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PACLOBUTRAZOL EFFECTS ON STRESS TOLERANCE OF SPRING CANOLA

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EXECUTIVE SUMMARY

Paclobutrazol (PBZ) is a growth regulator and fungicide which also protects plants against stress. PBZ was tested in a total of three field trials in 1992 and 1993 as a seed treatment for spring canola grown in Ontario, using both regular and slow-release formulations. The objective was to determine the efficacy of PBZ in protecting canola against stress. PBZ was a potent growth regulator when used as a seed treatment. It increased yields in 3 of the 6 comparisons at rates from 6.25 to 12.5 mg ha^{-1}. Yield increases, when they occurred, averaged 9.2% across three cultivars. Yield increases were larger (14.5%) with the stress-susceptible, triazine-tolerant cultivar Stallion than with Global or Kristina. At Elora in 1993, PBZ also increased seed oil concentration in Stallion (45.6% vs. 42.7 in the untreated control), decreased free fatty acids (0.46 vs. 1.12%), but increased meal allyl glucosinolate controls (5.7 vs 12.3 uM g^{-1} oil-free meal). Quality parameters were unaffected at other locations and with other cultivars. Both 1992 and 1993 were relatively low stress growing seasons in Ontario. In 1994, a field trial in southern Alberta is being included in the ongoing testing.

INTRODUCTION

Increasing the efficiency of agricultural production in the future will involve the ability of crop plants to withstand damage by environmental factors such as frost, drought, heat and atmospheric pollutants. A group of compounds known as triazoles, which exhibit varying degrees of both plant growth regulating and fungicidal activity, have been shown to increase the hardiness of plants to many types of environmental stresses. The majority of the research that has been done on triazole stress protection has been in the area of drought stress. Several studies have suggested that triazole-treated plants may be better able to withstand drought conditions.

Soil-applied triadimefon increased seed yield of soybean and pea when soil moisture was maintained at half field capacity (Fletcher and Nath, 1984). Studies with snap beans showed that triadimefon reduced transpiration and protected plants from drought (Asare-Boamah et al., 1986). Pretreatment of jack pine seedlings with root drenches of paclobutrazol increased resistance to drought stress. The treated seedlings exhibited rapid stomatal response, more favourable water potentials, enhanced water retention and increased survival compared with control seedlings (Marshall et al., 1991).

In addition to perhaps being more tolerant to water stress, triazole-treated plants appear to be more tolerant to a number of other stresses. Treatment of snap beans with soil applied paclobutrazol ameliorated the injurious effects of sulphur dioxide (Lee et al., 1985a). Asare-Boamah and Fletcher (1986) also found that triadimefon protected snap bean seedlings from high (50°c for 2 minutes) and low (1°c for 8 hours) temperature damage when soil applied one week prior to the stress treatments. Similar results were obtained by Lee et al. (1985b) with paclobutrazol treated snap beans. Treatment of cucumber seedlings with soil applied paclobutrazol delayed symptoms of chilling injury (Wang 1985). Furthermore, winter survival of autumn-sown peas (Silim et al., 1985) and cereal crops (Frogatt et al., 1982) in the field was increased by paclobutrazol treatment. The mechanisms by which triazoles confer increased tolerance to various environmental stresses is not clear. Researchers have suggested that increases in abscisic acid content and increased antioxidant activity, brought on by treatment with triazoles, may be responsible for the increased hardiness of plants to environmental stress.

Mode Of Action

Gibberellin Biosynthesis

Triazole compounds exhibit varying degrees of both plant growth regulating and fungicidal activity. The intensity of their biological activity is dependent on their isomeric form (Fletcher 1985, Fletcher et al., 1986). The growth retarding properties of triazoles are largely attributed to interference with gibberellin biosynthesis. Gibberellins are synthesized from mevalonic acid via the ioprenoid pathway, and the triazoles specifically inhibit the oxidation of kaurene, kaurenol and kaurenal which is catalyzed by kaurene oxidase, a cytochrome P-450 oxidase (Hedden and Graebe 1985; Izumi et al., 1985). Furthermore, triazole induced growth inhibition can be reversed by exogenous application of gibberellins (Lever 1986). These observations support the hypothesis that growth inhibition due to triazoles is primarily due to reduced gibberellin biosynthesis. The isoprenoid pathway is complex and interference with it by triazoles may influence other processes in addition to gibberellin biosynthesis.

Sterol Biosynthesis

The fungicidal activity of triazole derivatives has largely been attributed to interference with sterol metabolism. In plants sterols are thought to function as regulators of membrane permeability and possibly as hormones or hormone precursors. Triazoles have been found to interfere with sterol biosynthesis via the same mode of action in plants as they do in fungi.

