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Effects of Two Plant Growth Regulators, Terpal-C and Cerone, on Freezing Tolerance and Winter Survival of Canola (*Brassica napus* L.)

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ABSTRACT

One of the most attractive short-term possibilities for increasing freezing tolerance of winter crops may be the application of chemicals. This research was conducted to determine the effect of two plant growth regulators, Terpal-C and Cerone on freezing tolerance and winter survival of canola. Three cultivars were planted on the Michigan State University Agronomy Farm at East Lansing, MI. on Sept. 10, 1992 and 1993. Chemicals were applied to one-month-old plants when they reached the 5 leaf stage. Ion leakage tests for freezing tolerance were conducted before and after chemical treatment. Winter survival was evaluated by counting the plant standing in the fall and spring. Neither of the chemicals had any effect in increasing freezing tolerance and winter survival. Instead, one of the chemicals, Terpal-C, inhibited natural cold hardening.

Key words: freezing tolerance, winter survival, plant growth regulator, Terpal-C, Cerone

INTRODUCTION

Numerous efforts have been made to overcome the freezing problems of crop plants in agriculture, including breeding efforts to develop more hardy cultivars and modification of cultural practices. Most of the hardy cultivars of wheat grown today contain major genes for cold hardiness 6). Consequently, little or no progress has been made in enhancing the genetic potential of cold hardiness in winter wheat. Searches for cost-effective strategies in plant freezing protection have focused on short-term solutions because of the limited gene pool and difficulties in breeding procedures for cold hardiness (Fowler and Gusta, 1970).

One of the most attractive short-term methods for increasing freezing tolerance may be the application of chemicals (Marth, 1965; Chen and Li, 1976; Ketchie and Murren, 1976; Probsting and Mills, 1976; Raese, 1977; Gusta et al., 1982; Durner and Gianfagna, 1988; Morrison and Andrew, 1992). The development of freezing tolerance is controlled by plant hormones. ABA is known to be involved in cold acclimation of plants (Irving, 1969; Waldman et al., 1975; Chen et al., 1979; Lang et al., 1988; Orr et al., 1986; Reaney and Gusta, 1987; Rikin et al., 1987; Mohapatra et al., 1992) and, coincidently, with expression of certain cold-regulated genes (Thomashow, 1990). Another plant hormone involved in cold hardiness is gibberellic acid (GA) which has an antagonistic effect on freezing tolerance. Waldman et al. (1975) suggested that ABA induces cold acclimation by inhibiting the synthesis of GA, and demonstrated that the exogenous application of ABA dramatically reduced GA level in cold hardy alfalfa cultivars. The primary synthetic plant growth regulators that increase winter hardiness have either anti-gibberellin or ethylene-producing characteristics. Though the role of these plant growth regulators in winter hardiness at the molecular and cellular level is uncertain, it is assumed that they prevent the loss of freezing tolerance (Gusta et al., 1988).

Some of the early interest in the use of PGRs on oilseed rape occurred as a result of experiments in Poland which suggested that CCC improved cold hardiness of rapeseed in controlled environment chambers. The role of ABA in cold acclimation was studied in cell suspension cultures of *Brassica napus*(Orr et al., 1986; Orr et al., 1992). ABA could promote freezing tolerance in the absence of low temperatures. Recently, the effects of several plant growth regulators on winter hardiness of canola under field conditions were studied in Canada (Morison and Anderw, 1992). Some of the triazole PGRs reduced plant size by limiting cell expansion and increasing cell numbers. While cold hardiness and freezing tolerance were increased by certain growth regulators, these effects were not consistent over time and did not result in increased winter survival. This suggests that natural cold hardening may have eclipsed the PGR-induced hardening.

Terpal-C is a mixture of mepiquat chloride and ethephon, while Cerone contains ethephon. Mepiquat chloride belongs to a group of plant growth retardants known to inhibit GA biosynthesis. This group of compounds includes AMO-1618, CCC (chlormequat chloride), Phosphon D (chloroponium), and ancymydol, as well as mepiquat chloride. Of all the compounds in this group, only CCC and mepiquat chloride have found large-scale application in agriculture. Ethephon belongs to the group of compounds which, when applied to plant materials, release ethylene as they decompose. This group includes ethephon (2-chloroethyl phosphonic acid), silaid (2-chloroethyl methylbis phenylmethoxy silane), alsol (2-chloroethyl tris 2-methoxyethoxy silane) and ACC (1-aminocyclopropane-1-carboxylic acid).

The objective of this study was to determine the effects of two plant growth regulators, Terpal-C and Cerone, on freezing tolerance and winter survival of canola under field condition.

