The Effect of Two Growth Retardant Chemicals, Cycocel and B-Nine, on Certain Nitrogenous Components in Barley Seedlings

Linda Kinser

Follow this and additional works at: http://digitalcommons.wku.edu/theses

Part of the Agriculture Commons, Biology Commons, and the Plant Biology Commons

Recommended Citation

http://digitalcommons.wku.edu/theses/1776

This Thesis is brought to you for free and open access by TopSCHOLAR®. It has been accepted for inclusion in Masters Theses & Specialist Projects by an authorized administrator of TopSCHOLAR®. For more information, please contact topscholar@wku.edu.
THE EFFECT OF TWO GROWTH RETARDANT CHEMICALS, CYCOCEL AND B-NINE, ON CERTAIN NITROGENOUS COMPONENTS IN BARLEY SEEDLINGS

A Thesis
Presented to
the Faculty of the Department of Biology
Western Kentucky University
Bowling Green, Kentucky
242712

In Partial Fulfillment of the Requirements for the Degree Master of Science
by
Linda Linser
August 1969
THE EFFECT OF TWO GROWTH RETARDANT CHEMICALS, CYCOCEL AND B-NINE, ON CERTAIN NITROGENOUS COMPONENTS IN BARLEY SEEDLINGS

APPROVED August 7, 1969
(Date)

Frank A. Toman
Director of Thesis

Lowell W. Fank

James W. Steele

Dean of the Graduate School

James D. Meiner
ACKNOWLEDGEMENTS

The author expresses deep appreciation to her major professor, Dr. F. R. Toman, for the development of this study and for his thoughtful assistance throughout its duration. Sincere thanks is extended to Dr. E. O. Beal, Dr. J. H. Jenkins, Dr. L. W. Shank, and Dr. J. D. Skean for their invaluable criticism of the manuscript.

The author wishes to thank Dr. Elmer Gray for his help with statistical analysis. Appreciation is expressed to Dr. A. L. Applegate, Dr. J. D. Parker, and Mr. J. R. McCurry for their time spent in behalf of the thesis.

Acknowledgement also is given to the Agriculture Department of Western Kentucky University for supplying the barley seed used in this study and to the Uniroyal Chemical Company for furnishing the growth-retardant chemical, B-Nine.
TABLE OF CONTENTS

LIST OF TABLES ........................................... vi

LIST OF FIGURES ........................................ vii

INTRODUCTION ........................................... 1

REVIEW OF LITERATURE ................................ 3

MATERIALS AND METHODS ................................ 9
  Plant Materials ..................................... 9
  Nitrate Determination .............................. 9
  Nitrogen Determination ............................ 11
  Nitrate-Reductase Determination ............... 12
  Dry Weight Determination ......................... 13
  Statistical Analysis ................................ 13

RESULTS ................................................. 14
  Nitrate ............................................. 14
  Soluble Protein Nitrogen ......................... 17
  Nitrate-Reductase Activity ....................... 21
  Percent Water ..................................... 25
DISCUSSION .......................................................... 27
Nitrate ............................................................. 27
Soluble Protein Nitrogen ........................................... 28
Nitrate-Reductase Activity ....................................... 29
Percent Water ....................................................... 31
Plant Height ........................................................ 31
CONCLUSIONS ...................................................... 32
SUMMARY ........................................................ 34
LITERATURE CITED ................................................ 36
LIST OF TABLES

Table 1. Nitrate nitrogen in millimoles per gram dry weight of barley seedlings .......... 15
Table 2. Soluble protein nitrogen in milligrams per gram dry weight of barley seedlings ... 18
Table 3. Nitrate reductase activity in micromoles of nitrite produced per milligram nitrogen per hour of barley seedlings ............... 22
Table 4. Percent water of barley seedlings ........... 26
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Nitrate content per gram dry weight of barley seedlings</td>
<td>16</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Soluble protein nitrogen per gram dry weight of barley seedlings</td>
<td>19</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Nitrate reductase activity per milligram of nitrogen per hour of barley seedlings</td>
<td>23</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Average of replications: nitrate content, soluble protein content, and nitrate reductase activity of barley seedlings</td>
<td>33</td>
</tr>
</tbody>
</table>
INTRODUCTION

Certain chemicals are known to inhibit growth in many plant species. These chemicals possess a common trait, the ability to inhibit stem elongation by suppressing the activity of the subapical meristematic region (11). These growth retardants have been extensively studied during the past several years in an effort to determine the biochemical mechanism responsible for reduction in plant height. The characteristic effect of these chemical retardants on the growth pattern has been described as producing plants with shorter, thicker stems and broader, darker green leaves. Tolbert, however, noted that although plants treated with the plant growth retardant, Cycocel, (2-chloroethyltrimethylammoniumchloride) and some of its related compounds were shorter and exhibited the above characteristics, he also found there was no loss in weight of the treated plants (25). Thus it appears that these chemicals cause growth to be manifested in a manner slightly altered from the normal pattern for that species.