Burden et al. (1987) found that triazoles inhibited the enzyme responsible for the removal of the C-14 methyl group in the conversion from lanosterol to ergosterol. This also induced accumulation of abnormal sterols as well as changing the ratio of the "normal sterols" of campesterol to stigmasterol and sitosterol. They hypothesize that this may reflect changes in membrane architecture and may offer an explanation for increases in frost hardiness sometimes observed with triazole treated plants. This hypothesis stems from studies with plant root membranes which indicate that the control of permeability decreases in the order cholesterol, campesterol, stigmasterol and sitosterol. Any change in sterol balance toward a "more planar" cholesterol type structure might enhance the control of permeability, particularly at low temperature, and hence contribute to cold acclimation and frost hardiness.

Abscisic Acid Biosynthesis

The effect of triazoles on ABA is of interest because ABA, like the gibberellins, is synthesized via the isoprenoid pathway, and the two compounds often exhibit opposing physiological activities. The action of triazoles on ABA could be the source of stress protection that has been observed with triazoles. ABA is a natural plant growth regulator that has been implicated in plant acclimation and protection against environmental stress. Exogenous application of ABA has been shown to increase plant resistance to salinity, ozone, heat, chilling and freezing (Mackay et al. 1990). Mackay et al. (1990) demonstrated that uniconazole and other triazoles induced stress resistance and they also increased the endogenous concentrations of ABA in snap beans. Hauser et al. (1990) also demonstrated that triazoles considerably increased endogenous ABA levels in detached leaves and hydroponically grown seedlings of oilseed rape. ABA accumulated in proportion to triazole concentration. Mackay et al. (1990) also hypothesized that stress protection inferred by triazoles may in part be the result of their effect on endogenous concentrations of ABA. However both experiments showed that increases in ABA were short lived and eventually decreased to normal or below control levels. Hauser et al. (1990) hypothesized that this may be due to stimulated ABA catabolism and/or by an inhibition of its biosynthesis. Therefore, providing a continuous supply, over the growing season, of the triazoles could help to maintain higher levels of endogenous ABA and thereby prolong its stress-protecting effects.

Spring Canola

The overall objective of this experiment was to determine a practical system for protecting spring canola against environmental stress. High temperature and drought stress are the two major factors limiting the productivity of spring canola in Ontario. The crop is quite susceptible to these stresses, which result in both low yields and poor quality. Therefore it was hypothesized that treating spring canola with triazoles could alleviate the impact of heat and drought stress, resulting in increased yields.

There are several cultivars of spring canola available and some are more susceptible to heat and drought than others. Triazine-tolerant cultivars are particularly sensitive to stress and would make good model systems for monitoring the alleviation of stress by triazoles. The cultivars chosen for testing were: Global, a stress-sensitive, triazine-susceptible cultivar; Stallion, a triazine-tolerant but very stress-sensitive cultivar and Kristina, a relatively stress-tolerant and triazine-susceptible cultivar.

Triazoles

A number of triazoles were tested for their ability to protect the chosen cultivars against heat and drought stress during the winter of 1992. The ability to reduce stress effects were in the order uniconazole> paclobutrazol> triadimefon. Uniconazole is unlikely to get registration; however paclobutrazol is already registered for use in the United States and Europe and has currently been submitted for registration in Canada. Therefore we have chosen to work with paclobutrazol. Its potency as a triazole is relatively high and several experimenters have found it second only to uniconazole. In general, relatively low rates of triazole application are required to inhibit shoot growth compared to other types of growth retardants. For example, the recommended rate of application for paclobutrazol is approximately one one-thousandth that of chlormequat chloride, which is one of the most widely used commercial growth retardants.

Seed Treatments

Paclobutrazol has conventionally been applied to crop plants by either soil drench or foliar spray, however seed treatments were chosen for use in this experiment for several reasons:

1. Paclobutrazol is taken up passively through plant roots. Movement within the plant is acropetal, moving in the xylem to leaves and buds. There is very little uptake through foliar application and there is no phloem mobility.
2. Lever (1986) found that paclobutrazol is relatively immobile in the soil and uptake via roots is dependent upon relative proximity of the chemical to the roots.
3. Experiments using foliar sprays required up to 1000 times more material than seed treatments.

Therefore seed treatments would appear to be the method of choice because it uses much less chemical, results in less residue on the soil surface and the chemical is directly available to the root system.

Seed Treatment Formulations

Two different types of seed treatments were tested in growth room studies during the winter of 1992:

1. External Seed Treatment (as Bonsai, the commercial formulation of paclobutrazol)

The chemical was applied as an external seed coating in a minirotostat seed treater. The upper range of application was approximately 10 mg a.i. kg^{-1} of seed without impairing emergence.

