JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2008. Vol. **2**(1), p. 23-28

Influence of Temperature on Maize Tolerance to Alachlor, Metazachlor and Metolachlor

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(Received: 26 January 2008; accepted: 07 March 2008)

Temperature affects the performances of herbicides in general. A study was conducted to determine the influence of temperature on the tolerance of maize genotypes to alachlor, metazachlor and metolachlor. Experiments were conducted in growth chambers at the University of Pretoria experimental farm. Results showed significant influence of temperature on maize tolerance to all the herbicides. Low temperatures significantly reduced the growth of maize plants. Metazachlor performance was most influenced by temperature, indicating that temperature influence on maize tolerance to herbicides is herbicide-dependent. This necessitates the need to screen maize genotypes for tolerance to herbicides under varying temperatures. Genotypes that are tolerant to commonly used herbicides under varying temperatures could be recommended for areas where temperatures fluctuate a lot.

Key words: Maize genotypes, maize herbicides tolerance, visual injury, shoot dry mass.

Alachlor (2-chloro-*N*-(2,6-diethyl phenyl)-*N*-(methoxymethyl)acetamide), metazachlor (2-chloro-*N*-(pyrazol-1-ylmethyl)acet-2'-6'-xylidide) and metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acet-amide) are chloroacetamide herbicides that were developed primarily for selective control of mostly germinating grass and some broadleaf weeds in maize and some other crops (Tomlin, 1994). Metazachlor was, however, deregistered for use in maize in South Africa during 1999, at about the time of concluding this experiment, as a result of inadequate selectivity. Although these herbicides are considered safe for use in maize, some maize genotypes have been reported to be sensitive to these herbicides (Ashley, 1972; Francis and Hamill, 1980; Rowe, *et al.,* 1990). Similarly, Kanyomeka and Reinhardt (2000) reported that some maize genotypes showed outstanding tolerance to these herbicides while some were very sensitive. Differential tolerance of crop cultivars, including maize, to herbicides has been documented (Malan *et al.,* 1984; O'Sullivan *et al.,* 1998; Van Wychen, *et al.,* 1999; Wilson, 1999; O'Sullivan *et al.,* 2001).

Crop tolerance to herbicides is influenced by genetic composition, type of herbicide and environmental factors (Klingman and Ashton, 1982; Akobundu, 1987). These latter factors in particular may increase the activity or uptake of a herbicide and reduce the normal crop tolerance,

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thereby causing crop injury. Environmental factors that can affect the efficacy of herbicides are relative humidity, temperature, light intensity, rainfall and soil conditions. The efficacy of chloroacetamides is known to be influenced by temperature and soil moisture content. Temperature is an important environmental factor that influences plant growth and development by influencing respiration, transpiration, nutrient and water uptake, and other biochemical processes (Didwell, 1974; Eastin, *et al.,* 1982).

Temperature changes have been reported to affect herbicide selectivity. Tolerance of maize and peas to alachlor decreased with increasing soil moisture and decreasing soil temperatures (Putnam and Rice, 1979). Reinhardt and Nel (1982) reported that alachlor is more active at lower temperature regimes of 20/10°C and at higher ones of 20/30°C (day/night) than at 25/15°C. Skipper et al. (1977) showed that alachlor and metolachlor are more active at 22/13°C than at high temperature regimes of 27/18°C and 32/24°C. Metolachlor activity was reported to be greater at high temperature of 29°C than at low temperature of 18°C (Gerber *et al.,* 1974). Van Biljon (1991) reported that metolachlor was most injurious to maize at temperature regimes of 30/20°C day/night. Gauvrit and Gaillardon (1991) found that 2,4-D selectivity towards maize is drastically reduced under cold conditions. Low temperature increases spray retention per unit dry matter and retards 2, 4-D degradation. Similarly, McMullan (1994) reported that compared to diclofop-methyl and fenoxafop-p-ethyl, mixtures cause more injury to barley at lower temperatures. This shows that effect of temperature on crop tolerance varies from herbicide to herbicide.

