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Variable Increases in Cold Hardiness Induced in Winter Rape by Plant Growth Regulators

M. J. Morrison and C. J. Andrews

Agriculture Canada, Plant Research Centre, Central Experimental Farm Building 75, Ottawa, Ontario, Canada K1A 0C6

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Abstract. Triazole and conventional growth regulators were tested for their ability to extend cold hardiness and improve the winter survival of winter rape (*Brassica napus* L.). Winter rape plants were grown in the field (Ottawa 45°23′ N) and in growth cabinets. Plant growth regulators (PGRs) were applied during the early vegetative stage and the plants were allowed to cold harden. Cold-hardened plants from the field and cabinet were subjected to freezing and ice encasement tests in the laboratory. Some of the triazole PGRs reduced plant size by limiting cell expansion and increasing cell numbers. While cold hardiness and ice encasement tolerance were increased by some growth regulators, these effects were not consistent with time nor were they reflected in increased winter survival. Natural cold hardening may have eclipsed the PGR-induced hardening.

Many annual crop species can modify their physiology, by the process of acclimation, to withstand freezing temperatures during winter. Winter rape (*Brassica napus* L.) plants with the ability to cold-harden are sown in the fall, produce a rosette, overwinter, and grow to maturity the following spring. Winter rape cultivation in most regions of Eastern Canada is limited by winter killing caused by extreme temperature and the formation of ice that encases the plants. In an Ontario-wide test, few locations resulted in consistently high winter survival and yield (Beaulieu and Hume 1987). The inability to withstand freezing temperatures limits the northward extension of winter rape cultivations in Eastern Canada.

Roberts (1971) increased the hardiness and the survival of winter wheat (*Triticum aestivum* L.) to freezing temperatures using CCC (chlorocholine chloride). Voskerusa (1972) concluded that fall application of CCC to winter rape had no effect on plant survival, seed yield, or oil content. Protection from 0–3°C chilling stress was obtained in corn (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) with mefluidide (Tseng and Li 1984). The triazole, triadimefon, increased the survival and fresh weight of barley (*Hordeum vulgare* L.) seedlings subjected to freezing temperatures of −6°C (Fletcher and Hofstra 1985).

The objectives of this study were to examine the effects of several plant growth regulators (PGRs) on the cold tolerance of winter rape with the ultimate goal of extending northward the zone of adaptation in Eastern Canada.

Materials and Methods

In the field, winter rape cultivars Ceres and Touchdown were seeded on 88/08/21, 89/08/24, and 90/09/05 in a randomized complete block design on the Central Experimental Farm, Ottawa (Lat. 45°23′ N). Each field trial is referred to by the year in which it was planted. Individual plots consisting of eight rows, 18 cm apart and 6 m long, were replicated four to six times depending upon the year. Treatments consisted of five growth regulators and a control. The fields were treated with fall-applied granular trifluralin at recommended rates, and carbofuran insecticide was applied with the seed at 5 kg ha^{-1} to prevent damage from flea beetles (*Phyllotera* spp. and *Psylliodes* spp). After emergence, winter sampling areas were determined and marked with stakes that were visible above the snow line. Fall plant populations were counted and the rows marked for spring survival determination. Plants were sprayed with the growth regulators at the five-leaf stage with a compressed CO_2 sprayer. Rates, chemical nomenclature, and company of manufacture can be obtained from Table 1.

During the 1988 trial, plant samples at several different growth stages were removed from the field, separated into leaves and stems, stem heights measured, and dry weights determined. A 0.5-m row length, surrounded by other rows, was used for the

PGR contribution number 1381.

Table. 1 PGRs, rates of application, chemical nomenclature, and company names.

PGR	Rate[a]	Chemical name	Company
Cerone	620	Ethephon	Union Carbide
Terpal C	1840	Ethephon and chlormequat chloride	BASF
Uniconizole	12	(E)-1-(p-chlorophenyl)-4,4-dimethyl-2-(1,2,3-triazol-1-yl)-penten-3-ol	Chevron
HWG 1608 (tebuconzol; experimental)	1000	α-[2-(4-Chlorophenyl) ethyl]-α-(1,1-dimethylethyl)-1H-1,2,3-triazol-1-ethanol	Mobay
Paclobutrazol	180	(2RS + 3RS-chlorophenyl)-4,4-dimethyl-2-(1,2,3-triazol-1-yl)-penten-3-ol	Chipman

[a] g ai ha^{-1} = grams active ingredient per hectare.

growth analysis. An analysis of variance was done on the growth characteristics.

