7.3	7.3
Mean	9.0	8.8	4.7	7.5
LSD (R) = 1.4	LSD (C) = ns		LSD (R x C) = 4.3	
CV (%) = 21.0	SE = 0.78		$R^2 = 0.81$	

Main effects. The significant ratio main effect indicates that there were differences in the number of pods per plant due to changes in the $\text{NO}_3^-:\text{NH}_4^+$ ratio. The highest number of pods per plant (9.0) was produced by plants receiving the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio followed by plants receiving the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (8.8) and then those receiving the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio (4.7) (Table 5.7). Although the effect of nitrogen concentration in the nutrient solution was not significant, the highest number of pods per plant (7.8) was produced by plants receiving the half strength nutrient solution while those receiving the full strength and quarter strength nutrient solutions both produced 7.3 pods per plant.

Interaction. The significant ratio x concentration interaction effect reflects the differences in podset per plant due to different ratio x concentration treatment combinations. Different ratios at different nutrient solution concentrations produced different number of pods per plant. The highest number of pods per plant (12) was set by plants receiving the full strength nutrient solution containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio followed by those receiving the half strength nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio which produced 9.5 pods per plant.

The effect of nitrate / ammonium ratio and concentration on number of seeds per pod is shown in Table 5.8. Both the ratio and concentration main effects and the ratio x concentration interaction effects were not significant ($P \leq 0.05$).

Table 5.8 Effect of nitrate / ammonium ratio and concentration on number of seeds per pod of dry bean cultivar Kranskop at maturity (ANOVA: Appendix Table 8.6H)

Concentration (C)	Nitrate / Ammonium ratio (R)			Mean
	14:1	1:1	1:14	
Full strength	3.4	4.0	4.0	3.8
Half strength	3.5	4.0	3.8	3.8
Quarter strength	3.6	3.8	3.8	3.8
Mean	3.6	3.9	3.9	3.8
LSD (R) = ns	LSD (C) = ns	LSD (R x C) = ns		
CV (%) = 13.5	SE = 0.26	R ² = 0.14		

Main effects. The non significant main effects indicate that there were no differences in the number of seeds per pod set due to changes in the nitrate / ammonium ratio and nutrient solution concentration. Plants receiving the nutrient solutions containing the 1:1 and the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratios set relatively more seeds per pod (3.9) than those receiving the nutrient solution containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio (3.6). For some unclear reason, plants receiving the nutrient solution containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio produced somewhat less number of seeds per pod at all nutrient solution concentrations (Table 5.8).

Interaction. Just as with the main effects, no differences were observed in seedset among all the ratio x concentration interaction combinations. Relatively more seeds per pod (4 seeds) were set by plants receiving the nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio at the full strength and half strength and those receiving the full strength nutrient solution containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio. The lowest number of seeds per pod was set by plants receiving the nutrient solution containing the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio at all nutrient solution concentrations, with plants receiving the full strength nutrient solution setting the lowest (3.4 seeds per pod).

Table 5. 9 shows the effect of the nitrate / ammonium ratio and concentration on 100 seed mass (g) of dry bean. The ratio main effect and the ratio x concentration interaction effects were highly significant ($P \leq 0.01$) while the concentration main effect was not.

Table 5.9 Effect of nitrate / ammonium ratio and concentration on 100 seed mass (g) of dry bean cultivar Kranskop at maturity (ANOVA: Appendix Table 8.6I)

Concentration (C)	Nitrate / Ammonium ratio (R)			Mean
	14:1	1:1	1:14	
Full strength	60.5	57.7	59.0	59.1
Half strength	60.0	61.5	29.4	50.3
Quarter strength	55.6	65.7	45.6	55.6
Mean	58.7	61.6	44.7	55.0
LSD (R) = 7.9	LSD (C) = ns		LSD (R x C) = 24.6	
CV (%) = 16.2	SE = 4.5		$R^2 = 0.65$	

Main effects. The significant nitrate / ammonium ratio main effect shows that changes in the $\text{NO}_3^-:\text{NH}_4^+$ ratio affected the seed size of dry bean cultivar Kranskop. The largest seed size (61.6g/100 seed) was produced by plants receiving the nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio although this was not significantly different from the 58.7g/100 seed produced by plants receiving the nutrient solution containing the 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio. The smallest seed size (44.7g/100 seed) was produced by plants receiving the nutrient solution containing the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio. The non significant concentration main effect shows that changes in the nutrient solution concentration did not affect the seed size of dry bean in the experiment. Plants receiving the full strength nutrient solution produced a somewhat larger seed size (59.9g/100 seed) than those receiving the quarter strength (55.6g/100 seed) nutrient solution. The smallest seed size (50.3g/100 seed) was produced by plants receiving the half strength nutrient solution.

Interaction. The significant interaction effects indicate that the different ratio x concentration interaction treatment combinations affected seed size of dry bean differently. The largest seed size (65.7g/100 seed) was produced by plants receiving the quarter strength nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio followed by 61.5g/100 seed produced by plants receiving the half strength nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio too. The

significant ratio x concentration treatment combination could be attributed to the unexpected low seed size (29.4g/100 seed) produced by plants receiving the half strength nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio. This may have been due to the termination of assimilate supply during pod fill stage as almost all the leaves of plants in this treatment senesced early due to chlorosis. This prompted early harvest of the treatment.

Table 5. 10 shows the effect of nitrate / ammonium ratio and concentration on harvest index (%) of dry bean. Only the ratio main effect was highly significant ($P \leq 0.01$) while the concentration main effect and the ratio x concentration interaction effects were not.

