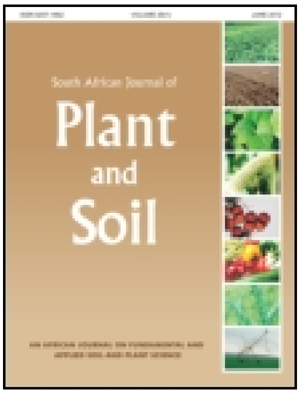

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Screening of South African sunflower (Helianthus annuus L.) cultivars for alachlor sensitivity

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Screening of South African sunflower (*Helianthus annuus* L.) cultivars for alachlor sensitivity

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Occasionally a herbicide that is considered safe to use at prescribed rates causes damage to crop plants at those same rates. Alachlor (Lasso EC®) is registered for use on sunflower at a rate of 1.5 to 1.92 kg ai ha^{-1}. Occasional injury to sunflower plants at these rates is usually caused by incorrect application or unsuitable weather conditions, but sometimes no explanation can be found. In many crops it has been shown that genetic differences can make specific cultivars more susceptible to alachlor injury. The objective of this study was to screen 22 of the available sunflower cultivars for sensitivity to alachlor in a glasshouse trial. Planting took place in pots filled with a sandy loam soil (20% clay). Five rates of alachlor (0, 0.96, 1.92, 3.84 and 7.68 kg ai ha^{-1}) were applied and leached into the soil with 100 ml of water. Pots were laid out in an air-conditioned glasshouse set to 28/18°C day/night in a randomised block design with three replicates. Plants were harvested 38 days after planting, at which stage plant height and mass were determined. The results indicated that cultivar differences with respect to alachlor tolerance were present. Plant height appeared to be the best predictor of alachlor activity in sunflower. Cultivars were divided into three classes (tolerant, intermediate and sensitive) based on their reaction to the herbicide.

Keywords: Alachlor, sensitivity, sunflower, tolerance

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Introduction

Sometimes a tried and tested herbicide, which is generally considered safe when applied at prescribed rates, damages crop plants at those same rates. This may be due to a variety of reasons, but it is known that different cultivars do not always react to a herbicide in the same way (Hodgeson, Thrasher & Eslick, 1964). Susceptibility and resistance within the same species has also been found with alachlor [2-chloro-N-(2,6-diethylphenyl)-N-methoxymethyl acetanilide] (Eastin, 1971; Voges & Nel, 1974; Narsaiah & Harvey, 1977; Unwin, Wilson & Mortensen, 1996). A plant's sensitivity to a herbicide depends upon the amount and rapidity of herbicide absorption, as well as its inherent ability to detoxify the herbicide (Ashton & Crafts, 1981). Therefore, any factor that influences the amount of herbicide absorbed would be likely to affect the plant's susceptibility to the herbicide (Le Court De Billot & Nel, 1977).

Large differences in alachlor tolerance have been found in both inbred maize lines and hybrids (Voges & Nel, 1974; Narsaiah & Harvey, 1977). Seed size appears to play a role in the sensitivity of maize to alachlor (Voges & Nel, 1974; Le Court de Billot & Nel, 1977).

The majority of work on cultivar differences with respect to alachlor tolerance of dicotyledonous crops has been carried out on dry beans. Differential response of bean varieties to alachlor has been reported by Penner & Graves (1972); Doersch *et al.* (1974); Meissner (1974) and Unwin *et al.* (1996). Meissner (1974) stated that these differences could be explained by seed size, as varieties with the largest seeds were unaffected.

The tolerance of common beans to alachlor is marginal and is dependent upon cultivar, herbicide placement, temperature, soil moisture and other soil factors (Rice & Putnam, 1980). Meissner (1982 - unpublished data) found that the effect of alachlor on 10 garden bean cultivars was not uniform. De Beer & Nel (1987) found that temperature plays a role in the effect of alachlor on dry beans, which agrees with the data of Penner & Graves (1972). De Beer & Nel (1987) also found that one of the three cultivars tested was sensitive to alachlor. Belote & Monaco (1977) worked with potatoes and reported that certain cultivars were sensitive to alachlor if the herbicide was applied immediately prior to emergence.