2. Slow Release Seed Treatment

The chemical was applied as an external seed coating, formulated as slow release microcapsules, in a minirotostat seed treater. The upper range of application was 10 mg a.i. kg^{-1} of seed without impairing emergence.

The Bonsai seed treatment would make the chemical available immediately after germination, while the slow release formulation would provide a slower, more controlled release of the chemical.

OBJECTIVES

1. To assess the effects of rates of paclobutrazol applications and seed treatment formulations on growth and yield of spring canola.
2. To determine cultivar responses to paclobutrazol.
3. To determine if paclobutrazol protected spring canola against environmental stress.

MATERIALS AND METHODS

In 1993 experiments were carried out at Elora and Cambridge Ontario. Both experiments involved 2 seed treatments, 3 cultivars and 4 rates of paclobutrazol arranged in a split-split plot design with four replications. In 1993 seed was coated, using equipment of Zeneca Agro, Stoney Creek, Ont., to apply 0, 1.25, 2.5, and 3.75 mg a.i. kg^{-1} of seed, either as Bonsai in inert carrier, or as a slow release seed treatment. In both experiments seeds of the cultivars Global, Kristina and Stallion were used. Seeds were planted at 5 kg ha^{-1} during the first two weeks of May. Treflan at 2.2 L ha^{-1} was used for weed control and 10 kg ha^{-1} Counter 5G was used for flea-beetle control.

The number of plants per m^2 was determined by counting the number of plants in a single row for each plot. Plant height at flowering and maturity were calculated by taking 3 measurements per plot of the representative mean height of the plants in the plot. Lodging at harvest was visually assessed using a 1-5 scale based on the severity of lodging and the proportion of the plot affected. The percentage of plants with *Sclerotinia* was measured by counting the number of infected plants in a single row from each plot and then dividing by the total number of plants in that row.

Each individual plot with an area of 1.5 x 5m was harvested for yield data. Plots were not swathed, but the plants were folded over into a windrow at normal swathing time, in order to decrease the possibility of shattering. Each individual plot was harvested with a plot combine, using a total area of 1.5 x 5 m. Plot samples were dried at 40°C, cleaned, weighed, and subsamples were retained for further analysis. Yields were adjusted to a 10% moisture basis.

Analyses

Oil content of the seed was measured using a Newport 4000 Nuclear Magnetic Resonance Spectrometer. Protein content was measured with a Technicon InfraAlyzer 450 near-infrared spectrometer calibrated against Kjeldahl standards, and using a 6.25 conversion factor to change N to protein. Both oil and protein contents were expressed on an 8.5% moisture basis. Protein contents were expressed as a percentage of the oil-free meal.

Glucosinolate contents were measured using Perkin-Elmer 8420 gas chromatographs equipped with automatic samplers, and the Nov. 30, 1990 amendment of the gas-liquid chromatography trimethylsilylether technique of the Canadian Grain Commission (method obtained from D. De Clercq).

Free fatty acids (FFA) were measured using the automated gas-liquid chromatographic method developed by May and Hume (J. Am. Oil Chem. Soc. 70:229-233).

Statistical Analysis

Analysis of variance was performed on all data using the general linear model procedure (ProcGLM) of SAS (SAS/Stat User's Guide, Software Version 6.06. SAS Institute Inc., Rary, N.C. 1989). When incremental rates of paclobutrazol were tested, the nature of the responses was determined by regression analysis.

RESULTS

Table 1. Effects of Rates and Formulations of Seed-Applied Paclobutrazol on Mean Yield and Stand of Three Spring Canola Cultivars Grown at Elora ON in 1992.

Paclobutrazol Rate	Formulation	Stand Counts	Seed Yield
mg ha^{-1}		pl m^{-2}	t ha^{-1} @ 10% H_2O
0.0	Normal (Bonsai)	32**	3.05*
12.5	"	31	3.33
25.0	"	25	2.81
50.0	"	21	2.80
0.0	Slow Release	38**	2.99*
12.5	"	34	3.29
25.0	"	37	3.20
50.0	"	21	2.56
C.V. (%)		23.8	11.1

* Significant quadratic response of rate on yield with both formulations ($P<0.05$).
** Significant linear response ($p<0.01$).

As background, the responses to paclobutrazol in 1992 are shown in Table 1. A significant interaction between rate and formulation was found and occurred because both the 12.5 and 25 mg ha^{-1} rates yielded higher than the control with the slow release formulation, but only the 12.5 mg ha^{-1} was higher than the control with the Bonsai formulation. The 12.5 mg ha^{-1} rate increased yields significantly over the controls by approximately 10% with both formulations. Stands were found to decrease significantly at the highest rate of application. All 3 cultivars responded the same way to both rates and formulations.