MATERIALS AND METHODS

Three winter canola cultivars, WRG86, Duobul, and KWC4113, which had a representative winter survival in previous experiments were used in this study. This study was conducted at the Michigan State University Agronomy Farm in East Lansing, Michigan. A split-plot design with cultivar as the main plot and growth regulators as subplots was used with three replications. Seed was planted at the rate of 5.6 kg/ha in a 5-row plot 6 m long and 92 cm wide. The planting date for the first year was Sept. 5, 1992 and for the second year, Sept. 6, 1993. Two plant growth regulators, Terpal-C (BASF) and Cerone (Union Carbide), were used for this experiment. Chemicals were applied with a compressed CO2 sprayer one month after planting (Oct. 13, 1993) when most of the plants reached the 5 leaf stage. The chemicals were diluted with distilled water at the rate of 300:1, then applied at the rate of 1840 g/ha for Terpal-C and 620 g/ha for Cerone. Control plots were sprayed with distilled water. In 1993-1994, freezing tests were conducted on plant samples collected just before chemical application (Oct. 10) and 25 days after chemical application (Nov. 5). Five different plants were collected from each plot in the field and immediately transferred to the laboratory. One leaf disc was taken from each plant and 3 leaf discs from the same plot were placed in a stoppered culture tube representing one replication. Freezing tests were conducted as described before (Song, 1994). Winter survival for both 1992-1993 and 1993-1994 studies were evaluated by counting the no. of plants both in fall and spring time.

RESULTS AND DISCUSSIONS

Freezing assay was based on ion leakage as a measure of freezing damage. A plot of temperature versus percent electrolyte leakage was used to determine the value for 50% eletrolyte leakage, which was defined as LT50, the degree of the freezing tolerance of cultivars tested. Typical freezing tolerances were developed from the two sensitive cultivars, WRG86 (Fig. 1) and Duobul (Fig. 2), and the hardy cultivar, KWC4113 (Fig. 3). All three cultivars showed similar responses to the growth regulator treatment. Cerone had little or no effect in increasing freezing tolerance in all the cultivars. However, Terpal-C treatment decreased the freezing tolerances of all cultivars measured on Nov. 5 compared to that of control on Nov. 5 (Table 1). Interestingly, this treatment seemed to

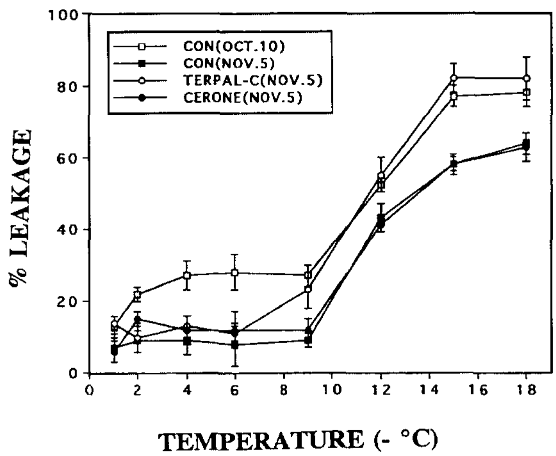

Figure 1. Effect of plant growth regulators, Terpal-C and Cerone, on freezing tolerance of winter canola cv.WRG86. Freezing tests were conducted before chemical treatment(Oct. 10) and 25 days after chemical treatment(Nov. 5). Vertical bar indicates mean±SE.

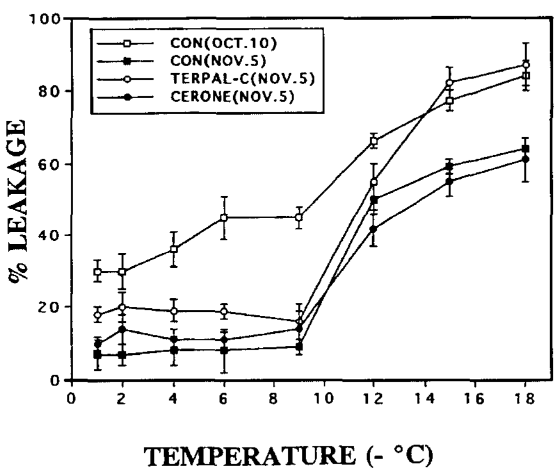

Figure 2. Effect of plant growth regulators, Terpal-C and Cerone, on freezing tolerance of winter canola cv.Duobul. Freezing tests were conducted before chemical treatment(Oct. 10) and 25 days after chemical treatment(Nov. 5). Vertical bar indicates mean±SE.

inhibit the normal development of freezing tolerance. No interaction was found between variety and chemical treatment.

Both Terpal-C and Cerone treatment had no significant effect on the winter survival of canola in either year (Table 2 and Table 3). The only effect on winter survival was

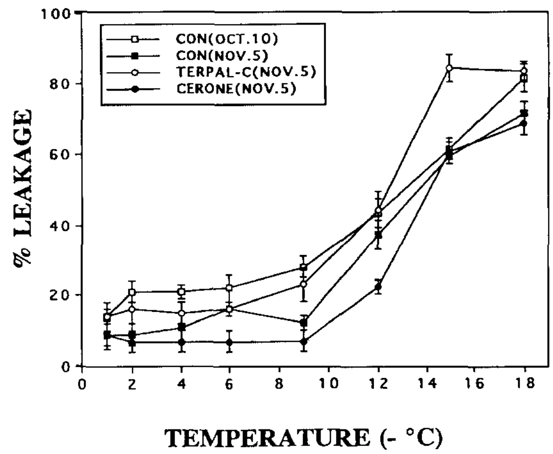

Figure 3. Effect of plant growth regulators, Terpal-C and Cerone, on freezing tolerance of winter canola cv.KWC4113. Freezing tests were conducted before chemical treatment(Oct. 10) and 25 days after chemical treatment(Nov. 5). Vertical bar indicates mean ± SE.