Table 5.10 Effect of nitrate / ammonium ratio and concentration on harvest index (HI) (%) of dry bean cultivar Kranskop at maturity (ANOVA: Appendix Table 8.6J)

N-Concentration (C)	Nitrate / Ammonium ratio (R)			Mean
	14:1	1:1 (7:7)	1:14	
Full strength	45.1	48.6	30.6	41.4
Half strength	46.1	50.0	40.3	45.5
Quarter strength	43.0	45.7	44.9	44.5
Mean	44.7	48.1	38.6	43.8
LSD (R) = 5.6	LSD (C) = ns		LSD (R x C) = ns	
CV (%) = 14.6	SE = 3.2		$R^2 = 0.50$	

Main effects. The significant ratio main effect shows that the different the $\text{NO}_3^-:\text{NH}_4^+$ ratios affected harvest index differently. The highest harvest index (48.1%) was observed among plants receiving the nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio. This was not significantly different from the harvest index observed among plants receiving the nutrient solution containing the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio (Table 5.10). No differences were observed among plants receiving the different nutrient solution concentrations. A somewhat higher harvest index (45.5%) was observed among plants receiving the half strength nutrient solution followed by that observed among plants receiving the quarter strength nutrient solution (44.5%) and the full strength nutrient solution (41.4%).

Interaction. The non significant ratio x concentration treatment interaction shows that the main effects affected the harvest index independent of each other. However, a somewhat

higher harvest index (50%) was obtained among plants receiving the half strength nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio followed by 48.6% produced by plants receiving the full strength nutrient solution containing the 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio.

The non significant ratio x concentration interaction effect for harvest index, while that of seed mass was significant, could be attributed to the loss of biomass in the half strength nutrient solution containing the 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio. The plants in this treatment lost the leaves due to chlorosis and suspected ammonium toxicity. As such, the high harvest index shown does not take into account this loss in biomass.

5.4 DISCUSSION

The main outcome of this experiment is that $\text{NO}_3^-:\text{NH}_4^+$ ratio and nutrient solution concentration affected growth, development and productivity of dry bean. More attention should be paid to $\text{NO}_3^-:\text{NH}_4^+$ ratio as it has an overriding effect on plant growth and productivity. Both vegetative and reproductive growth were enhanced with a combination of NO_3^- -N and NH_4^+ -N in the nutrient solution. More vegetative growth was associated with plants receiving either the nutrient solutions containing 93% NO_3^- -N (14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio) or 50% NO_3^- -N (1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio) in combination with NH_4^+ -N.

Theoretically, NH_4^+ is a more desirable form of nitrogen for plants as it is the form in which plants assimilate nitrogen directly into amides and amino acids (Davis *et al*, 1986). In contrast, NO_3^- -N requires a lot of energy for it to be reduced to NH_4^+ before assimilation. The toxicity observed among plants receiving high NH_4^+ -N could be attributed to limited use of the absorbed and assimilated NH_4^+ . Plants need to balance between the rates of uptake and detoxification during its utilisation as suggested by Ikeda & Tan (1998). They further observed a trend of NH_4^+ decreasing cation absorption and occasionally causing physiological disorders like Ca deficiency. The Association for Intensive Plant Production (1999) reports that high NH_4^+ -N concentration may result in NH_4^+ competing with cation uptake of especially K, Ca and Mg and that the rhizosphere acidifies to unacceptably low pH. Ikeda & Tan (1998) concluded that NH_4^+ -N is detrimental to potato growth regardless of stage of development if it is the sole source of nitrogen. They also state that nitrogen source influenced the mineral composition of potato tissue, particularly levels of P, Ca and Mg. Bernardo *et al* (1984 b) indicate that as the proportion of NH_4^+ in solution increased, K, Ca, Mn, and Zn concentrations decreased in the leaves, while Ca, Mg, Mn and Cu concentrations

decreased in roots.

In these experiments good plant growth was observed among plants receiving the nutrient solutions containing 14:1 and 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratios (93% NO_3^- -N with 7% NH_4^+ -N and 50% NO_3^- -N with 50% NH_4^+ -N respectively) and poor among plants receiving the nutrient solution containing 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio (7% NO_3^- -N with 93% NH_4^+ -N). More leaf area and total biomass (both fresh and dry) were produced by plants receiving the nutrient solution containing 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio treatment than those receiving the nutrient solution containing 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio treatment although the differences were not significant. Dry biomass production was poor among plants receiving the nutrient solution containing 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio treatment. Gashaw & Mugwira (1981) found similar results in their work with triticale in which a 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio produced more shoot and root dry matter than with the mixture of 1:3 $\text{NO}_3^-:\text{NH}_4^+$ ratio and sole NH_4^+ -N. They further report poor performance of wheat and rye when grown in solutions containing 0:4 than with 4:0, 3:1, 1:1 and 1:3 $\text{NO}_3^-:\text{NH}_4^+$ -N mixtures, indicating that high NH_4^+ -N in nutrient solutions affects plant growth. Poor growth of plants receiving the nutrient solution containing high NH_4^+ -N has also been reported for potato. Reports using potato meristem and stem culture show that NH_4^+ -N may be detrimental to potato growth. In studies of potatoes grown to maturity in solution and sand cultures, Davis *et al.* (1986) report that NH_4^+ -N reduced growth, caused leaf roll, suppressed Ca and Mg absorption and increased P and N accumulation.

Studies with maize have also shown the benefit of combining different nitrogen sources in nutrient solutions (Schrader, Domska, Jung & Peterson, 1972 and Below & Gentry, 1992). In all these experiments, results have shown that plant growth is usually greater at 25% and 50% NH_4^+ -N than with 75% NH_4^+ -N (Gamnore - Newmann & Kafkafi, 1980; Gashaw & Mugwira, 1981). Working with potatoes Cao & Tibbitts (1993) found that dry weights of whole plant and separate plant parts were significantly higher with all NO_3^- -N / NH_4^+ -N combinations from 4% to 20% NH_4^+ -N than with NO_3^- -N only.