In living systems growth can be correlated with an increase in protein content; and since plants must synthesize their own amino acids, the metabolism of nitrogen is a vital factor in determining the rate of growth.
Nitrogen is absorbed by the plant in the form of nitrate and must be converted into a usable form (14). The first step of this conversion is accomplished enzymatically by nitrate reductase (2).

Although Kahn and Faust (8) have determined the effect of Cycocel on the soluble protein level in barley seedlings, information concerning other aspects of nitrogen metabolism has not been reported in the literature. The purpose of this study was to determine the effects of two growth retardant chemicals, Cycocel and B-Nine (succinic acid 2, 2-dimethylhydrazide), upon the nitrate level, the soluble protein nitrogen content, and the activity of the nitrate reductase enzyme of young barley plants.
REVIEW OF LITERATURE

Although the exact mechanism of growth retardation has not been revealed, various effects of the chemicals Cycocel and B-Nine have been observed and their mode of action has been investigated. These chemicals are known to be effective over a wide range of concentrations and can be applied in various ways and still initiate their response. Reports show Cycocel to be effective at concentrations from $10^{-2}$ to $10^{-6} \text{M}$ when applied as a foliar spray or when seeds have been soaked in a solution containing the chemical prior to planting. In peas when one application was used, soil treatment was more effective than spray. Soil application in some plants was ineffective in bringing about the desired response (17). Experiments by Larter (11) revealed that treatments of $10^{-3} \text{M}$ were not effective in reducing plant height of barley. A concentration of $10^{-1} \text{M}$ did cause a reduction in plant height.

Various concentrations of Cycocel on pea plants showed that $10^{-5} \text{M}$ increased the length of internodes, and the weight of plants and seeds, while a concentration of $10^{-3} \text{M}$ reversed these results (17). It appears that the concentration used determines the effect that will be realized.
In addition to the reduction in stem growth produced by these chemicals, Cycocel has been found to affect the pigment content of young leaves, inhibit the synthesis of gibberellins in *Fusarium moniliforme*, delay flowering, reverse inhibition of seed germination and root growth, and alter the anatomical structure of the plant.

Foliar spray of Cycocel produces a greater reduction in pigment content than soil application. The ratio of chlorophyll a to chlorophyll b has been found to increase with foliar application but to decrease with soil application. The chlorophyll to carotenoid ratio remains unchanged (17).

The chemical effects of these substances upon plant hormones have been extensively investigated (6), (12), (15). No definite relationship between growth retardants and plant hormones has been established. Lockhart (12) suggested that since the molecular configuration of gibberellins and Cycocel are so different, the two could work independently of each other and not compete for an active site. His results, however, indicated that the gibberellins and the growth retardants (referred to as anti-gibberellins) do competitively interact, and Cycocel somehow blocks the system which supplies active gibberellins to the plant's growth mechanism.

Paleg, Ninnemann, and Lang (15) indicated that neither Cycocel nor B-Hine affected the gibberellic acid-induced release of reducing sugar from barley endosperm. Their results suggested that these chemicals be referred to as
growth retardants rather than anti-gibberellins, since they probably block the synthesis of gibberellins. Harada and Lang (6) noted that the characteristic reduction in growth, the deeper color, and suppression of flowering produced by growth retardants could be overcome by application of gibberellin. They agree with Paleg et al. that Cycoce l blocks the biosynthesis of gibberellins, rather than destroying it or competing with it for the active site. This is based on the result that the fungus, Fusarium moniliforme, does not produce gibberellic acid in the presence of Cycoce l.

Cycoce l was found to be an active inhibitor of gibberellic acid at concentrations of $10^{-5}$M but $10^{-3}$M was the concentration found to be most active. Cycoce l analogues produced no effect at a concentration of $2 \times 10^{-3}$M (6).