Table 2. Effects of Rates and Formulations of Paclobutrazol on Mean Yield and Stand of Three Spring Canola Cultivars Grown at Elora and Cambridge ON in 1993.

Paclobutrazol Rate	Formulation	Elora		Cambridge	
		Stand	Yield	Stand	Yield
mg ha^{-1}		pl m^{-2}	t ha^{-1}	pl m^{-2}	t ha^{-1}
0.00	Normal	43	2.12	61	1.96**
6.25	"	57	2.30*	49	1.95
12.50	"	42	2.30*	53	1.87
18.75	"	26	2.07	38	1.73
0.00	Slow Release	43	2.13	66**	2.16**
6.25	"	52	2.12	68	2.02
12.50	"	55	2.11	64	2.10
18.75	"	53	2.21	55	2.01
C.V. (%)		40.0	9.10	23.8	7.40

* Form x rate significant (p<0.05) at Elora. Quadratic response for normal formulation. Response for slow release formulation not significant. Values marked with * are significantly (P<0.05) higher than the untreated control.

** Rate significant (P<0.01) at Cambridge. Linear responses for both formulations.

In 1993, the rates used were half or less of the rates used in 1992. At Elora in 1993, there was again a significant formulation x rate interaction (Table 2). There was no effect of rates in the slow release formulation; however the normal Bonsai formulation showed the same quadratic response as in 1993. The 6.25 and 12.5 mg ha^{-1} rates increased yields approximately 5% over the control. There was no significant effect of rates on stands in 1993 at Elora, however a decreasing trend with increasing rates of paclobutrazol is apparent.

At Cambridge in 1993 there was a significant yield decrease at the highest rate of application with both formulations. In this experiment both seed treatment formulations showed the same linear response to rates. Stand counts were also significantly reduced at the highest rates.

Cultivars in 1993 did show a significant interaction with rates. At both locations the largest numeric yield increases from paclobutrazol seed treatments was found in the most stress susceptible cultivar, Stallion. These responses are discussed in more detail later.

Table 3. Effects of Rates of Paclobutrazol on Mean Agronomic Traits of Three Spring Canola Cultivars Grown at Elora ON in 1992. Values are averaged over two paclobutrazol formulations.

Rate	Days to F.F.[z]	Height at F.F.	Days to Mat.[y]	Height at Mat.	Lodg. (1-5)[x]	Plants with Sclerotinia
mg ha^{-1}		cm		cm		%
0.0	56	112	119	157	1.6	8.5
12.5	56	106	120	155	1.9	7.6
25.0	56	104	121	157	2.0	7.1
50.0	59	101	126	156	2.8	5.4
	**	**	**	n.s.	**	n.s.

[z] F.F. = full flower
[y] Mat. = maturity
[x] Lodg. = lodging at swathing measured on a scale of 1 = erect to 5 = flat
**, n.s. Significant at $p<0.01$ and not significant ($p>0.05$).

Again, as background, the agronomic responses to paclobutrazol in 1992 are shown in Table 3. There was no formulation by rate interaction, so values are shown averaged over the slow-release and normal (Bonsai) formulation. At Elora in 1992 the number of days to full flower was significantly increased at the highest rate of application. Height at full flower decreased significantly in a linear fashion. All rates were statistically different from each other. The number of days to maturity significantly increased in a linear fashion with the highest rate taking the longest to mature. There was no significant effect of rate on the height at maturity. Lodging was significantly increased in a linear fashion from least lodging at 0 to the most lodging at 50 mg ha^{-1}. However, it is assumed that this is more likely a stand effect than a chemical effect due to very poor stands in 1992. There was no significant difference in the percentage of plants infected with *Sclerotinia* although there is an apparent trend toward lower infection with higher paclobutrazol rates.

In addition, chlorophyll fluorescence measurements were taken to assess the effects of paclobutrazol on photosynthetic stress protection but no significant results were observed. The 1000 seed weights were not significantly affected and the delay in maturity in 1992 did not affect chlorophyll content in the seed (data not shown).

At Elora in 1993 the number of days to full flower was significantly increased at the highest rate; however there was no effect of rates on height at full flower. The number of days to maturity was significantly increased at the highest rate, but again there was no effect on height at maturity. Lodging was significantly decreased by all rates in comparison to the control. The percentage of plants infected with *Sclerotinia* was not significantly affected by rates of paclobutrazol applied.

Table 4. Effects of Rates of Paclobutrazol on Mean Agronomic Traits of Three Spring Canola Cultivars Grown at Elora ON in 1993. Values are averaged over two paclobutrazol formulations.