Plants from designated plots were removed from the soil in the winter using shovels and jackhammers. The first sampling was done in early December, just before the ground was completely frozen. Further winter sampling was limited to one date in mid-January 1989. While attempts were made to remove the plants in other years, the soil had frozen in horizontal layers that fractured when dug and cleaved the frozen plants making them unusable. Samples from the field were allowed to thaw overnight at 5°C before the plants were separated from the soil. At least 100 plants per plot were removed from the field. These were further subdivided into four replications consisting of five 5-plant subsamples subjected to different freezing temperatures or times in ice encasement under controlled environments.

Leaf samples from a similar position on the plant were removed from each treatment at the first field sampling. The leaves were preserved in a solution of formaldehyde acetic acid (100 ml 50% ethanol, 6.5 ml formalin, and 2.5 ml glacial acetic acid) until similar areas of the leaf blade were mounted in paraffin, cross-sections prepared, and photographed.

Winter rape (cvs. Ceres and Touchdown) was grown in growth cabinets for 2–3 weeks at 20/15°C (day/night) during 16 h of 525 μmol photon m^{-2} s^{-1} light intensity. Five plants per 13-cm diameter pot were grown in a 1:1:1 soil mix (soil:greenhouse mix: vermiculite). The temperature was reduced to 10/5°C for 1 week and then further reduced to 2/0°C for 7–10 weeks of cold acclimation before freezing. During the cold acclimation period, the day length was reduced to 12 h and the light intensity was 250 μmol photon m^{-2} s^{-1}. Plants were watered to field capacity daily and fertilized once per week with a solution of 20-20-20 (N:P:K). Plants at the five-leaf stage were sprayed with the growth regulators at approximately the same rates as were used in the field. Plants were tested for cold and ice encasement tolerance at the five to seven-leaf stage.

Plants from the field or growth cabinet were washed free of soil in cold water and trimmed so that there was vegetative material 2–3 cm above and below the crown tissue. Groups of five plants were placed in polyethylene bags, sealed, and put in a programmable freezer at 0°C. The freezer temperature was decreased by 1°C/h and the bags were removed at intervals through a temperature range expected to encompass the 50% kill point of the plants. The test unit for an ice encasement measurement was five plants in a 200 ml polyethylene beaker covered with crushed ice and chilled water. The beakers were placed in a freezer at −2°C for 12 h to induce freezing and then the temperature was raised to −1°C and the light period changed to 8 h of 100 μmol photon m^{-2} s^{-1}. The beakers were removed at daily intervals expected to encompass the 50% kill range and allowed to thaw. A full description of the freezing and ice-encasement test protocol can be found elsewhere (Andrews and Morrison 1992).

The plants from either test were thawed at 2°C, transplanted to moist vermiculite, and grown at 20/15°C during a 16-h day with a light intensity of 525 μmol photon m^{-2} s^{-1}. The transplants were watered daily with a dilute solution of 20-20-20 (N:P:K). Plant survival was rated according to the presence of both new leaves and active root tissue. LD_{50}s were calculated as the temperature- or time-duration when 50% of the treatment population were killed. An analysis of variance was done on the LD_{50} results.

To examine the residual effect of the growth regulators, plants from the third replication of the growth-cabinet experiment, which survived the freezing temperatures, were transplanted into pots and grown in a growth cabinet. Phenological development and plant height were observed until flowering. The percentage of development to physiological maturity (Morrison et al. 1988) was determined after 8 weeks of regrowth.

Results and Discussion

Climatic conditions were favorable for the fall establishment of winter rape in all 3 years of the experiment. Plant populations ranged from 80–100 plants m^{-2}. Spring survival and regrowth was negligible in the 3 years of the experiment. In 1989, rape plants were sampled from the field in early March, trimmed, and tested for regrowth. Percent survival ranged from 7–48% depending upon the growth regulator used (Table 2). Terpal and HWG 1608 resulted in a significant increase in percent survival above the control. However, in late April 1989, when the plants were rated for field survival, none had survived. March and April of 1989 were dry, and the plants with damaged or reduced root systems, resulting from winter kill, were subjected to desiccation. The 1989 and 1990 winters had numerous periods of warm temperatures and freezing rain resulting in several ice layers covering the plants. In early spring, when the plants were examined for winter survival, few plants had survived. Those plants at the edges of depressions survived, leading to the conclusion that in areas where the topography resulted in improved drainage and reduced ice encasement the plants could survive.