Under normal temperatures maize is able to metabolise the absorbed alachlor, metazachlor and metolachlor. Cool and hot environments probably cause changes in the plant physiology that could contribute to crop injury due to reduced rates of inactivation of these herbicides. Generally, most crop injury from chloroacetamide herbicides occurs on sandy soils with low organic matter, i.e., on soil with low adsorptive capacity for the compounds. Deep planting, cool wet conditions and soil crusting may also increase the potential for crop injury. These factors extend the time the emerging maize seedling is in contact with the soil-herbicide solution, and the plant is likely to ultimately take up more herbicide than expected. In addition, under those conditions, the plant may not be able to detoxify the absorbed herbicide effectively (Didwell, 1974; Akobundu, 1987; Butzen, 2000).

Many reports of maize injury from these herbicides have been linked to temperature changes, other than those mentioned above. Cool temperatures during or soon after herbicide application are more associated with this kind of injury (Ashton and Crafts, 1981; Akobundu, 1987; Butzen, 2000). It is therefore important to determine how temperature affects the tolerance of genotypes. The objective of this study was to determine the influence of temperature on the tolerance of maize genotypes to alachlor, metazachlor and metolachlor.

MATERIAL AND METHODS

Three separate experiments were conducted in growth chambers to determine the influence of temperature on the tolerance of maize genotypes to alachlor, metazachlor and metolachlor. The application rates for the formulated products Lasso (alachlor), Preecede (metazachlor), and Dual S GOLD (metolachlor) were 4.0, 1.5 and 0.9 L ha^{-1}, respectively. Herbicides were applied immediately after planting, since they are registered as pre-emergence herbicides in maize.

Maize plants were grown in pots containing 2.5 kg of soil for five weeks. Four maize inbreds were used in the experiments involving metolachlor and alachlor, while two inbreds and two hybrids were used in the metazachlor experiment. These genotypes included tolerant and susceptible types, which were selected based on results from earlier greenhouse and field experiments reported by Kanyomeka and Reinhardt (2000).

The experiments were conducted in growth chambers at the University of Pretoria phytotron. The night/day temperature regimes were 10/18°C, 15/25°C, 20/35°C and 10/30°C. The first experiment involving metazachlor did not include the 10/30°C temperature regime. The photoperiod was maintained at 12 hours for the day regime.

Pots were filled with 2.5 kg of soil, which was sieved through a 4 mm screen prior to filling. The soil consisted of 75.5 % sand, 8.2 % silt, 17.1 % clay with 0.4 % organic C, and had a pH (H_2O) of 6.5. The soil used was collected from the University of Pretoria experimental farm. The pots were fitted with plastic bags before filling them with soil in order to avoid herbicide leaching.

Five seeds of each genotype were planted in each pot at a depth of 30 mm. Three days after emergence; seedlings were thinned to three per pot. Watering was done to ensure that moisture was maintained at field capacity level. Pots were first weighed soon after planting and after the initial watering, and subsequently once per week to avoid under- or over-watering. After emergence, either a complete nutrient solution (Nitsch, 1972) or tap water was applied on alternate days. The same volume of nutrient solution (100 ml) was always applied to each pot, but the volume of tap water was dependent on the amount of water lost.

Three days after plant emergence, the rate of emergence was determined by taking plant counts. Visual injury rating (VIR) was done according to a 1-10 rating scale, where 1 indicates no effect and 10 indicates complete kill. Shoot dry mass (SDM) was measured five weeks after planting by cutting the plants at soil surface level and oven-drying them at 65°C for 48 hours. Data for shoot dry mass were expressed as percentage reduction from the untreated control for respective genotypes.

Data were subjected to analysis of variance. Treatment means were compared using Turkey's Least Significant Difference (LSD) test at the 5 % level of significance.

RESULTS AND DISCUSSION

Data on the influence of temperature on the tolerance of maize genotypes to alachlor, metazachlor and metolachlor are presented in Tables 1, 2, 3, 4, 5, and 6. There was no effect of herbicides on the rate of emergence (data not shown).