Rate	Days to F.F.[z]	Height at F.F.	Days to Mat.[y]	Height at Mat.	Lodg.[x] (1-5)[t]	Plants with Sclerotinia
mg ha^{-1}		cm		cm		%
0.0	42	106	93	128	3.0	18.7
6.25	42	108	93	129	2.6	16.0
12.5	42	107	93	130	2.4	15.1
18.75	43	106	94	129	2.7	17.7
	**	n.s.	*	n.s.	**	n.s.

[t] Lodging measured on a scale from 1 (erect) to 5 (flat).
*, **, n.s. Significant at $p<0.05$, significant at $p<0.01$ and not significant
[z] F.F. = full flower
[y] Mat. = maturity
[x] Lodg. = lodging at swathing measured on a scale of 1 = erect to 5 = flat

Chlorophyll fluorescence measurements at Elora in 1993 showed no significant effect of the chemical on photosynthetic stress protection. The 1000-seed weights were not significantly affected and the slight delay in maturity in 1992 did not affect chlorophyll content in the seed (data not shown).

At Cambridge in 1993 the number of days to full flower was significantly increased in a linear fashion with the highest rate taking the longest to reach full flower. The height at full flower was not significantly affected. The number of days to maturity was again significantly increased in a linear fashion with the highest rate taking the longest to reach maturity. Height at maturity was not significantly affected. Lodging was not a problem at Cambridge in 1993 and as such it was not significantly affected by rates of chemical applied. The percentage of plants infected with *Sclerotinia* was not significantly affected, although a decreasing trend of infection with increasing rates of application is apparent.

Table 5. Effects of Rates of Paclobutrazol on Mean Agronomic Traits of Three Spring Canola Cultivars Grown at Cambridge ON in 1993. Values are averaged over two paclobutrazol formulations.

Rate	Days to F.F.[z]	Height at F.F.	Days to Mat.[y]	Height at Mat.	Lodg.[x] (1-5)[t]	Plants with Sclerotinia
mg ha^{-1}		cm		cm		%
0.0	58	92	99	129	1.5	2.4
6.25	58	92	99	129	1.4	2.2
12.5	59	91	99	130	1.3	2.2
18.75	60	92	101	131	1.5	1.9
	**	n.s.	**	n.s.	n.s.	n.s.

[t] Lodging measured on a scale from 1 (erect) to 5 (flat).
*, **, n.s. Significant at $p<0.05$, sig. at $p<0.01$ and not significant
[z] F.F. = full flower
[y] Mat. = maturity
[x] Lodg. = lodging at swathing measured on a scale of 1 = erect to 5 = flat

Chlorophyll fluorescence measurements at Cambridge again showed no significant effect of the chemical on photosynthetic efficiency. The 1000-seed weights were significantly increased at the highest rate of application; however this was assumed to be the result of poor stands compensating with increased seed size and not a chemical effect. The slight delay in maturity did not affect chlorophyll content in the seed (data not shown).

Quality Analysis

Since we last reported, the samples from the 1992 trials have undergone laboratory analyses and we have just completed the 1993 sample analyses. The results for 1992 samples at Elora are shown in Table 6, averaged over the three cultivars, Global, Kristina and Stallion. High rates of paclobutrazol (note these are higher rates than in 1993) decreased stands and increased FFA with high rates of the Bonsai formulation. This response may have occurred simply because low stand counts normally result in increased FFA levels in Ontario. High FFA occurs in prematurely aborted seed and premature abortion occurs more on branches than on main racemes. Plants grown in thin stands have more branches and therefore may be susceptible to high FFA levels. The year 1992 was cool and moist, with little stress, and these are normal, acceptable FFA levels. High rates of paclobutrazol decreased seed oil content significantly. Seed protein and meal glucosinolate contents were unaffected.

Table 7 shows the responses at Elora in 1992 of the triazine-tolerant variety Stallion. We investigated Stallion in detail because it grows under more stress than the other varieties. In the triazine-tolerant varieties the flow of electrons through photosystem II (the first stages of the light reactions in photosynthesis) is impaired. The net result is that oxidizing free radicals get produced which can overwhelm the plant's protective free-radical scavenging systems. Under cool, moist, non-stress conditions, triazine-tolerant varieties yield at near-normal levels. However, under hot, dry stress conditions they are low in both yield and seed oil content because there is no reserve capacity of free radical scavenging systems to cope with the free radicals produced as a result of the weather stress. Therefore, the variety Stallion, which is consistently producing its own stress, is considered a useful indicator of what likely would happen to normal varieties under heat and drought stress.