B-Nine failed to inhibit the synthesis of gibberellins in the fungus although it was still present and could be recovered from the culture media. Harada and Lang (6) suggested that the chemical might not be able to penetrate the mycelium or that the enzymes regulating synthesis of gibberellic acid may be insensitive to B-Nine. Even though B-Nine did not prevent the synthesis of gibberellic acid, its growth retarding effects could be altered by application of this hormone. They suggested that B-Nine may exert its effect through a mechanism unrelated to the synthesis of gibberellic acid.
Investigations concerning the effect of Cycocel on auxin have shown the inhibition of leaf growth caused by Cycocel could be overcome by gibberellic acid and indoleacetic acid and that inhibition of coleoptile growth and retarded stem growth could be overcome only by auxin. The amount of diffusable auxin recovered from stems after their growth had been retarded by Cycocel was only one-half that of normal plants (10). It was indicated that the effects of growth retardants are due to a lowering of the auxin level. Cycocel and its analogues also reversed the inhibition of seed germination and root growth impinged upon them by auxins (9).

Cycocel treatment was found to increase the thickness of cell walls and increase the number of vascular bundles (4). Marth and Ray (13) found that plants treated with Cycocel were more tolerant to toxic levels of salts (in the form of fertilizers) than were untreated plants. Treated plants were also more resistant to wilting under drought conditions. Appleby et al. (1) reported that the length of the largest roots was not decreased by application of 0.5 percent Cycocel, indicating that the treated plants were at no disadvantage in obtaining water and minerals.

The biochemical mechanism of B-Nine is believed to be related to blocking oxidation of tryptamine to indoleacetaldehyde. Retardation of growth was attributed to the formation of 1, 1-dimethylhydrazine, which has been shown to inhibit tryptamine
oxidation in pea plants (16). Rothenburger (18) studied the translocation of these chemicals and found that B-Nine was readily absorbed and translocated when applied by spray or when added to the soil. When this chemical was spotted on the leaf, it moved first to the margin of the leaf, then into the roots, and finally upward to other parts of the plant. If applied to the roots, the chemical is transported upward throughout the plant in the xylem system. Plant extracts showed B-Nine to be present as the intact molecule three weeks after application, indicating that it is not broken down but causes its dwarfing effect in this form. The mode of action may be completely different from that of Cycocel, since the intact molecule appears to be bound to a specific enzymatic site.

Tolbert concluded that the high degree of specificity in structure required for biological activity of Cycocel suggests that these substances are bonding at a site of similar high specificity. Structurally, Cycocel is related to choline in that the hydroxyl group is replaced by a halogen. In animals choline was reported to inhibit the cholinesterase enzyme system, although this has not been found in plants. Choline is also involved in lipid metabolism and methylation reactions. If these reactions are blocked, then alteration in cell development seems inevitable and subsequent reduction in plant height could follow (25).
Toman and Mitchell (22) isolated five soluble protein fractions from wheat and were able to show that treatment with B-Nine had no effect in altering these soluble protein fractions.

Gohlke and Tompert (3) noted that Cycocel inhibited phosphorous translocation by 75-95 percent. Spencer (19) found that inorganic phosphate was required for maximum activity of the nitrate-reductase enzyme. Thus, it appears that these chemicals, especially Cycocel, may be influencing growth through some mechanism related to the enzymatic conversion of nitrates to a usable form. This hypothesis appears to be reinforced by findings of Hewitt and Notton (7) who described the effects of the growth-retarding chemical, L-azetidine-2-carboxylic acid, upon nitrate reductase. They found that this chemical inhibited induction of the enzyme and acted as a competitor of L-proline in the biosynthesis of proteins. They successfully demonstrated that it operates during the specific formation of nitrate reductase. They concluded that the chemical interferes early with the incorporation of a single amino acid, proline, by transfer-RNA.
MATERIALS AND METHODS

Plant Material

Harrison barley (Hordeum vulgare L.) seeds were planted approximately 2" deep in vermiculite. Plants were supplied Hoagland's Number One nutrient solution and grown in Biotronette Mark III environmental chambers modified by installation of an air conditioner. The chambers were maintained on a 12-hr photoperiod at a temperature of 73 to 76 F. Seventeen days after planting, the plants in each pan (9 1/2 X 11") received 25 ml of 0.1M B-Nine or Cycocel from a spray atomizer. This concentration was found by Larter (11) to be the most effective in reducing plant height in barley seedlings. One pan of chemically untreated plants in each chamber served as a control. The plants were an average of 12 cm in height at the time of treatment and were in the two-leaf stage. Fourteen and twenty-eight days after treatment with the chemicals, plant extracts were assayed in the following manner.