Allemann (1993) evaluated 22 of the then commercially available sunflower cultivars and showed that these differed with respect to their tolerance to alachlor (Lasso EC – alachlor 384 g $^{-1}$ EC). Since the work conducted by Allemann (1993) was carried out there have been many changes in the field of sunflower cultivation in South Africa. A capsule suspension of alachor (Lasso Micro Tech or Alanex 480 CS) is now available on the market, and the cultivars tested in the previous research are no longer available commercially. According to producers alachlor injury to some cultivars has been observed in the field during the past few seasons (Dr J. Saayman-du Toit, ARC-Grain Crops Institute, Potchefstroom, 2004 – personal communication). Herbicides have to be shown to be safe on crops at the rates recommended prior to registration in South Africa (Anon., 2000). However, when new cultivars are released there is no requirement for the breeder to prove that the new cultivar is not sensitive to the herbicides that are already registered on that specific crop.

It would be useful from a producer's viewpoint, as well as for the chemical companies, to determine whether marketed cultivars show drastic differences in sensitivity to alachlor, in which case the weed control practices for sensitive cultivars should be adapted. Information of this type could also be of value in screening programmes for new herbicides, or new cultivars. The objective of this trial was to determine if the commercially available sunflower cultivars exhibited differences with respect to their tolerance to alachlor.

Material and methods

The trial was conducted during October in a temperature controlled glasshouse at the University of the Free State. The temperature in the glasshouse was set to a 28/18°C day/night temperature regime, but the daylength of approximately 13 hours was not altered by supplemental lighting. During this trial 22 of the commercially available sunflower cultivars (Table 1) in South Africa were screened for their tolerance to alachlor. The trial was laid out in a randomised block design with three replicates. Nine sunflower seeds (achenes) were planted in polyethylene pots (150 mm in diameter and 120 mm high) which were lined with plastic bags to prevent contamination from the sides of the pot and filled. Each pot contained 1.8 kg of a sandy-loam soil (20% clay, 0.2% C and $pH_{(KCl)}$ 4.32) collected from the University's Paradys Experimental Farm.

Alachlor was applied at five rates, *viz.* 0 (control), 0.96, 1.92 (recommended application rate), 3.84 and 7.68 kg ai ha^{-1}. The herbicide was applied to the surface of the potted soil using a laboratory spraying apparatus constructed at the University of the Free State. The spraying apparatus consisted of a travelling boom with two Tee-Jet 8003-E nozzles mounted on it. This boom was set to move at a constant speed of 2.88 km h^{-1} during the spraying operation. The pots were placed on the floor below the boom, with the soil surface 300 mm below the nozzle tips. The system delivered a spray mixture of 200 L ha^{-1} at a pressure of 1.9 bar.

Herbicide was applied two days after planting. Prior to treatment, pots were watered to within 100 ml of the volume required to wet the soil to field capacity. After herbicide application 100 ml of distilled water (6 mm of rainfall) was applied to each pot in order to leach the herbicide into the soil. The soil water content at field capacity (19% m/m) was determined gravimetrically. After water was applied the plastic bags were knotted in order to prevent loss of water from the pot, so preventing crusting of the soil surface. Once the plants started to emerge the bags were opened.

Two weeks after seeding the plants were thinned out, so that only three plants remained in each pot. Pots received between 50 and 100 ml of distilled water as required. Plants were harvested 38 days after treatment and plant height determined prior to cutting the plants off at the soil surface and determining their fresh mass. The dry mass of plants from each treatment combination was determined after they were dried to constant mass in an oven at 70°C. Data were analysed using the NCSS 2000 statistical package (Hintze, 1998). The data were also expressed as a percentage of the control treatments before statistical analysis in order to negate inherent growth differences between cultivars. Statistical analyses were carried out on both the original data and the transformed data. Significant differences at the 5% level of significance were determined using Tukeys' Least Significant Difference test as described by Steel & Torrie (1980).