The study demonstrated that temperature has an influence on maize tolerance to alachlor, metazachlor and metolachlor. Both susceptible

Table 1. Percentage shoot dry mass reduction, from the untreated control of maize genotypes treated with metazachlor

Genotype	Temperature regimes (night/day)			
	10/18°C	15/25°C	20/35°C	Mean
CV5 (susceptible)	35.9	22.6	31.4	30.0
CV1 (tolerant)	22.9	0	14.1	12.3
P1 (susceptible)	46.0	25.9	40.1	37.3
P2 (tolerant)	33.5	0	13.1	15.5
Mean	34.6	12.1	24.6	

$LSD_{T(0.05)}$ Genotype x Temperature regimes = 16.3 SE = 7.2

Table 2. Visual injury rating of maize genotypes treated with metazachlor

Genotype	Temperature regimes (night/day)			
	10/18°C	15/25°C	20/35°C	Mean
CV5 (susceptible)	7	2	5	4
CV1 (tolerant)	4	1	2	2
P1 (susceptible)	6	5	6	6
P2 (tolerant)	7	2	3	3
Mean	8	2	4	

Scale used: 1-10, indicating: 1=no effect, 2-3=slight effect, 4-5=medium effect, 6-7=severe effect, 8-9=very severe effect, 10=plants dead.

and tolerant maize genotypes shoot dry mass was significantly reduced from the untreated control by metazachlor at 10/18°C (night/day). However, at 15/25°C and 20/35°C temperature regimes, only SDM for susceptible genotypes, cultivars CV5 and P1, were significantly reduced (Table 1). Similarly, all genotypes treated with metazachlor showed visual injury symptoms ranging from severe to very severe at the low temperature regime of 10/18°C. At higher temperature regimes of 15/25°C and 20/35°C severe effects of metazachlor were only observed on susceptible genotypes (Table 2).

The tolerance of maize to alachlor was reduced at the low temperature regime of 10/18°C. At this temperature regime the SDM, for both susceptible and tolerant genotypes, was significantly reduced from the untreated control (Table 3). The tolerance of maize to alachlor was not affected significantly at both the 15/25°C and 20/35°C temperature regimes. Even at relatively low and high temperatures of 10°C (night) and 30°C (day) the tolerance of maize to alachlor was not affected. Visual injury symptoms caused by alachlor were only observed at the low temperature regime of 10/18°C for both tolerant and susceptible genotypes (Table 4).

Similarly, the tolerance of maize genotypes to metolachlor was only affected at low temperature conditions of 10/18°C (Table 5). The SDM, for both susceptible and tolerant genotypes, was significantly reduced from the

Table 3. Percentage shoot dry mass reduction, from the untreated control, of maize genotypes treated with alachlor

Genotype	Temperature regimes (night/day)				
	10/18°C	15/25°C	20/35°C	10/30°C	Mean
P2 (tolerant)	30	0	10	6	8
P3 (susceptible)	38	12	15	11	19
Mean	34	6	13	8	

LSD $_{(T=0.05)}$: Genotypes = 11, Temperature regimes = 20 SE =7.2

Table 4. Visual injury rating of alachlor effect on maize

Genotype	Temperature regimes (night/day)				Mean
	10/18°C	15/25°C	20/35°C	10/30°C	
P2 (tolerant)	3	1	1	1	2
P3 (susceptible)	3	1	1	1	2
Mean	3	1	1	1	

Scale used: 1-10, indicating: 1=no effect, 2-3=slight effect, 4-5=medium effect, 6-7=severe effect, 8-9=very severe effect, 10=plants dead.

Table 5. Percentage shoot dry mass reduction, from the untreated control, of maize genotypes treated with metolachlor

Genotype	Temperature regimes (night/day)				Mean
	10/18°C	15/25°C	20/35°C	10/30°C	
P2 (tolerant)	22	0	0	7	7.2
P31 (susceptible)	24	19	13	13	17.2
Mean	23	9.5	6.5	10	

LSD $_{(T=0.05)}$ Genotype x Temperature regimes = 14 SE = 6 untreated control at the low temperature regime. Only at this regime was visual injury caused by metolachlor observed for both tolerant and susceptible genotypes (Table 6).

The observed influence of temperature on the efficacy of herbicides is in accordance with the findings of Putnam and Rice (1979) who reported that snap bean tolerance to alachlor was reduced at low temperatures and low rainfall. Similarly, McWillian (1967), and Hodgins and Van Huystee (1976) found low maize tolerance to 2,4-D at low temperatures of less than 18°C. Mulder and Nalewaja (1978) reported that atrazine and alachlor toxicity to oats increased with decreasing temperatures. Nel and Reinhardt (1984) reported that both low and high temperatures influence the toxicity of atrazine to maize.