Table 6. The Effects of Rates and Formulation of Seed Applied Paclobutrazol on Stands, Free Fatty Acids, Oils, Proteins and Glucosinolates of Three Spring Canola Cultivars Grown at Elora ON 1992.

Pac. Rate mg ha^{-1}	Seed Tmt. Formulation	Stand pl m^{-2}	FFA (% of oil)	Oil (% @ 8.5% H_2O	Protein (% on an oil-free basis @ 8.5% H_2O)	Glucs. (µM @ 8.5% H_2O)
0	Bonsai	32**	0.23*	41.2*	38.0 n.s.	6.30 n.s.
12.5	"	31	0.21	40.9	38.0	6.90
25.0	"	25	0.24	40.2	37.9	6.41
50.0	"	21	0.28	39.0	37.4	6.71
0	Slow Release	38**	0.25 n.s.	40.9 n.s.	38.3 n.s.	5.63 n.s.
12.5	"	34	0.25	40.8	38.0	6.12
25.0	"	37	0.23	41.1	38.2	5.68
50.0	"	21	0.31	39.9	38.6	6.77
C.V. (%)		23.8	31.4	5.3	3.7	24.4

*, ** Significant linear response at P<0.05 and P<0.01 respectively
n.s. Result are not significant
Cultivars grown were Global, Kristina and Stallion.

In 1992 at Elora, Stallion yields increased by 7% over the controls with Bonsai (which was about the same as for Global and Kristina, see Table 1) and by 13% with the slow-release formulation. Stands decreased similarly to the other cultivars. Free fatty acid levels were slightly higher in Stallion than the average for the three cultivars (cf. Table 6). Triazine-tolerant varieties usually have higher FFA levels than normal varieties. The increase in FFA with paclobutrazol rates were similar, however, among all three cultivars. Oil content in Stallion did not drop, compared to the controls, for either the slow-release or Bonsai formulation of paclobutrazol, suggesting some protection against stress. Protein content in the meal increased with higher Bonsai rates. Glucosinolate levels decreased at intermediate rates with Bonsai but increased with the slow-release formulation. We interpret these results to indicate no real effect. Overall, low rates of paclobutrazol appeared to increase yields in this stress-susceptible variety without deleterious effects on quality.

Table 7. The Effects of Rates and Formulations of Seed Applied Paclobutrazol on Yield, Stands, Free Fatty Acids, Oils, Proteins and Glucosinolates of Stallion Spring Canola Grown at Elora ON 1992.

Pac. Rate mg ha^{-1}	Seed Tmt. Formulation	Yield t ha^{-1} @ 10% H_2O	Stand pl m^{-2}	FFA (% of oil)	Oil (% @ 8.5% H_2O)	Protein (% on an oil-free basis @ 8.5% H_2O)	Glucs. (µM @ 8.5% H_2O)
0	Bonsai	2.90*	32***	0.25 n.s.	39.9 n.s.	37.6***	4.43**
12.5	"	3.10	26	0.26	39.5	38.3	3.99
25.0	"	2.55	30	0.30	39.0	38.4	3.90
50.0	"	2.60	24	0.34	39.9	39.0	4.90
0	Slow Release	2.70*	38***	0.29 n.s.	38.9 n.s.	38.2 n.s.	3.17 *
12.5	"	2.75	37	0.30	39.8	39.2	3.79
25.0	"	3.05	39	0.23	39.3	37.8	3.98
50.0	"	2.35	25	0.35	38.8	38.7	3.62
C.V. (%)		10.4	21.3	33.7	2.2	1.6	11.3

*, ** Significant quadratic response at $p<0.05$ and $p<0.01$ respectively
*** Significant linear response at $p<0.05$
n.s. Results are not significant

Table 8 shows the response of quality parameters to the two formulations of paclobutrazol in 1993. The values are averaged across the varieties Global, Kristina and

Stallion, grown at the Elora Research Station. The Elora site is a silt loam with very high water-holding capacity, high yield potential, and low heat units. Low rates (6.25 and 12.5 mg ha^{-1}) of Bonsai applied as seed treatments at this site did not reduce stands, decreased FFA's, increased oils, had no effect on protein, but increased glucosinolates. At the same low rates of the slow-release formulation, there were essentially no differences from the controls.

Table 8. The Effects of Rates and Formulations of Seed Applied Paclobutrazol on Stands, Free Fatty Acids, Oils, Proteins and Glucosinolates of Three Spring Canola Cultivars Grown at Elora ON 1993.