The PGRs tested reduced leaf weight per plant at both fall sampling dates with the triazole compounds HWG 1608 and paclobutrazol resulting in

Table 2. Early and late spring survival of winter rape as affected by fall application of PGRs.

PGR	Percent survival	
	Early spring[a]	Late spring[b]
Control	11	0
Cerone	7	0
HWG 1608	48	0
Paclobutrazol	14	0
Terpal	35	0
Uniconazole	15	0
$LSD_{0.05}$	10.8	—

[a] Plants removed from the field, trimmed, and regrown in a greenhouse.
[b] Observed in situ.

Table 3. Effect of PGRs on leaf and stem dry weight (g plant^{-1}) and stem height (mm) at two growth stages.

PGR	Leaf weight	Stem weight	Stem height
Seven-leaf stage			
Control	1.38	0.13	15.2
Cerone	1.24	0.14	13.8
HWG 1608	0.64	0.08	14.0
Paclobutrazol	0.91	0.14	12.3
Terpal	1.15	0.15	15.8
Uniconazole	1.00	0.12	16.5
$LSD_{0.05}$	0.36	0.05	NS
Eight-leaf stage			
Control	1.89	0.21	16.7
Cerone	1.75	0.22	15.0
HWG 1608	1.19	0.17	12.0
Paclobutrazol	1.41	0.24	12.3
Terpal	1.49	0.21	18.3
Uniconazole	1.96	0.26	13.8
$LSD_{0.05}$	NS	NS	NS

NS, not significant.

the largest decrease (Table 3). The triazole compounds produced plants with leaves adpressed to the soil surface. HWG 1608 also produced plants with lower stem weights than the control. The other growth regulator treatments did not result in significant stem weight or height differences from that of the control.

Plants treated with the PGRs showed considerable differences in overall plant morphology and leaf anatomy. Paclobutrazol and HWG 1608 treatments reduced petiole length and leaf size, whereas terpal and uniconazole had little effect (Fig. 1). Paclobutrazol-, uniconazole-, and HWG 1608-treated plants showed increases in leaf thickness up to 35% above that of the control. Paclobutrazol and HWG 1608 treatments increased the number of leaf palisade layers to four or five, whereas the other PGR treatments had about three. The density of the mesophyll layer was also considerably greater in PGR-treated leaves than the control.

The reduction in leaf size and the increase in leaf thickness by the PGRs are similar to observations made by Asare-Boamah and Fletcher (1986) and Gao and Fletcher (1988). The decrease in cell size and the increase in cell numbers in some of the triazole-treated leaves are associated with a reduction in GA (gibberellin) content and a transient rise in the ABA (abscisic acid) concentration (Asare-Boamah et al. 1986). Limin and Fowler (1989) showed that decreased cell size, in some species, was highly correlated with increased cold tolerance due to decreased cellular water content and reduced cell contraction under dehydrative freezing stress.

In early December, cold hardiness levels of the control plants ranged from -14.1 to -17.0 during the 3 years of the experiment (Table 4). In 2 of the 3 years, some of the growth regulators increased the freezing tolerance of the plants above the control. Only one sampling was done successfully in mid-January and by that time hardiness levels for all but the control and the cerone treatment had increased from the December sampling. When the December samplings were analyzed across years there was a significant year by growth regulator interaction. Maximum hardiness was elicited by a different growth regulator in each year. The greatest increases over the control in December resulted from HWG 1608 and terpal. While some growth regulators improved the cold hardiness of the field-grown rape plants, the results are inconsistent across time and improvements in cold hardiness were not reflected in increased survival.

Despite similar morphological changes, growth-cabinet-grown plants did not exhibit the same treatment differences in cold hardiness as field-hardened plants (Table 5). Three replications of the experiment were conducted, but significant treatment differences were observed only when the cerone treatment resulted in a significantly lower cold hardiness than the control.