Nitrate Determination

The extraction procedure was a modification of the method of Hageman and Flesher (5). Fresh leaf and stem tissue was removed at the soil level. Five g were cut into smaller pieces, macerated in 20 ml of cold deionized water for 1 min
at highest speed in a Sorvall omni-mixer. The blending cup was immersed in an ice bath, the homogenate filtered through a plastic strainer, and samples centrifuged at 20,000 X g for 30 min in an IEC B-35 ultracentrifuge at 3 C. The supernatant was decanted and assayed. All extracts were incubated in an ice bath throughout the analysis, which was completed within 2 to 3 hr. Nitrate concentrations were determined by the colorimetric method of Wooley, Hicks, and Hageman (27). Nitrate determination depends on the reduction of nitrate to nitrite. When nitrite diazotizes sulfanilic acid and couples it with 1-naphthyl amine, a red color is formed. Preliminary work determined that the original extract required a dilution of 1 to 25 to obtain nitrate concentrations that fell within the values of the standard curve. One ml of this diluted extract was added to 9 ml of 20 percent acetic acid (containing 0.2 ppm Cu as CuSO₄). About 0.5 g of reducing powder containing 100 g barium sulfate, 75 g citric acid, 2 g powdered zinc, and 2 g of 1-naphthyl amine was added. The powder was mixed by rolling the reagents in an air-tight jar to keep out moisture. Results were the same whether 0.4 or 0.6 g was used. After addition of the powder, the samples were shaken 3 times for 15 sec at 3 min intervals. They were then centrifuged at 5,000 rpm for 5 min in an IEC Model HT Centrifuge. The supernatant was decanted and absorbance read on a Bausch and Lomb Precision Spectrophotometer at a wavelength
Concentrations were determined from a standard curve prepared from known concentrations of potassium nitrate. Nitrate determinations by this method also includes the nitrite present in the plant tissues. However, according to Toman and Pauli (23), these levels were so low in comparison to nitrate that no correction was made. Results are reported as mM of nitrate nitrogen per g dry weight.

Nitrogen Determination

Soluble protein nitrogen was determined by Toman's (24) modification of the nesslerization method developed by Thompson and Morrison (21). The extract used for nitrate determinations was also used for nitrogen determinations. One ml of the extract was digested in 4 ml of 3N sulfuric acid by heating in a micro Kjeldahl flask until dense white fumes evolved. Heating was continued several minutes longer, flasks were cooled, and two drops of 30 percent hydrogen peroxide were added. The sample was heated until the solution was clear. Each was quantitatively transferred to a 100-ml volumetric flask and brought to volume with distilled water. A 25-ml aliquot was neutralized with NaOH and placed in a 50-ml volumetric flask to which 1 ml of saturated ethylenediaminetetraacetic acid (EDTA) was added. Color was developed for 20 min after addition of 1 ml of commercial Koch and McMeekin Nessler's reagent. The solutions were mixed and the volume immediately brought to 50 ml. Absorbance was read at 420 nm on a Bausch and Lomb Precision Spectrophotometer calibrated with a blank composed of water, EDTA, and Nessler's
reagent. Concentration was determined from a standard curve prepared from known concentrations of ammonium sulfate. Two samples of the plant extract were digested for nitrogen determination. From each digest 2 samples were nesslerized and the average of these was used for calculations. Results are expressed as mg of nitrogen per g dry weight.

\textbf{Nitrate Reductase Determinations}

Extraction of nitrate reductase was the same as for the nitrate and nitrogen except that the blending medium consisted of 0.1M tris hydroxymethyl aminomethane (TRIS) 0.01M cysteine, and 0.0003M EDTA.

Measurement of enzyme activity utilized a modified procedure reported by Hageman and Flesher (5). The assay mixtures contained 1 ml of 0.1M phosphate buffer, 0.2 m'a of 0.1M potassium nitrate 0.5 ml of 1.36 \times 10^{-3} M reduced nicotinamide adenine dinucleotide (NADH + H\textsuperscript{+}) and 0.3 ml of enzyme. Fresh NADH + H\textsuperscript{+} was added to the assay mixture immediately before the enzyme. The mixture was incubated at 27°C for 20 min and the reaction stopped by adding 1 ml of 1 percent w/v sulfanilic acid in 1.5N hydrochloric acid. One ml of 0.02 percent w/v N-(1-naphthyl) ethylene diamine hydrochloride reagent was added and color developed for 5 min. Absorbance was read on a Bausch and Lomb Precision Spectrophotometer at a wavelength of 540 nm. Each sample was read against a blank complete except for the NADH + H\textsuperscript{+}. 
A standard curve for nitrate reductase was prepared by adding known concentrations of potassium nitrite to tubes containing the assay mixture. Results are expressed as µM nitrite produced per mg nitrogen per hr.