While a number of studies have been undertaken to determine the effect of $\text{NO}_3^-:\text{NH}_4^+$ ratio on different crops, many have mostly focussed on vegetative growth and have been terminated before physiological maturity. In the first experiment there were no differences in yields and yield components due to changes in the $\text{NO}_3^-:\text{NH}_4^+$ ratio. The non significant yield and yield components among plants receiving the nutrient solutions containing 14:1 and 1:1

$\text{NO}_3^-:\text{NH}_4^+$ ratios shows that either of the two ratios can be used without any compromise on seed yield quantity (Table 5.4). Differences were observed in the second experiment in which significantly higher seed yields per plant (21.2g and 19.1g) were produced by plants receiving the nutrient solutions containing 1:1 and 14:1 $\text{NO}_3^-:\text{NH}_4^+$ ratios respectively than the 7.5g seed yield per plant produced by plants receiving the nutrient solution containing 1:14 $\text{NO}_3^-:\text{NH}_4^+$ ratio. The use and benefits of combined nitrogen sources in crop production have been reported for potatoes (Hendrickson *et al.*, 1978), wheat and rye (Gashaw & Mugwira, 1981) and tomatoes (Ikeda & Tan, 1998) under hydroponic systems. Solanaceous crops such as tobacco and tomato have also been reported to prefer a high $\text{NO}_3^-:\text{NH}_4^+$ -N ratio (Davis *et al.*, 1986).

The concentration of nitrogen in the nutrient solution plays a role in determining seed yield and yield components of dry bean. In the first experiment there was no advantage in seed yields and yield components among plants receiving the full strength nutrient solution over those receiving the half strength nutrient solution concentration. For the nutrient solutions containing 14:1 and 1:1 $\text{NO}_3^-:\text{NH}_4^+$ ratios, the concentration of the nutrient solution does not matter. Both the full strength and half strength nutrient solution concentrations may be used with minimal yield differences.

If a high NH_4^+ -N source is to be used, there may be need to reduce the concentration of the nutrient solution. NH_4^+ -N source is reported to be less toxic at low concentration. As plants require large quantities of nitrogen, this level of nitrogen may not be adequate for plant growth. Nitrogen deficiency symptoms may be observed as the case in this study. Plants receiving the quarter strength nutrient solution in all the three $\text{NO}_3^-:\text{NH}_4^+$ ratios developed nitrogen deficiency symptoms. Plants developed pale yellow coloration. The quarter strength nutrient solution with high NH_4^+ -N treatment (7% NO_3^- -N with 93% NH_4^+ -N) showed severe deficiency with age and some brownish blotches on the leaf edges. This has been associated with Ca deficiency (Ikeda & Tan, 1998 and Association for Intensive Plant Production, 1999). Similar results have been reported for tomato plants too (Kirkby & Mengel, 1967).

5.5 CONCLUSION

A combination of NO_3^- -N and NH_4^+ -N in nutrient solutions is suitable for dry bean production. Limiting the NH_4^+ -N to between 7 - 50% in combination with NO_3^- -N would enhance vegetative growth and provide adequate nutrients for good growth and seed yield. Both the full strength and half strength concentrations produced similar seed yields indicating possibility of cost saving by using the half strength nutrient solution. These combined NH_4^+ and NO_3^- -N sources have also been associated with more stable nutrient solution pH due to their relatively high buffering capacities as observed by Clark (1982) and Bernardo, *et al.* (1984a & b).

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EFFECT OF A CYTOKININ-CONTAINING GROWTH REGULATOR ON SEED YIELD AND YIELD COMPONENTS OF DRY BEAN

6.1 INTRODUCTION

Seed yield per plant is determined by number of flowers formed per plant, percentage podset, number of seeds per pod and seed size. Dry beans form more flowers than mature pods. The difference between number of flowers and number of pods set has been attributed to abscission of both flowers and immature pods (Binnie & Clifford, 1981) and this may be one of the possible reasons for not maximising seed yield in dry beans. According to Tamas, Ozbun, Wallace, Powell & Engels (1979) abscission of pods appears to be the last step in the process of fruit abortion, which is characterised by cessation of seed development, flattening of pod walls and loss of green colour. These processes have been said to be under hormonal control and events leading to pod abortion have been associated with a decrease in the concentration of auxins (Luckwill, 1953, 1981) and an increase in the concentration of ethylene and abscisic acid (ABA) (Davis & Addicott, 1972; Lipe & Morgan 1972).

Although it cannot be stated categorically that overcoming abscission would result in yield increase, information concerning flower and pod abscission and the possible causes of the phenomenon is vital (Van Schaik & Probst, 1958 and Ojehomon, 1970). There is a need to evaluate available growth regulators to identify products with the potential to improve the productivity of dry bean by reducing flower and pod abortion. This information could provide a basis for possible intervention to control the phenomenon. Application of exogenous growth regulators offers one possibility of intervening in the process of abscission (Keller & Belluci, 1983).

The objective of this experiment was to establish whether a cytokinin-containing growth regulator (trade name Marinure), a seaweed extract containing 15ml per litre cytokinin and 22ml per litre auxin (Canyon Report, 1998), affects vegetative growth and yield of dry bean by limiting abscission and enhancing dry matter partitioning to the reproductive organs.

Plant material and growth conditions

Seed of two dry bean cultivars, Teebus and Kranskop, were planted in a pot experiment in a greenhouse on 10th August 2000 at the University of Pretoria Experimental Farm (Lat. 25° 45'S, Long. 28° 16'E, elevation 1372masl). Five litre capacity pots were filled with sterilised sand and three seeds were planted per pot and thinned to one plant per pot five days after emergence. The Nitsch nutrient solution (Nitsch, 1972) was applied at a rate of 600 ml per application three times a week. Tap water was supplied on the other days to leach the sand and hence avoid salt accumulation.

Aldicarb (Temik), a systemic insecticide and Triforine (Fungitex), a systemic fungicide, were applied for control of aphids and fungal infection respectively. Tetradifon, a red spidercide was also applied once weekly for three weeks to control spidermite infection.