After 8 weeks of regrowth in pots there were no significant differences in plant height or phenology among growth regulators for growth cabinet plants frozen to -16°C (Table 6). However, after exposure to -18°C, uniconazole-treated plants, while not demonstrating superior cold hardiness, were the only plants to reach the bud stage of development after 8 weeks regrowth. Paclobutrazol-treated plants also had significantly higher rates of recovery than the control. Cerone-treated plants were shorter and had significantly slower rates of development than the controls.

Field-hardened plants from December 1988 and

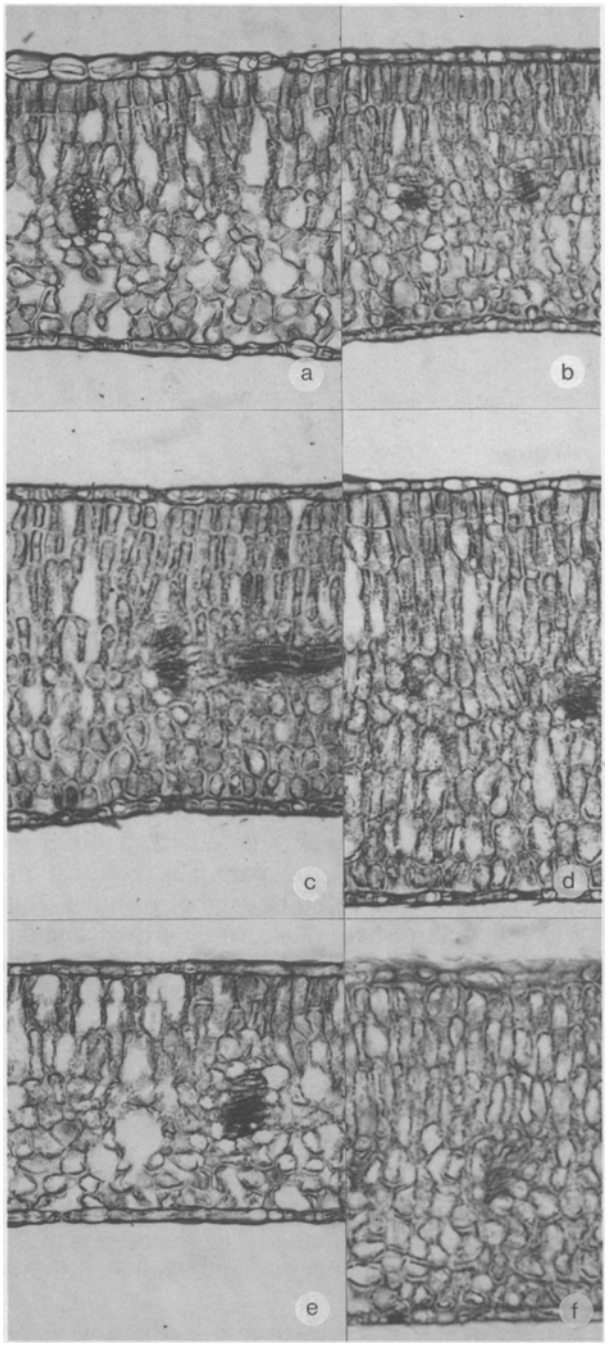

Fig. 1. Leaf cross-sections of cold-hardened winter rape plants treated with PGRs. (**a**) Control, (**b**) cerone, (**c**) HWG 1608, (**d**) paclobutrazol, (**e**) terpal C, and (**f**) uniconazole. Original magnification, ×100.

1990 were tested for ice encasement tolerance (Table 7). Winter rape plants were very susceptible to ice encasement. On average, 50% of the field-hardened control plants were dead after 12 days encased in ice. Uniconazole- and paclobutrazol-treated plants were more tolerant to ice encasement

Table 4. Cold hardiness test results (LD_{50} °C) of winter rape as affected by the fall applications of PGRs.

PGR	1988 Dec.	1988 Jan.	1989 Dec.	1990 Dec.	Dec. Mean
Control	−16.1	−16.2	−14.1	−17.0	−15.7
Cerone	−16.3	−15.9	−15.1	−16.9	−16.1
HWG 1608	−16.1	−18.0	−18.0	−16.2	−16.7
Paclobutrazol	−17.2	−19.5	−14.8	−16.4	−16.1
Terpal	−16.2	−17.4	−16.5	−19.6	−17.4
Uniconazole	−15.9	−18.7	−16.2	−16.7	−16.2
$LSD_{0.05}$	NS	2.3	1.9	1.7	1.0

NS, not significant.