**Dry Weight Determinations**

Percentage of water was based on the average of 2 samples in each treatment. Dry weights were determined after samples were dried 20-24 hr at 80°C.

**Statistical Analysis**

In order to determine if the observed differences were significant, statistical analyses were made. A completely randomized design (20) was used to determine whether differences among treatments and differences between sampling dates were significant.
RESULTS

Nitrate

Results of nitrate determinations are in Table 1 and are illustrated in Figure 1. Although the concentrations varied from the first date to the second, the variability was not statistically significant. Therefore, results for both sample dates in each replication were averaged and are illustrated in this form. In the first replication, nitrate levels decreased on the second date in both the control and Cycocel-treated plants. Average values for the two dates were equal in control and Cycocel-treated plants. There was no B-Nine treatment in the first replication.

The second replication shows that the initial nitrate levels were lower in chemically treated plants than in the controls. These differences were lower on the second date, when the concentration had declined in the control plants but had increased in the treated ones. However, when the results were averaged, (Figure 1), differences between treated plants and controls were marked with B-Nine-treated plants exhibiting the greatest difference. In replication three nitrate levels in the control plants remained constant on the two sampling dates. However, plants sprayed with Cycocel
<table>
<thead>
<tr>
<th>Replication</th>
<th>Sampling Date</th>
<th>Control</th>
<th>Cycocel</th>
<th>B-Nine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>November 28, 1968</td>
<td>1.06</td>
<td>1.08</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>December 13, 1968</td>
<td>1.01</td>
<td>0.99</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>December 20, 1968</td>
<td>1.15</td>
<td>0.97</td>
<td>0.83</td>
</tr>
<tr>
<td>2</td>
<td>January 3, 1969</td>
<td>1.12</td>
<td>1.06</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>February 28, 1969</td>
<td>0.87</td>
<td>0.75</td>
<td>0.84</td>
</tr>
<tr>
<td>3</td>
<td>March 14, 1969</td>
<td>0.87</td>
<td>0.99</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>April 11, 1969</td>
<td>1.17</td>
<td>1.30</td>
<td>1.05</td>
</tr>
<tr>
<td>4</td>
<td>April 25, 1969</td>
<td>0.99</td>
<td>0.72</td>
<td>0.65</td>
</tr>
</tbody>
</table>
Figure 1. Nitrate content per gram dry weight of barley seedlings, 1968-1969.
increased while plants sprayed with B-Nine decreased on 
the second date. Figure 1 illustrates that concentrations 
in control plants did not differ from Cycocel-treated 
plants. B-Nine-treated plants again showed lower levels of 
nitrate than either control or Cycocel-treated plants. The 
nitrate content in the B-Nine-treated plants was not as low 
in comparison with the control as it was in the previous 
replication. No difference between values for Cycocel 
treatment and control was demonstrated. Initial samples 
in the fourth replication showed that concentrations of 
nitrate in Cycocel-treated plants were higher than in 
controls or plants treated with B-Nine. Again, levels were 
lowest in the B-Nine-treated plants. On the second sampling 
date, plants receiving either treatment had lower concentrations 
than the controls, with B-Nine treatment having the lowest. 
These results (Figure 1) agree with the other replications. 

Figure 1 illustrates that nitrate concentrations were 
either equal or slightly higher in controls than in the 
Cycocel-treated plants. Statistically, the difference in 
nitrate content was not significant. In every case the 
amount of nitrate in the plants treated with B-Nine was 
less than either controls or Cycocel-treated plants. Differ- 
ences between B-Nine-treated plants and controls were 
statistically significant.

**Soluble Protein Nitrogen**

Levels of nitrogen are reported in Table 2 and are 
graphed in Figure 2. Initial concentrations of nitrogen in
### TABLE 2