Cytokinin - containing growth regulator treatments

Three treatments of a cytokinin-containing growth regulator (trade name Marinure), a sea weed extract, were used in the experiment. A control (without growth regulator) and two growth regulator treatments namely the recommended rate of 8ml growth regulator per litre nutrient solution (Canyon Report, 1998) and double the recommended rate at 16ml growth regulator per litre nutrient solution, were applied in the experiment. The seaweed extract is composed of 15ml per litre cytokinin and 22ml per litre auxin. The cytokinin-containing seaweed extract was mixed with the full strength Nitsch nutrient solution and applied twice fortnightly as a full cover spray.

Harvest and analysis

During the growing period three plants were harvested fortnightly from each treatment to monitor biomass accumulation, leaf area development, shoot and root development. The experiment was arranged in a completely randomised design with three replications. Three replicates of each treatment were left to reach maturity and were harvested on 25th November 2000. Data on seed yield and yield components were recorded. The seed yield and yield components data was subjected to statistical analysis using the SAS statistical package (SAS

Institute, 1989) with cultivar and growth regulator treatments as main effects in the ANOVA. The separation of means was done by means of the Duncan Multiple Range Test.

6.3 RESULTS AND DISCUSSION

Vegetative growth

The effect of a cytokinin-containing growth regulator on biomass accumulation is presented in Figure 6.1. Biomass accumulation in all the treatments increased exponentially as days after emergence (DAE) increased. Differences were observed for cultivar Teebus in which plants treated with the double rate cytokinin-containing growth regulator (T2) developed at a slower rate than plants treated with the control (T0) and the recommended rate (T1). No differences were observed for cultivar Kranskop, although plants receiving the double rate treatment had a somewhat larger biomass accumulation than those treated with the other two treatments especially during the first five weeks of growth.

Figure 6.2 shows the effect of the cytokinin-containing growth regulator treatment on leaf area development. No differences were observed among plants treated with different levels of cytokinin-containing growth regulator in the first six weeks for both Kranskop and Teebus cultivars. Cultivar differences in leaf area was observed after six weeks of growth. Cultivar Kranskop had a larger and more vigorous leaf area development than cultivar Teebus over all growth regulator treatments. Plants receiving both the recommended rate and the double rate treatments developed a somewhat higher leaf area than those in the control for cultivar Kranskop. For cultivar Teebus, plants receiving both the recommended and double rate treatments had a smaller leaf area than those of the control treatment.

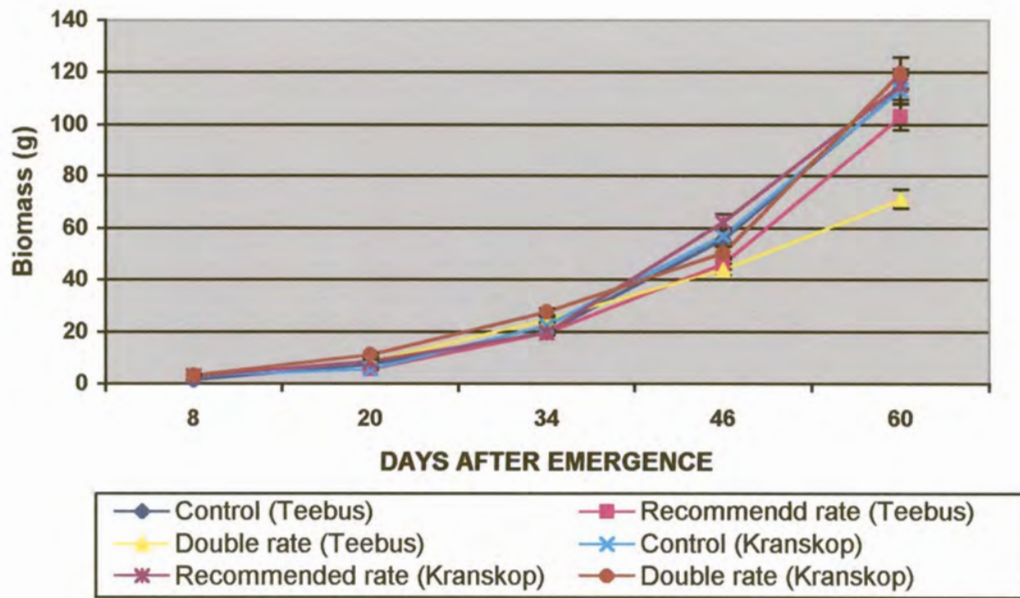


Figure 6.1 Effect of the cytokinin-containing growth regulator on fresh biomass (g) of dry bean, cultivars Kranskop and Teebus

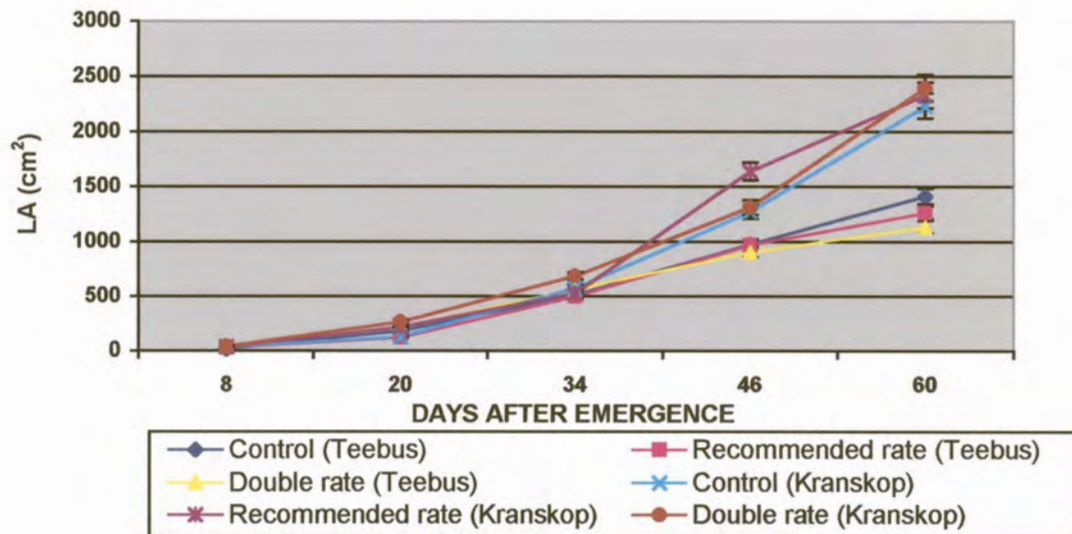


Figure 6.2 Effect of the cytokinin-containing growth regulator on the leaf area (LA) of dry bean cultivars Kranskop and Teebus