Table 6. Visual injury rating for metolachlor effect on maize

Genotype	Temperature regimes (night/day)				Mean
	10/18°C	15/25°C	20/35°C	10/30°C	
P2 (tolerant)	3	1	1	1	2
P31 (susceptible)	3	1	1	1	2
Mean	3	1	1	1	

Scale used: 1-10, indicating: 1=no effect, 2-3=slight effect, 4-5=medium effect, 6-7=severe effect, 8-9=very severe effect, 10=plants dead.

The effect of temperature on maize genotype tolerance to selected chloroacetamide herbicides was found to be significant. Generally, low temperatures reduced the tolerance of maize to these herbicides, to such an extent in the case of metazachlor that even genotypes that were highly tolerant at normal maize growing temperature regimes were susceptible at lower regimes. Cool temperatures tend to reduce the plant metabolic processes, thereby impeding the process of detoxifying herbicides to safe metabolites (Thompson *et al.,* 1970; Didwell, 1974; Gerber*, et al.,* 1974; Narsaiah and Harvey, 1977; Putnam and Rice, 1979; Ritter and Coble, 1981; Klingman and Ashton, 1982; Blair *et al.,* 1983; Pillmoor, 1985; Akobundu, 1987; Polge and Barrett, 1997; Harrison and Peterson, 1999. Butzen, 2000). Rate of plant metabolism may be slowed down when conditions are cool, and when wet conditions coincide with this, as is often the case early in the summer growing season, plants may not adequately inactivate such high amount of herbicide. Plant metabolic processes are probably not seriously affected at intermediate and high temperature regimes, and therefore, tolerant genotypes were able to detoxify the herbicides at this temperature.

Among the herbicides tested, maize tolerance to metazachlor was more dependent on temperature than the tolerance towards metolachlor or alachlor. The results suggest that in cases where tolerance to certain herbicides are known to be temperature-dependent, maize genotypes should preferably be screened under varying temperatures in an controlled environment. The requirement of specialized research facilities, which this approach necessitates, could be circumvented by using excessive herbicide rates (>1-X amounts) to simulate the role of environmental factors, which promote the accumulation of phytotoxic amounts of herbicide at the site of action in the plant system.

REFERENCES

1. Akobundu, I.O., Weed Science in the tropics: Principles and practices. John Wiley & Sons, New York 1987.
2. Ashley, R.A., Varietal response of sweet maize to alachlor and alachlor combinations. *Proc. Northeast Weed Control Conf.* 1972; **26**: 22-26.
3. Ashton, F.M. and Crafts, A.S., Mode of action of herbicides. John Wiley & Sons, New York 1981.
4. Blair, A.M. Richardson, W.G. and West, T.W. The influence of climatic factors on metoxuron activity on *Bromus sterilis* L. *Weed Sci.* 1983; **23**: 259-265.