**SOLUBLE PROTEIN NITROGEN IN MILLIGRAMS PER GRAM DRY WEIGHT OF BARLEY SEEDLINGS**

<table>
<thead>
<tr>
<th>Replication</th>
<th>Sampling Date</th>
<th>Control</th>
<th>Cycocel</th>
<th>B-Nine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>November 28, 1968</td>
<td>21.98</td>
<td>21.98</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>December 13, 1968</td>
<td>32.87</td>
<td>33.76</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>December 20, 1968</td>
<td>21.52</td>
<td>20.99</td>
<td>19.65</td>
</tr>
<tr>
<td>2</td>
<td>January 3, 1969</td>
<td>23.90</td>
<td>20.25</td>
<td>18.10</td>
</tr>
<tr>
<td></td>
<td>April 11, 1969</td>
<td>27.86</td>
<td>28.14</td>
<td>20.78</td>
</tr>
<tr>
<td>4</td>
<td>April 25, 1969</td>
<td>19.48</td>
<td>19.49</td>
<td>14.32</td>
</tr>
</tbody>
</table>
Figure 2. Soluble protein nitrogen per gram dry weight of barley seedlings, 1968-1969.
the first replication were equal for both the control and Cycocel-treated plants. By the second date, levels of nitrogen had increased more in the Cycocel-treated plants than in controls. Since results were not significantly different between dates, average values were graphed in Figure 2 which indicates that concentrations were approximately equal. Again there was no B-Nine treatment in this replication. In the second replication treated plants contained lower amounts of nitrogen than controls on the first and second sampling dates. Although the concentration in controls increased, concentration in treated plants decreased by the second sample date. Averaged results (Figure 2) illustrate these differences, emphasizing the low level in B-Nine-treated plants. Replication three shows that results in Cycocel-treated and control plants were similar to the first replication. Initial concentrations of nitrogen were almost equal in control and Cycocel-treated plants. Plants treated with B-Nine contained typically lower levels of nitrogen. Concentrations had increased in all plants on the second sampling date, and control plants contained a higher amount of nitrogen than did the chemically-treated plants. Plants treated with Cycocel had slightly lower concentrations than the controls while B-Nine-treated plants were considerably lower in nitrogen levels on this date. Figure 2, showing the average for these two dates, indicates equal levels of nitrogen in the control and very low levels in B-Nine-treated plants. Results in
replication four are very similar to those found in the previous replication. Although initial concentrations showed the same relationship between chemical treatments and control, the nitrogen level had decreased in all the plants on the second date. Again, low levels are exhibited by the plants sprayed with B-Nine on both sampling dates. In this replication, the amounts of nitrogen in the plants treated with Cycocel were equal to those in controls on the second date. Figure 2 shows that average concentrations in replications one, three, and four were about equal in the control and Cycocel-treated plants while replication two indicated Cycocel-treated plants were lower than controls. However, differences between Cycocel treatments and controls were not statistically significant. Replications involving B-Nine treatments show characteristic low levels of nitrogen that were significantly different from the control.

**Nitrate Reductase Activity**

Activity of the nitrate reductase enzyme is recorded in Table 3 and illustrated in Figure 3. On the first sampling date, enzyme activity in the plants treated with Cycocel was slightly higher than in the controls. However, by the second date, activity in the Cycocel-treated plants had declined while that in the control plants had increased. Statistical analysis indicated that there was no significant difference in activity between sampling dates. Therefore, the activities on the two dates were averaged and presented in
TABLE 3
NITRATE REDUCTASE ACTIVITY IN MICROMOLES OF NITRITE PRODUCED PER MILLIGRAM NITROGEN PER HOUR OF BARLEY SEEDLINGS

<table>
<thead>
<tr>
<th>Replication</th>
<th>Sampling Date</th>
<th>Control</th>
<th>Cycocel</th>
<th>B-Nine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>November 28, 1968</td>
<td>0.62</td>
<td>0.67</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>December 13, 1968</td>
<td>0.68</td>
<td>0.57</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>December 20, 1968</td>
<td>0.78</td>
<td>0.71</td>
<td>0.68</td>
</tr>
<tr>
<td>2</td>
<td>January 3, 1969</td>
<td>0.33</td>
<td>0.52</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>February 28, 1969</td>
<td>0.79</td>
<td>0.94</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>March 14, 1969</td>
<td>0.60</td>
<td>0.62</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>April 11, 1969</td>
<td>0.44</td>
<td>0.67</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>April 25, 1969</td>
<td>1.12</td>
<td>1.18</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Figure 3. Nitrate reductase activity per milligram of nitrogen per hour, 1968-1969.
Figure 3. Treatment with Cycocel produced slightly lower enzyme activity than the control. Plants treated with B-Nine were not available for analysis on this date. In the second replication all plants showed a decrease in activity from the first date to the second. Chemically-treated plants were lower than the controls at the start, with B-Nine-treated plants having the lowest activity.