Pac. Rate mg ha^{-1}	Seed Tmt. Formulation	Stand pl m^{-2}	FFA (% of oil)	Oil (% @ 8.5% H_2O)	Protein (% on oil-free basis @ 8.5% H_2O)	Glucs. (µM @ 8.5% H_2O)
0	Bonsai	43*	0.78*	44.8*	37.6 n.s.	10.78**
6.25	"	57	0.55	45.5	38.2	13.68
12.50	"	42	0.63	45.7	38.2	12.20
18.75	"	26	0.65	45.4	37.9	14.39
0	Slow Release	43 n.s.	0.80*	44.9 n.s.	37.9 n.s.	10.73 n.s.
6.25	"	52	0.82	44.9	38.2	12.03
12.50	"	55	0.87	45.0	38.0	11.14
18.75	"	53	0.70	45.0	38.0	11.75
C.V. (%)		40.0	16.4	1.2	2.1	19.4

* Significant quadratic response at $p<0.05$
** Significant linear response at $p<0.01$
n.s. Results are not significant
Cultivars grown were Global, Kristina and Stallion.

In Table 9, however, it is clear that most of the changes with Bonsai treatment at Elora occurred because of its effect on the Stallion variety. The lowest rate of Bonsai increased yields by 24%. Some of this increase may have been due to the increased stand density, with the 6.25 mg ha^{-1} Bonsai formulation. Stallion canola treated with 12.5 mg ha^{-1} of Bonsai formulation still outyielded the untreated control by 17%, however, free fatty acids dropped significantly with Bonsai treatment and oil content increased to levels similar to those for Global and Kristina. Protein levels were unaffected. Glucosinolates increased substantially in Stallion (Table 9) but not in the other cultivars (compare Table 9 results with those in Table 8). Most of the trends which occurred with the Bonsai treatment also appeared to be occurring with the slow-release formulation, but higher rates of the slow-release paclobutrazol were required to elicit a response.

Table 9. The Effects of Rates and Formulations of Seed Applied Paclobutrazol on Yield, Stands, Free Fatty Acids, Oils, Proteins and Glucosinolates of Stallion Spring Canola Grown at Elora ON 1993.

Pac. Rate mg ha^{-1}	Seed Tmt. Formulation	Yield t ha^{-1} @ 10% H_2O	Stand pl m^{-2}	FFA (% of oil)	Oil (% @ 8.5% H_2O)	Protein (% on an oil-free basis @ 8.5% H_2O)	Glucs. (µM @ 8.5% H_2O)
0	Bonsai	1.86**	38*	1.14**	42.7**	38.2 n.s.	5.68*
6.25	"	2.30	68	0.46	45.6	38.7	12.33
12.5	"	2.18	39	0.50	45.8	38.4	12.30
18.75	"	2.08	34	0.58	45.6	38.8	13.76
0	Slow Release	1.81 n.s.	48 n.s.	1.20 n.s.	42.8 n.s.	38.2 n.s.	5.48***
6.25	"	1.94	46	1.11	42.9	38.7	5.29
12.50	"	1.78	64	1.28	43.1	38.4	5.79
18.75	"	2.21	50	0.98	43.8	38.4	8.40
C.V. (%)		9.9	33.5	14.9	1.6	2.0	20.9

*,** Significant quadratic response at $p<0.05$ and $p<0.01$ respectively
*** Significant linear response at $p<0.05$
n.s. Results are not significant

The same experiment was conducted at the Cambridge Research Station in 1993. This is a light, sandy loam vegetable research station and was chosen because canola here was more likely to suffer moisture stress than was canola at the Elora site. In fact, at Cambridge there was relatively little moisture stress. Values shown in Table 10 are averaged across the three varieties, Global, Kristina and Stallion. Stand counts at Cambridge (Table 10) were decreased linearly with higher rates of paclobutrazol, with a greater effect from the Bonsai than from the slow-release formulation. Free fatty acid levels were quite high but unchanged by paclobutrazol. Oil contents were unchanged and protein decreased at low levels of Bonsai, but recovered at higher levels. Protein appeared to continue to increase with higher rates of the slow-release formulation. Glucosinolates increased slightly with higher Bonsai rates, but not with increasing rates of the slow-release formulation.

The same parameters measured on just the variety Stallion at Cambridge are shown in Table 11. Unlike at Elora, there were essentially no effects of either paclobutrazol treatment at any rate tested, with the exceptions that yields were increased slightly at the lowest rate of the Bonsai treatment and that glucosinolates were increased by the highest rates of the Bonsai treatment.