The effect of the cytokinin-containing growth regulator on shoot dry weight of cultivars Kranskop and Teebus is presented in Figure 6.3. The shoot dry weight during the first six weeks of growth did not differ significantly between both cultivar and growth regulator

treatments. Differences were observed six weeks after emergence where cultivar Kranskop had a relatively higher shoot dry weight than cultivar Teebus. Plants in the two growth regulator treatments did not perform any better than those receiving control treatment for cultivar Kranskop. For cultivar Teebus the plants receiving the recommended rate treatment had a somewhat larger shoot dry weight than those receiving control and double rate treatments.

Figure 6.4 shows the effect of the cytokinin-containing growth regulator on root dry weight of cultivars Kranskop and Teebus. No clear trend was observed in the development of root dry weight between both cultivars and growth regulator treatments. For cultivar Teebus, plants receiving the control treatment had a somewhat larger root dry weight than plants receiving growth regulator treatment while for cultivar Kranskop, plants receiving the double rate treatment had a somewhat larger root dry weight than the other treatments.

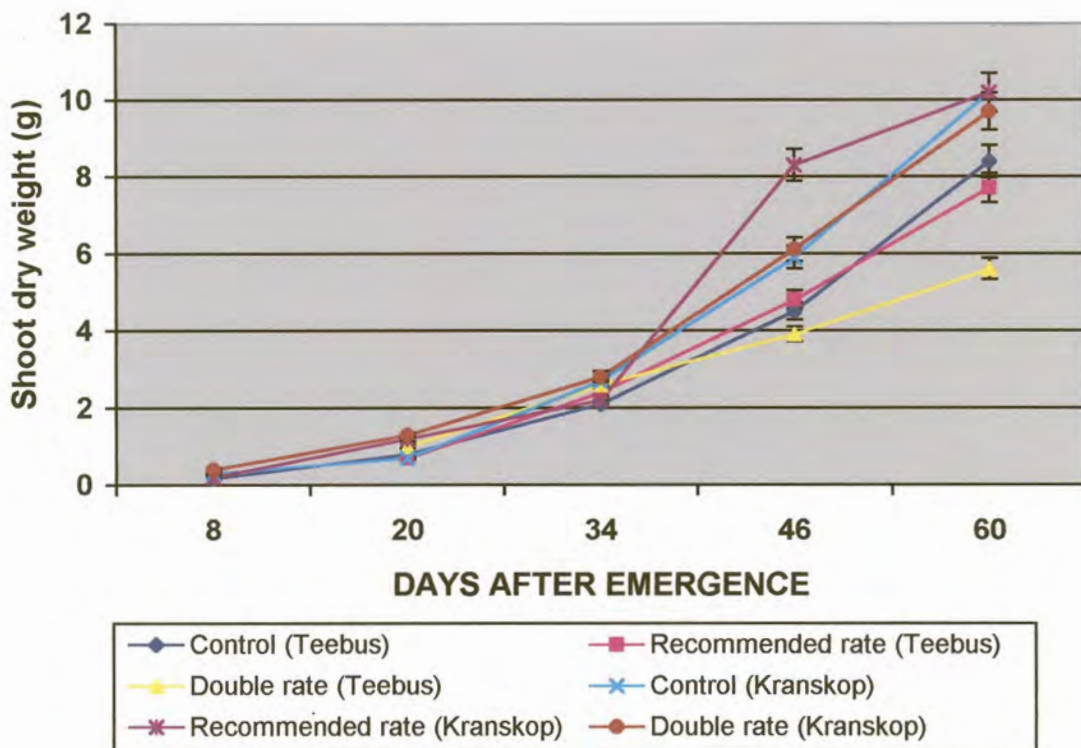


Figure 6.3 Effect of the cytokinin-containing growth regulator on the shoot dry weight of dry bean cultivars Kranskop and Teebus

