Effects of Cycocel and B-Nine on Cold Hardiness of Wheat Plants

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Cold hardiness measurements by a specific conductivity method indicated that Minturki wheat was hardier than Ponca. Crown tissues of both B-Nine– and Cycocel-treated Minturki wheat plants showed small decreases in per cent conductance compared with untreated plants, indicating that cold hardiness was increased to some extent. Effects of these two retardants were less on Ponca wheat than on Minturki. The soluble protein fractions from B-Nine–treated Minturki wheat crowns were compared with fractions from untreated Minturki. No new fractions were found, and the shapes of the elution curves were similar for the treated and untreated plants. Little difference was noted in percentages of the total areas for each fraction, indicating that treatment with B-Nine did not alter the soluble protein fractions of Minturki.

Cycocel and B-Nine growth-retardant chemicals have received much attention as regulators of plant growth and flowering. Each has been found active over a wide range of plant species (Cathey, 1964).

Tolbert (1960) reported that Cycocel in concentrations of 10^{-2} to $10^{-6}M$ retarded the growth of wheat. Shorter and thicker stems, with broader and greener leaves, were among the characteristics he reported. Marth and Frank (1961) reported that plants treated with retardants were more resistant than controls to loss of water. Marth (1965) later reported that cabbage plants sprayed with Cycocel and B-Nine showed increased frost resistance.

This study was concerned with the effect of B-Nine and Cycocel on the cold hardiness of winter wheat crowns and on the soluble protein fractions of winter wheat crown tissue. The latter were investigated because of a reported correlation between winter hardiness and soluble protein content of wheat crown tissue (Zech and Pauli, 1960).

MATERIALS AND METHODS

Minturki and Ponca winter wheats were planted on the University Agronomy Farm, September 29, 1965. The source of seed for both varieties was plants grown on the farm the previous year. These particular genotypes were chosen because Minturki is a cold-hardy variety and Ponca is moderate in hardiness (Zech and Pauli, 1960).

On October 26, 1965, Cycocel (2-chloroethyl trimethylammonium chloride) was applied to field plots of Minturki and Ponca winter wheats that were planted at the same time and were adjacent to the wheat used as controls. B-Nine (*N*-dimethylaminosuccinamic acid) was applied to other Minturki and Ponca plots October 27, 1965. The chemicals were applied by spraying the growing wheat plants to runoff with 500-p.p.m. solutions.

Collection of crown tissue started November 21, 1965, and continued at approximate 2-week intervals until March 16, 1966. The plants were brought from the field to the laboratory, where they were washed free of soil with cold tap water. The crowns, defined as that portion of tissue above the roots and below the soil surface, were removed, washed with cold tap water, and rinsed with distilled water. Excess surface water was removed by blotting the crowns between towels.

Essential to this study was a rapid method for measuring the cold hardiness of the plants. Dexter *et al.* (1932) developed a conductivity method and showed it to be a reliable measure of cold hardiness by comparing it with survival of plants exposed to low temperatures. The wheat crowns in this study were tested for cold hardiness by this method.

Five grams of fresh crown tissue were placed in an uncovered beaker and frozen for 4 hours at −12° C. in a controlled temperature chamber. The tissue was removed, placed in 50 ml. of double-distilled water, and allowed to stand at least 12 hours in a chamber maintained at approximately 5° C. A Wheatstone bridge was used for resistance readings at 20° C. on the solution containing the exosmosed solutes from the cells damaged by freezing. The crown tissue then was blended to macerate the tissues, extracting all the cell solutes. Resistance readings were taken on solutions containing the total cell electrolytes. Specific conductance was calculated from the resistance readings. Results were expressed as percentages of maximum conductance, reflecting the percentage of total electrolyte exosmosed following freezing. These were calculated by dividing the specific conductance of the solution following freezing by the specific conductance of the solution containing the total cell electrolytes and multiplying by 100. Average values for four samples per treatment were used. The maximum variation within each treatment was 2%, indicating good reproducibility of the method. Lower percentages of maximum conductance indicate greater cold hardiness, since fewer cells of hardy plants are damaged during the freezing period.

Soluble proteins were extracted and fractionated as described by Toman and Mitchell (1968). Total nitrogen contents of the protein extracts were determined by a modification of the nesslerization method of Thompson and Morrison (1951).

RESULTS AND DISCUSSION

Per cent conductances for untreated plants indicated that Minturki lost fewer solutes than Ponca after freezing, reflecting its greater cold hardiness as reported earlier (Toman and Mitchell, 1968).