5. Butzen, S., Crop management and agronomics-weed management and herbicide resistance: Herbicide injury to maize. Crop insights. Pioneer HI_BRED International Inc. Iowa 2000.
6. Didwell, R.G.S., *Plant physiology*. Macmillan Publishing Co., New York 1974.
7. Eastin, J.D., Haskins, F. A. Sullivan, C.Y. and Van Bavel, C.H.M., Physiological aspects of crop yield. American Society of Agronomy, Madison, 1982.
8. Francis, T.R. and Hamill, A.S., Inheritance of maize seedling tolerance to alachlor. *Canadian J. Plant Sci.* 1980; **60**: 1045-1047.
9. Gauvrit, C. and Gaillardon, P.M., Effect of low temperatures on 2,4-D behaviour in maize plants. *Weed Res.* 1991; **31**: 135-142.
10. Gerber, H.R., Moller, G., and Ebner, L., CGA 24705, a new grass killer herbicide. *Proc. 12th. Br. Weed Control Conf.* 1974; **3**: 787-794.
11. Harrison, H.F. and Peterson, J.K., Effect of temperature and cultivar on the response of Brocoli and Collard (*Brassica oleracea*) to oxyfluorfen. *Weed Technol.* 1999; **13**: 726-730.
12. Hodgins, R. and Van Huystee, R.B., Porphyrin metabolism in chill stressed maize. *J. Plant Physiology* 1986; **125**: 325-336.
13. Kanyomeka, L. and Reinhardt, C.F., Maize genotypes show distinctive growth responses to various herbicides. *J. New Seeds* 2000; **2**: 13-28.
14. Klingman, G.C. and Ashton, F.M., Weed Science: Principles and Practices. John Wiley & Sons, New York 1982.
15. Malan, C., Visser, J.H. and Van De Venter, H.A., Screening for DIMBOA (benzoxazinone) concentration among South African inbred maize lines and sorghum cultivars. *S. Afr. J. Plant Soil.* 1984; **1**: 99-102 .
16. McMullan, P.M., The influence of temperature on barley tolerance to diclofop-methyl or fenoxaprop-p-ethyl mixtures. *Weed Res.* 1994; **43**: 23-28.
17. McWilliam, J.R., and Naylor, A.W., Temperature and plant adaptations. I. Interaction of temperature and light in the synthesis of chlorophyll in maize. *J. Plant Physiology* 1967; **42**: 1711-1715.
18. Mulder, C.E.G. and Nalewaja, J.D., Temperature effect on phytotoxicity of Soil applied herbicides. *Weed Sci.* 1978; **26**: 566-570.
19. Narsalah, B.D. and Harvey, R.G., Alachlor placement in soil as related to phytotoxicity to maize seedlings. Weed Res. 1977A; **17**: 163-168.
20. Nel, P.C. and Reinhardt, C.F., Factors affecting the activity of atrazine in Plants and soil. *S. Afr. J. Plant Soil* 1984; **1**: 67-72.
21. Nitsch, J.P., Phytotrons: Past achievements and future needs: In: R. Rees, K. E. Cockshull, D.W. Hand & D.G. Hurd (eds). Crop processes in controlled environments. Academic Press, London 1972.
22. O' Sullivan, J., Thomas, R.J. and Bouw, W.J., Tolerance of sweet maize (*Zea mays* L.) cultivars to Rimsulfuron. *Weed Technol.* 1998; **12**: 258-261.
23. O'Sillivan, J., Thomas, R.J. and Sikkema, P., Sweet maize cultivar sensitivity to RPA 201772. *Weed Technol.* 2001; **15**: 332-336.
24. Pillmoor, J.B., Influence of temperature on the activity of AC222,293 against A*vena fatua* L. and *Alopecurus myosuroides* Huds. *Weed Res.* 1985; **25**: 433-442.
25. Polge, N.D., and Barrett, M., Temperature effects on imazaquin soil bioavailability, uptake and metabolism in maize (*Zea mays*). *Weed Sci.* 1997; **45**: 198-204.
26. Putnam, A.R., and Rice, JR. R.P., Environmental and edaphic influences on the selectivity of alachlor on snap beans (*Phaseolus vulgaris). Weed Sci.* 1979; **25**: 570-574.
27. Reinhardt, C.F. and Nel, P.C., Die rol van grondeinnskappe lugtemperatuur en plantdiepte by die aktiwitet van alachlor. *Crop Prod.* 1982; **9**: 154-158.
28. Ritter, R.L., and Coble, H.D., Influence of temperature and relative humidity on the activity of Aciflurofen. *Weed Sci.* 1981; **29**: 480-485.
29. Rowe, L., Rossman, E. and Penner, D., Differential response of maize hybrids and inbreds to metolachlor. *Weed Sci.* 1990; **38**: 563-566.
30. Skipper, H.D., Allen, J.L. and Toler, J.E., Environmental factors affecting phyto-activity of metolachlor and alachlor. *Abstr. Weed Sci.* Soc. America. 1977; 106
31. Tomlin, C. (ed). The Pesticide Manual. Crop protection publication, Cambridge 1994.
32. Thompson, T., Slife, F.W., and Butler, H.S., Environmental influence on the tolerance of maize to atrazine. *Weed Sci.* 1970; **18**: 509-514.
33. Van Biljon, J.J., Factors affecting the selectivity of metolachlor in maize. *Ph D thesis,* University of Pretoria, South Africa 1991.
34. Van Wychen, L.R., Harvey, R.G., Rabay, T.L. and Bach, D.J., Tolerance of sweet maize (*Zea mays* L) hybrids to RPA201772. *Weed Technol.* 1999; **13**: 221-226.
35. Wilson, R.G., Response of nine sugarbeet cultivars to Post-emergence herbicide applications. *Weed Technol.* 1999; **13**: 25-28.