Results and discussion

Symptoms of phytotoxicity were readily apparent at the two highest rates of alachlor applied (3.84 and 7.68 kg ai ha^{-1}). The occurrence and severity of the symptoms appeared to be correlated to cultivar, but this data was not collected during this trial. Alachlor phytotoxicity manifested as stunted plants with small, dark green shrivelled or badly crinkled leaves that appeared to be thickened when compared with leaves from untreated plants. The darker colour, reduction in size and thickening was also noted on cotyledons. Occasionally fusion of leaf margins was found at the highest rate of alachlor application. These symptoms are characteristic of alachlor injury (Silk *et al.*, 1977), and similar to those found on potato and snap beans (Belote & Monaco, 1977; Putnam & Rice, 1979).

In this experiment it was found that both height and dry mass of the sunflower plants appeared to be fairly good indicators of alachlor activity. Reinhardt (1985) found that dry mass was the best indicator of alachlor activity in other crops, but Winarsih & Moenandir (1986) stated that plant elongation appeared to be more sensitive to alachlor than dry mass increase. A similar result was obtained by Van Rensburg & Van Dyk (1986), who found that hypocotyl-epicotyl growth was reduced by the acetanilides. In the present study, plants treated with higher rates of alachlor tended to be both shorter and thicker than the untreated controls, but no marked symptoms of phytotoxicity were noted at the recommended application rate. Although this was not measured, the internodes of treated plants, while being shorter than their untreated counterparts, were visibly thicker. Stem swelling has also been observed in alachlor treated potatoes (Belote & Monaco, 1977). This is an aspect that might deserve further study in a trial which includes both resistant and sensitive dicotyledonous plants. In the present study the highest R^2 values were obtained for plant height (0.79) and dry mass (0.41), consequently only these parameters will be discussed.

Deal & Hess (1980) noted that growth inhibition of plants exposed to alachlor resulted from the inhibition of both cell division and cell enlargement. Plant height should therefore

Table 1 Sunflower cultivars used in the cultivar evaluation trials

Seed Marketing Company				
AGRICOL	MONSANTO	ADVANTA	PANNAR	PIONEER
Agsun 5551	CRN 1414	Hysun 333	PAN 7010	PHB6488
Agsun 8251	DK 4040	Hysun 334	PAN 7351	PHB65A02
Agsun 8751	DKF 68-27	Hysun 338	PAN 7355	
HV 3037	DKF 68-22	Hysun 350	PAN 7371	
Mono Sun 150	SNK 28			
Nonyana				
Sunstripe				

be very sensitive to alachlor treatment, a contention that appears to be correct as Allemann & Reinhardt (1995) found that increasing alachlor concentration in nutrient solution had no effect on the dry mass of the top growth of sunflower seedlings, although plant height was significantly affected.

Plant height

When the original (un-transformed) data were analysed a significant interaction effect between alachlor application rate and cultivar was noted, indicating that the differences between cultivars increased as the application rate increased (data not presented). The interaction effect for plant height (% of control) was not significant, although both the main effects of cultivar and alachlor rate were highly significant (P<0.01). Cultivar differences were noted when data were analysed over all rates of alachlor (Table 2). Although the differences were not significant, nine cultivars (Mono Sun 150, Nonyana, SNK 28, PHB 6488, Hysun 350, PAN 7351, Agsun 8751, DKF 68-27 and PAN 7371) exhibited a tendency to grow taller than the control treatments at the lower rate of alachlor application (0.96 kg ai ha^{-1}), suggesting that growth stimulation might have occurred at sub-inhibitory alachlor rates. This phenomenon, termed hormesis, was first postulated in 1888 and has been shown to occur with several herbicides, including alachlor and propachlor, another acetanilide herbicide (Wiedeman & Appleby, 1972; Chang, Marsh & Jennings, 1975; Deal & Hess, 1980). This is a development that might warrant further study.