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Minturki plants treated with B-Nine and Cycocel (Figure 1) showed small decreases in per cent conductance as compared with untreated plants, indicating that hardiness in the treated plants may have been increased somewhat, as reported by Marth with cabbage (1965). Lower conductance values were observed on all dates except the last two, when all values for each date were essentially the same. The greatest difference between treated and untreated plants occurred December 27, 1965, when the plants were about half hardened. Of the six dates on which treated plants had lower conductance values than control, B-Nine was lower than Cycocel on four. However, differences between the retardants were small and probably both retardants have comparable potency. Although the differences in per cent conductance between treated and untreated Minturki were relatively small (Figure 1), they approached the magnitude of the differences between untreated Minturki and Ponca (Figures 1 and 2), which consistently exhibit differences in hardiness.

The effects of B-Nine and Cycocel on Ponca wheat (Figure 2) were somewhat different from those on Minturki. In the early stages of hardening, the retardants had little effect on the hardiness of Ponca, but at the time of maximum hardiness, treated plants were hardier than untreated plants. The slower development of hardiness of treated Ponca compared to treated Minturki indicates that these chemicals were somewhat less effective with Ponca and suggests varietal differences in response.

The soluble proteins of untreated and B-Nine–treated Minturki wheat crown tissue, harvested January 31, 1966, were extracted and fractionated as described by Toman and Mitchell (1968). Fractionation was accomplished with DEAE-cellulose columns and echelon elution with NaCl in 0.005*M* phosphate buffer at pH 7.3.

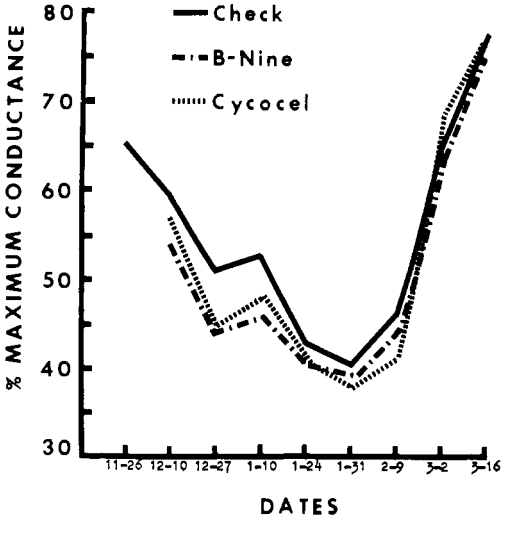

Figure 1. Per cent maximum conductance of Minturki wheat crown tissue treated with growth retardants, 1965–66

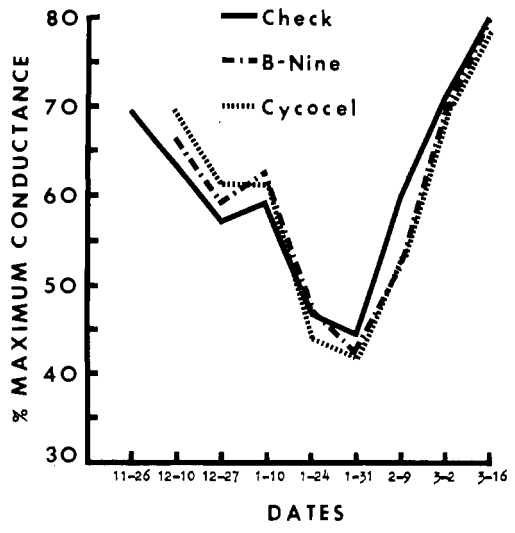

Figure 2. Per cent maximum conductance of Ponca wheat crown tissue treated with growth retardants, 1965–66

Table I. Total Area of Each NaCl Fraction Eluted from DEAE-Cellulose Columns

	Area, %				
	0.05*M* NaCl	0.1*M* NaCl	0.2*M* NaCl	0.3*M* NaCl	1*M* NaCl
Untreated Minturki	26.3	17.5	21.5	19.2	15.3
B-Nine–treated Minturki	23.7	18.5	22.0	20.1	15.8

The shapes of the elution curves for the treated and untreated plants were essentially the same. No new peaks were found, and the shapes of corresponding peaks were similar. Comparisons of the percentages of the total area for each fraction (Table I) showed little difference between treated and untreated plants. The authors concluded that treatment with B-Nine did not alter the soluble protein fractions obtained in this experiment.

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