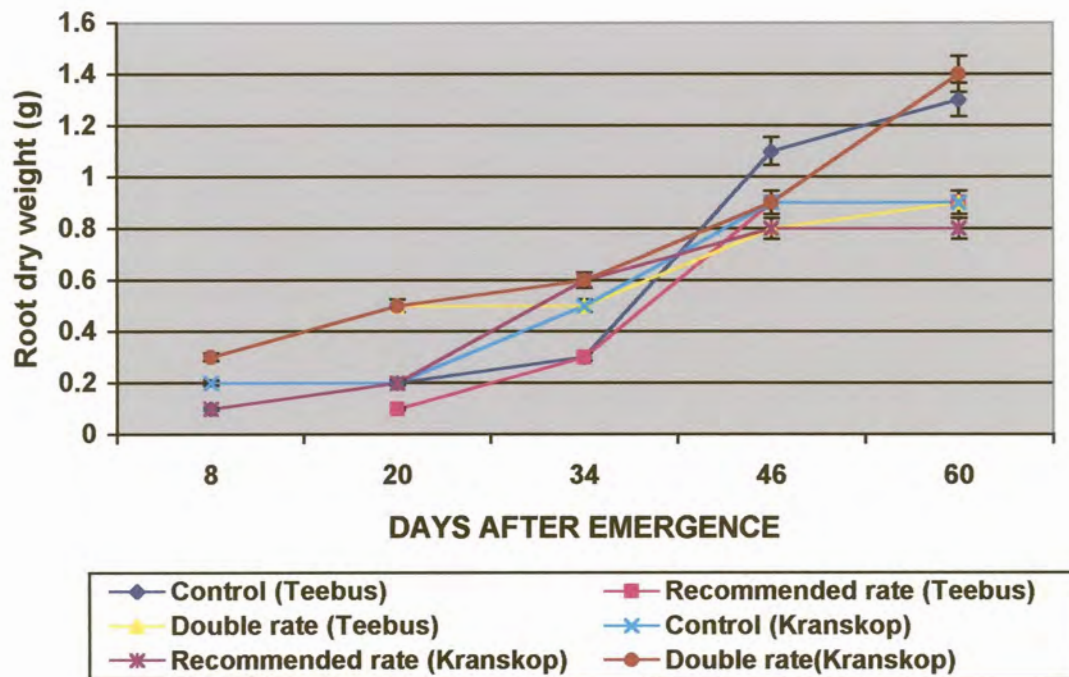


Figure 6.4 Effect of the cytokinin-containing growth regulator on the root dry weight of dry bean cultivars Kranskop and Teebus

Seed yield and yield components

The effect of the cytokinin-containing growth regulator on seed yield and yield components of dry bean cultivars Kranskop and Teebus are given in Tables 6.1 to 6.5.

Seed yield

Table 6.1 shows the effect of cultivar and cytokinin-containing growth regulator on seed yield per plant. Only the cultivar main effect was significant while the growth regulator main effect and the cultivar x growth regulator interaction were not.

Main effects. Significant seed yield differences were observed between cultivar Kranskop and cultivar Teebus over all growth regulator treatments (Table 6.1). Cultivar Kranskop produced a higher seed mass per plant (15.4g) than cultivar Teebus (11.0g). No differences were observed among growth regulator treatments for either of the cultivars.

Interaction. The non significant cultivar x growth regulator interaction indicates that both cultivar and growth regulator treatments influenced seed mass independent of each other.

Table 6.1 Effect of the cytokinin-containing growth regulator on seed yield per plant of dry bean, cultivars Teebus and Kranskop (ANOVA: Appendix Table 8.7A)

Growth regulator treatment	Cultivar		Mean
	Teebus	Kranskop	
Control	11.3	14.4	12.8
Recommended rate	11.4	16.3	13.9
Double rate	10.2	15.5	12.9
Mean	11.0	15.4	13.2

LSD (C) ($P \leq 0.05$) = 3.6 CV(%) = 26.2 $R^2 = 0.40$ SE = 2.0

LSD values given only where effects are significant.

Number of pods per plant

Data for the effect of cultivar and growth regulator on number of pods per plant is shown in Table 6.2. The cultivar main effect was highly significant while both the growth regulator main effect and the cultivar x growth regulator interaction were not.

Main effect. The significant cultivar main effect indicates that the cultivars differed in the production of pods per plant. Cultivar Teebus produced a significantly higher number of pods per plant (15.4) than cultivar Kranskop (9.0). The non-significant growth regulator main effect observed shows that the three treatments did not influence the number of pods per plant differently. This means that there was no advantage in using growth regulator treatments over control treatment for improving number of pods per plant. However, the recommended rate treatment produced a somewhat higher number of pods per plant (13.3) than both the control and double rate treatments which produced 11.7 pods.

Interaction. The non significant cultivar x growth regulator interaction indicates that the growth regulator treatments did not affect the two cultivars differentially.

Table 6.2 Effect of the cytokinin-containing growth regulator on the number of pods per plant of dry bean cultivars Teebus and Kranskop (ANOVA: Appendix Table 8.7B)

Growth regulator treatment	Cultivar		Mean
	Teebus	Kranskop	
Control	16.3	7.0	11.7
Recommended rate	16.3	10.3	13.3
Double rate	13.7	9.7	11.7
Mean	15.4	9.0	12.2
LSD (C) ($P \leq 0.05$) = 2.2 CV(%) = 17.2 $R^2 = 0.80$ SE = 1.2			

LSD values given only where effects are significant.

Number of seeds per pod

Effect of cultivar and growth regulator on number of seeds per pod is presented in Table 6.3. Only the cultivar main effect was significant while the growth regulator main effect and the cultivar x growth regulator interaction were not.

Main effects. The significant cultivar main effect indicates that both cultivars performed differently over all growth regulator treatments. Number of seeds per pod was higher for cultivar Teebus (4.7) than for cultivar Kranskop (3.7) (Table 6.3). The non significant growth regulator main treatment effect highlights the fact that the treatments applied did not affect the number of seeds per pod over both cultivars. Nevertheless, a somewhat decreasing trend in the number of seeds per pod with increasing growth regulator treatments was observed. The control had a somewhat higher number of seeds per pod (4.4) than the recommended rate (4.2) and the double rate (4.1) treatments.

Interaction. The cultivar x growth regulator interaction was not significant showing that the cultivars were not affected differently by the growth regulator treatments.

Table 6.3 Effect of the cytokinin-containing growth regulator on the number of seeds per pod of dry bean cultivars Teebus and Kranskop (ANOVA: Appendix Table 8.7C)

Growth regulator treatment	Cultivar		Mean
	Teebus	Kranskop	
Control	4.7	4.0	4.4
Recommended rate	4.8	3.6	4.2
Double rate	4.7	3.5	4.1
Mean	4.7	3.7	4.2

LSD (C) ($P \leq 0.05$) = 0.5 CV(%) = 11.2 $R^2 = 0.64$ SE = 0.3

LSD values given only where effects are significant

Seed size (100 seed mass)

The data for the effect of cultivar and growth regulator on seed size is given in Table 6.4. Only the cultivar main effect was significant while the growth regulator main effect and the cultivar x growth regulator interaction were not.