Table 5. Cold hardiness test results (LD_{50}°C) of cabinet-grown winter rape treated with PGRs.

PGR	Experiment A (r = 4)[a]	B (r = 4)	C (r = 2)	Mean
Control	−14.7	−18.8	−17.2	−16.9
Cerone	−15.5	−17.2	−17.3	−16.6
HWG 1608	−15.1	−19.0	−17.3	−16.6
Paclobutrazol	−15.3	−17.8	−15.9	−16.3
Terpal	−14.4	−19.4	−17.4	−17.0
Uniconazole	−14.3	−17.5	−15.2	−15.6
$LSD_{0.05}$	NS	1.2	NS	

NS, not significant.
[a] r = number of replications.

than the control, whereas cerone and HWG 1608 plants were more susceptible.

The chemical properties of PGRs that cause the inhibition of cell elongation and growth may also influence the freezing tolerance of some plants. Within plant cells, ethephon growth regulators are converted to ethylene that may inhibit the movement of auxin in stem tissues thereby reducing elongation (Noguchi 1987). Triazole growth regulators and fungicides inhibit GA synthesis (Asare-Boamah and Fletcher 1986). The inhibition of GA may make more substrate available for the synthesis of ABA and cytokinins (Fletcher and Hofstra 1985). Studies have observed transient increases in ABA after treatment with triazole growth regulators (Asare-Boamah et al. 1986, Mackay et al. 1990). A similar transient increase in endogenous ABA concentration has been observed in plants naturally cold hardened in field conditions (Zeevaart and Creelman 1988). Exogenous application of ABA and analogues of ABA were found to increase cold hardiness and survival of winter wheat (Chen and Gusta 1983, Flores et al. 1988). The exact mechanisms by which ABA increases freezing resistance are not

Table 6. Percentage development to physiological maturity (%DPM) and plant height (cm) of cabinet-grown, PGR-treated winter rape plants attained after 8 weeks of regrowth following two levels of freezing stress (−16° and −18°C).

	%DPM		Plant height	
PGR	−16	−18	−16	−18
Control	45.7	31.5	37.5	10.5
Cerone	42.6	26.3	31.2	4.8
HWG 1608	46.1	35.3	35.5	15.0
Paclobutrazol	44.6	39.7	31.7	19.4
Terpal	40.8	31.2	28.2	10.6
Uniconazole	42.3	40.1	26.4	38.8
$LSD_{0.05}$	NS	7.8	NS	12.4

NS, not significant.

Table 7. Ice encasement test results (LD_{50} days in ice) of winter rape as affected by the fall application of PGRs.

PGR	December 1988	December 1990
Control	11.3	12.5
Cerone	11.2	10.1
HWG 1608	10.5	8.8
Paclobutrazol	12.0	13.9
Terpal	13.0	11.4
Uniconazole	12.3	14.9
$LSD_{0.05}$	NS	1.4

NS, not significant.

known. ABA may strengthen the microtubular network associated with membranes, as well as increase the intracellular concentration of cryoprotective proteins (Flores et al. 1988, Lark and Dörffling 1985).

The results of the current experiment in controlled environments appear to be in disagreement with those in the literature that demonstrate improved freezing tolerance with the application of PGRs, ABA, or analogues of ABA (Chen and Gusta 1983, Fletcher and Hofstra 1985, Flores et al. 1988). In the above experiments, the authors used either unhardened plants, or cold-hardened plants which were treated directly before being subjected to freezing stress. In the current experiment the plants were treated with the growth regulators at the five-leaf stage and cold hardened for 8–10 more weeks before being frozen. Therefore, the transient increase in ABA due to the application of PGR, as reported in the literature, may have been eclipsed by a natural increase in ABA due to the cold-hardening process.

The degree of winter stress to which the rape plants were subjected in this experiment was very severe. It is possible that PGRs would improve winter survival in milder climatic regions by improving fall frost and winter cold tolerances. There is the added benefit that most triazole growth regulators are also fungitoxic (Asare-Boamah et al. 1986) and may reduce fall and winter pathogens thereby improving the health of the plants going into winter.

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