Table 2 Plant height of sunflower seedlings of various cultivars as affected by alachlor application (% of control

Cultivar (C)	Alachlor application rate (A) (kg ai ha^{-1}) 0.96	1.92	3.84	7.68	Mean
Mono Sun 150	121.46	92.75	78.38	43.65	84.06
Hysun 350	108.13	96.21	80.14	53.95	82.11
Nonyana	115.68	75.82	79.28	51.98	80.69
PAN 7351	107.69	87.67	61.70	37.88	73.74
CRN 1414	99.62	86.36	73.31	34.21	73.37
Hysun 334	95.16	82.29	63.62	52.29	73.34
PAN 7371	101.99	82.65	64.86	42.83	73.08
PAN 7010	94.32	86.68	69.89	49.32	72.80
PAN 7355	91.34	87.59	39.27	41.86	72.51
Sunstripe	91.12	83.45	69.85	38.99	70.85
DK 4040	83.69	91.34	58.05	50.13	70.80
SNK 28	115.31	71.93	49.82	39.55	69.15
Hysun 333	91.32	84.84	71.88	26.63	68.67
PHB 6488	109.06	83.68	55.33	23.61	67.92
HV 3037	90.08	85.04	59.37	34.16	67.16
Agsun 8751	104.45	80.97	56.06	22.88	66.09
Agsun 8251	81.92	84.05	60.72	19.79	61.62
Agsun 5551	86.81	70.15	54.39	33.36	61.18
DKF 68-27	102.19	79.41	48.50	14.49	61.15
Hysun 338	85.16	71.99	43.89	34.82	58.96
PHB 65A02	91.51	78.34	39.80	12.45	55.53
DKF 68-22	77.57	77.14	32.57	23.28	51.89
Mean	97.53	82.74	60.94	34.55	
$LSD_{T(0.05)}$	C = 26.55		A = 8.10	C × A = ns	

ns = not significant

As can be seen from Table 2, plants of most sunflower cultivars tended to be more sensitive to alachlor at rates higher than the one recommended (1.92 kg ai ha^{-1}), although Nonyana was an exception. Also obvious from the data is that the cultivars differed in their relative sensitivity to alachlor, as shown by plant height, when data were averaged over all rates of alachlor (Table 2), which was indicated by the relative positions of the cultivars. The top two cultivars (Mono Sun 150 and Hysun 350) were both significantly taller than the bottom two cultivars (PHB 65A02 and DKF 68-22). As a result these cultivars were classified as being either tolerant or sensitive to the herbicide. The remainder of the cultivars in Table 2 did not differ significantly from either these "tolerant" or "sensitive" types, and were then classified as being intermediate in tolerance to alachlor.

The effects of various alachlor rates on the mean height of selected tolerant and sensitive cultivars are shown in Figure 1. This figure indicates height tendencies averaged over the tolerant and sensitive classes. Tolerant cultivars, such as Mono Sun 150 and Hysun 350, showed little reduction in plant height with increasing alachlor rate until a fairly high rate of application, double or even four times the recommended rate of 1.92 kg ai ha^{-1}, when a dramatic decrease in height occurred. On the other hand, less tolerant cultivars, such as PHB 65A02 and DKF 68-22, exhibited a marked reduction in height at the first rate of alachlor application (half the recommended rate of 1.92 kg ai ha^{-1}), and plant height was further decreased with each increase in alachlor rate.

From these results it can be seen that increasing alachlor application rates resulted in severe stunting of certain sunflower cultivars. This reaction of sunflower to increasing alachlor application rates is similar to that noted on various monocotyledonous species as well as on potato (Belote & Monaco, 1977; Deal & Hess, 1980). In potato this decrease in plant height was accompanied with a decrease in fresh mass, something that was also noted in this trial (data not presented).