Table 1. Effect of plant growth regulators, Terpal-C and Cerone, on freezing tolerance of three canola cultivars, WRG86, Duobul and KWC4113.

Chemical	LT_{50}(-°C)			
	WRG86	Duobul	KWC4113	Mean
Control†(Oct. 10)	10.3	9.5	12.1	10.6b
Control(Nov. 5)	11.9	12.0	13.8	12.6a
Terpal-C(Nov. 5)	9.2	10.4	11.4	10.3b
Cerone(Nov. 5)	11.7	12.6	13.6	12.3a
Mean	10.8c	11.2b	12.7a	
F-test:	Variety(A)	11.74(**)‡		
	Chemical (B)	12.75(**)		
	A×B	0.75(ns)		

† Two controls as before chemical treatment(Oct. 10) and after chemical treatment(Nov. 5)

‡ (*) and (**): significant at 0.05 and 0.01 probability levels, respectively. (ns) not significant at 0.05 probability level.

among cultivars, and only during the second year. No interaction occurred between growth regulator treatment and cultivar in either year.

The possible mechanisms of the PGRs in winter hardiness have been illustrated by several studies in winter crops : 1) Some PGRs prevent the dehardening of plants in mid-winter. The ultimate level of freezing tolerance in November was not increased by application of the chemical. However, they did reduce the loss of freezing tolerance

Table 2. Effect of plant growth regulators, Terpal-C and Cerone, on winter survival of three canola cultivars in 1992-1993.

Chemical	Winter survival(%)			
	WRG86	Duobul	KWC4113	Mean
Control	82.0	80.3	86.0	83.0
Terpal-C	92.0	81.0	90.3	88.0
Cerone	83.0	91.0	95.6	89.0
Mean	80.5	84.3	90.8	
F-test:	Variety(A)		1.160(ns)†	
	Chemical (B)		0.270(ns)	
	A × B		1.470(ns)	

† (*) and (**): significant at 0.05 and 0.01 probability levels, respectively. (ns) not significant at 0.05 probability level.

Table 3. Effect of plant growth regulators, Terpal-C and Cerone, on winter survival of three canola cultivars in 1993-1994.

Chemical	Winter survival(%)			
	WRG86	Duobul	KWC4113	Mean
Control	60.0	65.0	76.5	67.0
Terpal-C	63.5	58.0	73.5	65.0
Cerone	58.0	59.0	78.5	65.1
Mean	60.3b	60.6b	76.1a	
F-test:	Variety(A)		3.200(*)†	
	Chemical (B)		0.270(ns)	
	A × B		1.470(ns)	

† (*) and (**): significant at 0.05 and 0.01 probability levels, respectively. (ns) not significant at 0.05 probability level.

in mid-winter, which is considered to be the most critical period (Gusta et al., 1990). 2) Certain PGRs promote regrowth of roots in the spring, even if most of the root system is winter-killed (Robertson et al., 1992). 3) Some PGRs reduce the elongation of the subcrown internodes, thereby avoiding exposure to freezing air temperatures. 4) Some PGRs stimulate photosynthesis, resulting in increased starch accumulation and enhanced winter hardiness. 5) Certain PGRs inhibit degradation of endogenous ABA, and thus, indirectly affect cold hardiness (Fowler and Gusta, 1970).

All of these effects are inconsistent from year to year (Robertson et al., 1992). The reason for this inconsistency is not known, but possible reasons include lack of uptake, improper growth stage when applied, temperature at the time of application, and severity of winter. A period of relatively warm temperatures is generally required following application to allow the PGRs to result in the desired chemical change.

The results from the freezing tests in this experiment are not consistent with those of Morrison and Andrews (1992) who reported that Terpal-C increased freezing tolerance. Terpal-C application to canola in the field decreased the freezing tolerance of canola by 1-2 °C in December compared to the control. Whether decrease in freezing tolerance by Terpal-C treatment in this experiment is due to its effect on the retention of physiological immaturity or the inhibition of natural cold-hardening by the chemical l application should be explored in future studies. In spite of the decrease of freezing tolerance by Terpal-C, it did not affect winter survival. This may be additional evidence that its effect is not the increase in freezing tolerance but the prevention in loss of freezing tolerance during the middle of severe winters (Gusta et al., 1982).

The finding that application of Cerone had no effect on increasing freezing tolerance and winter survival of canola is not consistent with other results (Raese, 1977). The reason for this could be that the natural cold acclimation under field conditions masked the effect of chemicals. This assertion is possible in that most of the data for the effect of the chemicals on freezing tolerance, not winter survival, were measured in laboratory condition. Also, this inconsistency could have resulted from the lack of winter severity to reveal the effects of chemical treatment: lack of chemical uptake, or improper developmental stages of plant for chemical application could be considered. A split application of the chemicals at various stages of development in the fall might be considered for future experiments.

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