Table 10. The Effects of Rates and Formulations of Seed Applied Paclobutrazol on Stands, Free Fatty Acids, Oils, Proteins and Glucosinolates of Three Spring Canola Cultivars Grown at Cambridge, ON 1993

Pac. Rate mg ha^{-1}	Seed Tmt. Formulation	Stand pl m^{-2}	FFA (% of oil)	Oil (% @ 8.5% H_2O)	Protein (% on an oil-free basis @ 8.5% H_2O)	Glucs. (µM @ 8.5% H_2O)
0	Bonsai	61**	1.03 n.s.	41.8 n.s.	42.2***	3.65**
6.25	"	49	1.07	41.4	41.2	4.33
12.50	"	53	0.94	42.0	41.6	3.66
18.75	"	38	1.04	41.5	42.0	5.37
0	Slow Release	66**	1.11 n.s.	41.7 n.s.	41.4***	3.13 n.s.
6.25	"	68	1.08	41.7	41.6	3.22
12.50	"	64	0.98	41.8	41.9	3.40
18.75	"	55	1.04	42.1	42.3	3.66
C.V. (%)		23.8	16.1	1.6	2.0	19.2

** Significant linear response at p<0.01
*** Significant quadratic response at p<0.05
n.s. Results are not significant
Cultivars grown were Global, Kristina and Stallion.

The distinct differences in the response of the triazine-tolerant Stallion canola at Elora and Cambridge are difficult to understand. We conducted the laboratory analyses on many of the Elora samples a second time and confirmed the initial results.

Table 11. The Effects of Rates and Formulations of Seed Applied Paclobutrazol on Yield, Stands, Free Fatty Acids, Oils, Proteins and Glucosinolates of Stallion Spring Canola Grown at Cambridge On 1993.

Pac. Rate mg ha^{-1}	Seed Tmt. Formulation	Yield t ha^{-1} @ 10% H_2O	Stand pl m^{-2}	FFA (% of oil)	Oil (% @ 8.5% H_2O)	Protein (% on a dry basis @ 8.5% H_2O)	Glucs. (µM @ 8.5% H_2O)
0	Bonsai	1.55 n.s.	46 n.s.	1.12 n.s.	40.5 n.s.	42.4 n.s.	2.62 n.s.
6.25	"	1.68	68	1.18	40.0	41.9	2.91
12.50	"	1.47	54	1.17	40.5	42.4	2.68
18.75	:	1.67	48	1.44	40.4	42.8	2.72
0	Slow Release	1.94 n.s.	64 n.s.	1.34 n.s.	40.4 n.s.	41.8 n.s.	2.18 n.s.
6.25	"	4.96	54	1.14	40.4	42.2	2.42
12.50	"	1.93	50	0.97	40.7	42.3	2.56
18.75	"	1.69	42	1.16	41.3	43.5	2.25
C.V. (%)		9.2	27.8	18.7	2.1	2.1	

n.s. Results are not significant.

At Elora, the results with the variety Stallion in 1993 are consistent with the hypothesis of Kraus and Fletcher (1994) that the chloroplast is the primary site of damage caused by oxygen free radicals and that paclobutrazol can cause protection from damage by these free radicals. Such protection would be expected to increase yields and oil contents and decrease free fatty acids more significantly in a triazine-tolerant variety like Stallion than in varieties like Global and Kristina, which have normal, unimpaired photosynthetic systems.

These results suggest that low rates of paclobutrazol seed treatment can protect against stress. In 1992 and 1993 there has been very little stress on canola in Ontario. We have initiated a series of trials for 1994, as described in the proposal for 1994. These trials include one being conducted by Dr. Hans Henning-Muendel near Lethbridge, AB. It is anticipated that this site will experience more stress and give a better understanding of how normal cultivars react to paclobutrazol seed treatment when grown under environmental stress. One of the useful lessons learned in the 1993 experiments was that useful responses to normal Bonsai formulations of paclobutrazol occurred at rates low enough so that stand establishment was not impaired. From these results there appears to be no need to use slow-release formulations of paclobutrazol.

CONCLUSIONS

1. Paclobutrazol seed treatment has increased yields in 3 of the 6 comparisons conducted over the past 2 years. Results are more consistent with the stress-susceptible, triazine-tolerant variety Stallion than with triazine-susceptible cultivars.
2. Paclobutrazol is a potent plant growth regulator. In 1993, most yield increases occurred with rates of active ingredient between 6.25 and 12.5 mg ha^{-1}.
3. Seed treatment appears to be an effective way of delivering paclobutrazol to the crop. Slow release formulations of paclobutrazol appear unnecessary.
4. At Elora in 1993, paclobutrazol seed treatment on Stallion canola increased yields and oil content, but also increased glucosinolate contents.
5. Both 1992 and 1993 were low-stress years in Ontario. In 1994, a trial at Lethbridge is being included to determine paclobutrazol effects under Alberta conditions.

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