Main effects. The significant cultivar main effect shows that the two cultivars differed in seed size. Cultivar Kranskop produced a significantly larger seed (46.6g) than cultivar Teebus (22.9g). The non significant growth regulator main treatment effect shows that the three treatments did not affect seed size differently over both cultivars. No clear trend was observed in seed size as growth regulator treatment increased. The largest seed size over both cultivars was observed in plants receiving the control treatment (36.7g) followed by those receiving the double rate treatment (35.5g) and the recommended rate treatment (32.6g).

Interaction. No significant interactive effect was observed in seed size between cultivar and growth regulator treatments. This indicates that the effect of the growth regulator on the two cultivars was similar. The trend showed an increase in seed size from 22.1g to 23.9g for cultivar Teebus as growth regulator treatment increased from the control treatment to the

double rate treatment. No clear trend was observed for cultivar Kranskop, producing the largest seed size by plants receiving the control treatment (51.3g) and the smallest size among plants receiving the recommended rate treatment. Plants receiving the double rate treatment were intermediate.

Table 6.4 Effect of the cytokinin-containing growth regulator on the seed size (g) of dry bean cultivars Teebus and Kranskop (ANOVA: Appendix Table 8.7D)

Growth regulator treatment	Cultivar		Mean
	Teebus	Kranskop	
Control	22.1	51.2	36.7
Recommended rate	22.8	42.4	32.6
Double rate	23.9	46.2	35.0
Mean	22.9	46.6	34.8
LSD (C) ($P \leq 0.05$) = 5.7 CV(%) = 15.8 $R^2 = 0.88$ SE = 3.2			

LSD values given only where effects are significant.

Harvest index (%)

Table 6.5 shows the effect of cultivar and growth regulator on harvest index of dry bean. No significant cultivar main effect was observed, while the growth regulator main effect and the cultivar x growth regulator interaction were significant.

Main effects. The non significant cultivar main effect shows that no difference in harvest index was observed between the two cultivars over all growth regulator treatments, averaging 48.8% for cultivar Teebus and 46.3% for cultivar Kranskop. The growth regulator main effect was highly significant showing that the different treatments affected harvest index for both cultivars. The highest harvest index was observed among plants receiving the recommended rate treatment (54.1%) and lowest among plants receiving the control treatment (36.9%) while those receiving the double rate treatment were intermediate (51.7%).

Interaction. The significant cultivar x growth regulator interaction indicates that the harvest index for both cultivars were affected differently by the different growth regulator treatments. For cultivar Teebus the highest harvest index (59.0%) was observed for plants receiving the recommended rate of the growth regulator, followed by those receiving the double rate treatment (46.7%) and plants receiving the control treatment had the lowest harvest index (40.6%). For cultivar Kranskop, the highest harvest index (56.7%) was observed for plants receiving the double rate treatment followed by those receiving the recommended rate treatment (49.1%) while plants receiving the control treatment had the lowest harvest index (36.9%).

Table 6. 5 Effect of the cytokinin-containing growth regulator on the harvest index (%) of dry bean cultivars Teebus and Kranskop (ANOVA: Appendix Table 8.7E).

Growth regulator (GR) treatment	Cultivar (C)		Mean
	Teebus	Kranskop	
Control	40.6	33.1	36.9
Recommended rate	59.0	49.1	54.1
Double rate	46.7	56.7	51.7
Mean	48.8	46.3	47.6

LSD (GR) ($P \leq 0.05$) = 10.2 LSD (C x GR) ($P \leq 0.05$) = 11.8
 CV(%) = 13.9 $R^2 = 0.73$ SE = 3.8

LSD values given only where effects are significant.

According to Donald (1968) harvest index is the ratio of seed yield to total shoot dry matter, which reflects the partitioning of photosynthate to seed. The results of the experiment clearly show that a cytokinin-containing growth regulator had some positive effect on harvest index of the two cultivars. While there were no differences in seed yield and yield components due to different growth regulator treatments, the significant harvest index indicates the possible influence of growth regulator on the reproductive sink of dry bean.

More assimilates have been allocated to the sink (the seed) than other plant parts as reported by Clifford, Pentland & Baylis (1992) who indicated the role of growth regulators in the

control of photosynthate competition between reproductive and vegetative sinks. However, Cipollini (1997) suggests to the contrary that due to the numerous effects that exogenously applied hormones can have on plant growth, it is impossible that a particular plant hormone treatment can alter a plants assimilatory capacity and / or resource allocation pattern (such as root/shoot ratio). It may therefore be possible that the improved harvest index observed may be associated with limited vegetative growth of plants in the cytokinin-containing growth regulator treatments.

6.4 CONCLUSION

There may be a possibility of improving bean production with the growth regulator used in this experiment, a cytokinin-containing seaweed extract (trade name marinure) treatment, and that different cultivars may be affected differently. Somewhat higher seed yield and yield component values were obtained with the recommended rate of application.

As the results are not conclusive, further research is required. The cytokinin-containing growth regulator evaluated in this trial did not improve seed set and seed development and can not be recommended for dry bean seed production under greenhouse conditions.

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GENERAL DISCUSSION AND CONCLUSION

The objective of this study was to evaluate ways of optimising dry bean seed multiplication under greenhouse conditions. This is with a view to improve the commercial dry bean seed multiplication programme by increasing the quantity of disease-free seed produced in the greenhouse multiplication phase.

Three factors, (i) plant density, (ii) nitrate/ammonium ratio and concentration and (iii) the use of a cytokinin-containing growth regulator were investigated.

7.1 PLANT DENSITY

This investigation has shown that dry bean seed yield per unit area can be increased by planting at very high plant densities. A plant population of 139 plants m^{-2} , achieved by a spacing of 12 x 6cm produced a seed yield of 822g m^{-2} in the greenhouse, which is equivalent to 8.2 tons ha^{-1} . Such high plant densities have been reported by many authors (Mack & Hatch, 1968; Crandall, 1971; Cooper, 1977), with an equidistant spacing being more beneficial than a rectangular arrangement (Crothers & Westermann, 1976). While in most of these reports there has not been an indication of seed yield per plant, there is a tendency of a negative relationship between seed yield per plant and seed yield per unit area. An increase in seed yield per unit area in our investigation was associated with reduced seed yield per plant. It seems that 139 plants m^{-2} approaches the threshold plant population for cultivar Kranskop indicating that there is no benefit in increasing the plant density beyond this threshold level. Plant morphology and / or growth habit has been reported to influence seed yields at very high plant densities as suggested by Crothers & Westermann (1976). This indicates that different cultivars may require different spacings.