On the second date, enzyme activity was highest in Cycocel-treated plants and lowest in the controls. However, when data of the different sampling dates were averaged, Cycocel-treated plants were highest in enzyme activity, and B-Nine-treated the lowest. On both sampling dates in the third replication, activity was again highest in Cycocel-treated plants and lowest in B-Nine-treated plants. Greater differences in activity between Cycocel-treated plants and control plants were demonstrated on the first date, while differences between the B-Nine and control were greater on the second date. However, when values for the two dates were averaged, the difference in activity between Cycocel-treated plants and controls (Figure 3) was less than differences between B-Nine-treated plants and controls. In the fourth replication activity was higher in all three samples. From the first to the second sample date, enzyme activity increased. Average values (Figure 3) show that plants treated with Cycocel exhibit the highest levels of activity and those treated with B-Nine the lowest. The greatest effect of the growth retardant chemicals upon the
nitrate reductase activity is shown by the fourth replication, although the other replications demonstrate this trend repeatedly. However, statistically, none of the differences was significant.

Percent Water

The water content of the plants was determined to ascertain if the chemicals were affecting the uptake of water. Since the amount of water varied between sampling dates and among individual samples, all determinations are presented on a dry weight basis to eliminate this variability. Percentages of water are reported in Table 4.
<table>
<thead>
<tr>
<th>Replication</th>
<th>Sampling Date</th>
<th>Control</th>
<th>Cycocel</th>
<th>B-Nine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>November 28, 1968</td>
<td>89.4</td>
<td>89.4</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>December 13, 1968</td>
<td>90.3</td>
<td>90.3</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>December 20, 1968</td>
<td>90.3</td>
<td>90.4</td>
<td>89.4</td>
</tr>
<tr>
<td>2</td>
<td>January 3, 1969</td>
<td>90.7</td>
<td>90.5</td>
<td>69.9</td>
</tr>
<tr>
<td></td>
<td>February 28, 1969</td>
<td>90.6</td>
<td>89.9</td>
<td>89.7</td>
</tr>
<tr>
<td>3</td>
<td>March 14, 1969</td>
<td>90.4</td>
<td>90.5</td>
<td>90.1</td>
</tr>
<tr>
<td></td>
<td>April 11, 1969</td>
<td>93.1</td>
<td>93.2</td>
<td>92.6</td>
</tr>
<tr>
<td>4</td>
<td>April 25, 1969</td>
<td>90.6</td>
<td>91.1</td>
<td>88.4</td>
</tr>
</tbody>
</table>
DISCUSSION

Nitrate

The variability in nitrate content between sampling dates was not statistically significant, therefore, fluctuations in direction from one date to another are not critical. Generally, within a replication the plants responded similarly to both treatments (Table 1). Changes in nitrate levels from one sampling date to another were probably due to plants receiving unequal amounts of nutrient solution when watered and to experimental error involved in measurement of nitrate. Average values for the sampling dates (Figure 1) illustrate the major trends.

Variation among the control, Cycocel, and B-Nine treatments was significant at the 0.10 level. Differences between B-Nine treatments and control were significant at the 0.025 level, while variation between Cycocel treatments and the control were non-significant. It is concluded that treatment with B-Nine lowered the nitrate content in barley seedlings, while Cycocel treatment has little or no effect. When the nitrate contents for chemical treatments were pooled and compared to those for the controls, there was no significant difference. This is expected since the lower concentrations in plants treated with B-Nine was counteracted by the high levels in Cycocel treatments. Nitrate levels of Cycocel and B-Nine treatments were not
significantly different. Although nitrate concentrations in Cycocel-treated plants were slightly lower than in controls, they were not significantly different.

Consistently lower concentrations of nitrate in B-Nine treatments could be due to a reduced ability to absorb the nutrient or to greater reduction of the nitrate by nitrate reductase. Hageman and Flesher (5) have reported that higher enzyme activity resulted in lower levels of nitrate. This seems to support the first hypothesis since the enzyme level in these plants was always lower than in controls (Figure 3).

**Soluble Protein Nitrogen**

Nitrogen levels in either control or chemically-treated plants did not differ significantly. The differences from date to date were probably due to experimental error and to the genetic variability of individual plants. The variance among the treatments was significant. When each chemical treatment was compared with the control, differences between B-Nine-treated plants and controls were significant, but no significance was found in the differences between the control and Cycocel-treated plants. This indicates that B-Nine decreases the soluble protein nitrogen level in barley seedlings, while Cycocel has no effect, as illustrated in Figure 2. Nitrate levels exhibited the same trend. As in analysis of the nitrate content, pooled data for chemical treatments showed no difference from the control. This is because the B-Nine treatment contains the major difference which is not expressed when treatments are pooled.
The variability in nitrogen levels between Cycocel and B-Nine treatments was significant at the .01 level, indicating that B-Nine affects the nitrogen level of plants differently from Cycocel. There is no apparent relationship between nitrate content and nitrogen levels on a particular sampling date. This is reasonable since nitrate must furnish nitrogen to all the nitrogenous compounds in the plant and this analysis measured only certain soluble fractions. The basic conclusion is that B-Nine somehow affects the soluble protein nitrogen levels in barley plants. On every sampling date, these values were much lower than those for either the controls or Cycocel treatments. Perhaps the dwarfing effect of this particular growth retardant is related to lower levels of the nitrogen fractions assayed. These results do not agree with Kahn and Faust (8) who found a rise in soluble protein content of plants treated with Cycocel. Their results, however, were expressed per g fresh weight, while determinations in this study were expressed per g dry weight.

*Nitrate-Reductase Activity*

Enzyme activity between sampling dates in the control or the chemically treated plants did not differ significantly. Fluctuations among sampling dates (Table 3) were probably due to experimental error and the individual differences inherent in the plants. Low enzyme activity on January 3, 1969 was due, in part, to a rise in temperature, producing wilted plants.
The increase in temperature was caused by malfunction in the cooling system of the environmental chambers.

Analysis of the data showed no significant difference between control, Cycocel, or B-Nine. Although the differences were not significant, they may be important. In all replications except the first, the same relationship exists between chemically-treated plants and the controls. Cycocel-treated plants always contained a higher level of activity and B-Nine-treated plants a lower level than the controls (Figure 3). The greater activity of the enzyme in Cycocel-treated plants could account for their lower amounts of nitrate.

Higher levels of nitrate would be expected due to the lower nitrate reductase activity in B-Nine-treated plants. Since B-Nine treatment also decreased the nitrate concentration, the effects of B-Nine upon nitrate and enzyme must be independent of each other.

If the B-Nine is affecting growth of the plant by decreasing activity of the nitrate reductase enzyme, this would support findings by Tolbert (25) and Hewitt and Notton (7) that its biochemical mechanism involves some active site. Likewise, results on Cycocel-treated plants tend to support Rothenburger's theory that B-Nine and Cycocel have different modes of action (18).
Percent Water

The growth retardant chemicals did not affect water content of the plants. This agrees with Appleby et al. (1) who found that Cycocel did not affect the length of roots or their ability to absorb water and nutrients.

Plant Height

Plant height was not noticeably affected by treatment with Cycocel, however, the plants may not demonstrate the dwarfed condition in the seedling stage. Plants treated with B-Nine did exhibit the shorter, denser growth, characteristic of plants treated with these chemicals.
CONCLUSIONS

Although the mechanism by which Cycocel and B-Nine inhibit plant growth was not determined in this study, it appears that they have different modes of action. The data (Figure 4) showed that B-Nine decreased the concentration of nitrate, soluble protein nitrogen, and activity of the nitrate-reductase enzyme. This may be responsible for the reduction in plant growth. Cycocel did not affect these nitrogenous fractions.
Figure 4. Average of replications, nitrate content, soluble protein content, and nitrate reductase activity of barley seedlings, 1968-1969.
SUMMARY

This study was conducted to determine the effect of the growth retardant chemicals, Cycocel and B-Nine, on some nitrosoeous fractions in barley. It was hypothesized that these chemicals could inhibit activity of the nitrate reductase enzyme, which is the rate-determining step in the conversion of nitrate to ammonia.

Barley (Hordeum vulgare L.) seedlings were grown in light and temperature-controlled environmental chambers. They were supported in vermiculite and supplied nutrient solution. Plants were sprayed with Cycocel or B-Nine seventeen days after planting. One group of plants was untreated and served as the control. Fourteen and twenty-eight days after spraying, the plants were assayed for nitrate, soluble protein nitrogen, nitrate reductase activity, and water content.

Cycocel-treated plants did not differ significantly from the controls in any of the determinations made, but B-Nine-treated plants did differ significantly from the control plants in concentration of nitrate and soluble protein nitrogen. No significant difference was found in nitrate reductase activity between B-Nine and control plants, although in all replications, the B-Nine-treated plants showed lower levels of enzyme activity than either the control or Cycocel-treated plants. Therefore,
the lower enzyme activity caused by treatment with B-Nine may be important. Neither of the chemicals affected the water content of the plants.

It is concluded that B-Nine lowered the concentrations of nitrate, soluble protein nitrogen, and activity of the nitrate reductase enzyme in barley seedlings. Cycocel had no significant effect upon these nitrogeneous fractions.