Large differences in yield per plant for comparable plant densities were observed between the pot trial, the first crate trial and the field trial, indicating the important role of other environmental factors (water supply, nutrition, climate, pests, growing period etc) in the determination of yield. This emphasises that the optimum plant density will differ between

different growing conditions.

The number of pods per plant seems to be the determining factor of seed yield per plant. The number of pods per plant showed a positive relationship with seed yield per plant, decreasing with increasing plant density. The number of seeds per pod and seed size remained relatively stable even at high plant densities. This is in agreement with Leakey (1972) and Crothers & Westermann (1976) who indicated a direct relationship between seed yield and number of pods per plant. They also stated that the number of pods per unit area was the major seed yield component influencing seed yield, with little influence of either seed size or seeds per pod on seed yield per unit area.

The greenhouse trials showed that relatively high seed yields of up to 822g m⁻² could be obtained using the cultivar Kranskop when seed was used as planting material. Provided similar results can be obtained with explants, this indicates that greenhouse multiplication can be a viable procedure in a seed multiplication programme. The highest yield was obtained with a high population of 139 plants m⁻². In a commercial situation the growing conditions and convenience of crop management practices will affect the optimum plant density. The optimum density will be determined by the unit cost of explants and the production cost per unit area of greenhouse space.

Although in practice explants from meristem cultures multiplied *in vitro* will be the main source of planting material in the greenhouse multiplication phase, plants in this investigation were produced from seed. No comparison between the performance of plants derived from *in vitro* culture and from seed was attempted. Based on the appearance of plants derived from *in vitro* plantlets in greenhouses of the Dry Bean Producers Organisation it was assumed that growth habit, reaction to plant density, nutritional requirements and other growth reactions are similar for plants from seed. Provided seed can be multiplied economically in greenhouses or other protective structures, multiplication for more than one generation after the *in vitro* phase with seed as planting material may also be a viable proposition.

7.2 NITROGEN SOURCE AND CONCENTRATION

Nitrogen plays a vital role in the growth and development of dry bean and determining seed yields and yield components. Experiments were conducted to determine the effect of the source of nitrogen (NH₄⁺-N, NO₃⁻-N or a combination of the two) as well as the

concentration of nitrogen in the nutrient solution on seed yield. Good plant growth and similar seed yields were observed among plants receiving the full strength and half strength nutrient solutions containing either 7% $\text{NH}_4^+\text{-N}$ with 93% $\text{NO}_3^-\text{-N}$ (1:14) or 50% $\text{NH}_4^+\text{-N}$ with 50% $\text{NO}_3^-\text{-N}$ (1:1). A nitrate / ammonium ratio in the nutrient solution with more ammonium than nitrate detrimentally affected yield. The high $\text{NH}_4^+\text{-N}$ (93% $\text{NH}_4^+\text{-N}$ with 7% $\text{NO}_3^-\text{-N}$) treatment caused stunted growth and chlorotic lesions on leaves. This was more pronounced in plants receiving the full strength and half strength nutrient solution, and almost all plants were dead by the time of harvest maturity due to ammonium toxicity. Similar findings have been reported for potatoes by Cao & Tibbitts (1993) where enhanced growth was observed when 80 to 92% of the nitrogen in the solution was in the nitrate form. Hydroponic studies with corn (Gashaw & Mugwira, 1981; Below & Gentry, 1992; Shrader *et al.* 1972), tomato (Gamnore-Newmann & Kafkafi, 1980) and wheat (Wang & Below, 1992) have shown better plant growth with 25% and 50% $\text{NH}_4^+\text{-N}$ than with 75% $\text{NH}_4^+\text{-N}$. Barker & Mills, 1980; Below & Gentry (1992) and Pilbeam & Kirkby (1992) state that enhanced growth when both ammonium and nitrate is supplied, results from increased nitrogen accumulation in the plants, even though the physiological basis of the benefit is yet to be established.

The results indicate that good seed yields can be obtained with nitrate/ammonium ratios of either 14:1 or 1:1. As reported in Chapter 5 it seems that a nutrient solution containing a 1:1 nitrate/ammonium ratio may be advantageous for dry bean production. This ratio enhanced the green colouration in the bean plants and resulted in a somewhat larger leaf area and stronger shoot growth at 40 DAE (Table 5.4a). This ratio also results in a more stable nutrient solution pH due to its relatively high buffering capacity according to Clark (1982) and Bernardo, *et al.* (1984a & b).

Similar seed yields were produced when either the full strength or half strength nutrient solution concentrations were supplied, indicating possible cost savings by applying diluted nutrient solutions.

7.3 CYTOKININ-CONTAINING GROWTH REGULATOR

No yield benefit was obtained by applying a cytokinin-containing growth regulator to the nutrient solution in a pot trial. The use of plant growth regulants to limit abscission of flowers and pods deserves more research attention.

7.4 FUTURE RESEARCH

Aspects not included in this investigation and deserving further research include;

- i. Comparison of the growth, development and yield of *in vitro* plantlets to that of plants derived from seed.
- ii. Evaluation of the reaction of other important dry bean cultivars, as this research focussed on cultivars Kranskop and Teebus.
- iii. Confirmation of the results on a larger (semi-commercial) scale. This should include a treatment where the cultivar Kranskop is grown at approximately 139 plants m⁻² and supplied with a half strength nutrient solution containing 1:1 NO₃⁻: NH₄⁺ ratio.
- iv. Evaluation of other plant growth regulators to limit abscission of flowers and young pods.

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