Dry mass

A number of researchers, including Reinhardt (1985), have

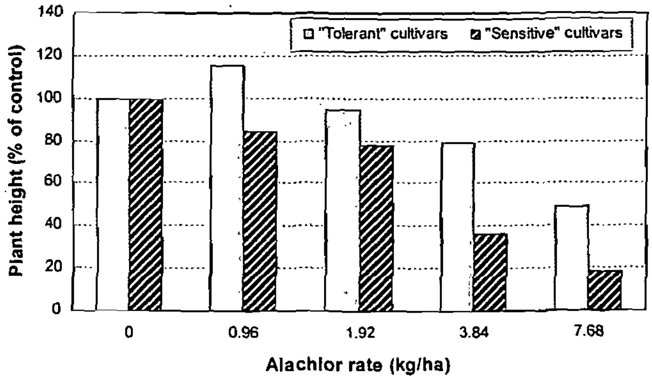

Figure 1 Effect of increasing alachlor rate on the height of sunflower seedlings (LSD_A = 8.1)

found that plant dry mass was well correlated with alachlor activity. In this experiment it was found that, although plant height had a higher R^2 value, the R^2 value for dry mass was high enough to warrant further scrutiny. Once again the analysis of the original data indicated that the interaction effect between alachlor application rate and cultivar was significant, showing differences in cultivar reaction to increasing amounts of the herbicide (data not presented). However, as this could be due to inherent growth differences between cultivars, this data was not presented.

Highly significant differences ($P<0.01$) were obtained for main effects, cultivar and alachlor rate when the data were analysed as a percentage of the control treatment. The interaction effect was, however, not significant, giving an indication of similar reactions to increasing alachlor application rates for the majority of cultivars tested, i.e. a reduction in mass with increasing herbicide concentration.

Although plant dry mass (over all cultivars) was reduced by alachlor application rates higher than the recommended rate, no significant differences were noted between the recommended application rate of 1.92 kg ai ha^{-1} and twice the recommended rate (Table 3). Plants at both of these rates performed better than plants at the highest application rate of 7.68 kg ai ha^{-1} (four times the recommended application rate). Nine cultivars exhibited an increase in dry mass at half the recommended rate of alachlor application (0.96 kg ai ha^{-1}), and two of these (Mono Sun 150 and Nonyana) also showed increased dry mass at the recommended rate of application (1.92 kg ai ha^{-1}). This could also be ascribed to hormesis, as was noted for plant height.

Table 3 Effect of alachlor application on the dry mass of sunflower seedlings of various cultivars (% of control)

Cultivar	Alachlor application rate (A) (kg ai ha^{-1})				
(C)	0.96	1.92	3.84	7.68	Mean
Mono Sun 150	159.99	143.66	136.19	120.73	140.14
CRN 1414	118.38	92.13	97.47	97.50	101.37
PAN 7371	118.91	88.82	100.80	73.67	95.55
Hysun 338	102.02	95.94	85.19	81.29	91.11
PAN 7355	106.37	96.74	80.25	80.32	90.92
Nonyana	135.42	102.36	60.56	63.69	90.51
Hysun 350	97.16	105.65	89.42	66.00	89.56
PAN 7351	105.39	88.05	81.62	75.35	87.58
Hysun 334	92.28	84.72	78.99	81.61	84.40
Hysun 333	80.92	87.77	81.82	67.60	79.53
PAN 7010	77.31	69.38	90.45	80.69	79.46
SNK 28	116.65	84.34	54.35	55.84	77.80
PHB 6488	99.40	96.86	90.67	22.79	77.43
Agsun 5551	90.71	80.27	84.65	51.57	76.80
Agsun 8251	87.96	95.92	81.18	38.51	75.89
Agsun 8751	90.84	99.27	74.04	39.31	75.86
DK 4040	79.04	104.14	62.96	44.21	72.59
HV 3037	66.86	95.07	66.14	56.83	71.23
DKF 68-27	101.29	74.39	65.75	26.87	67.08
DKF 68-22	92.49	90.55	37.05	36.47	64.14
Sunstripe	73.03	59.35	75.00	48.03	63.85
PHB 65A02	87.51	66.84	47.94	12.14	53.61
Mean	99.08	91.01	78.29	60.05	
$LSD_{T(0.05)}$	C = 45.10		A = 13.75		C x A = ns

ns = not significant

From the data presented in Table 3 it can be seen that the various cultivars showed differences with respect to their relative tolerance to alachlor when data were analysed over all rates of alachlor. For example, the cultivar CRN 1414 exhibited little or no decline in dry mass with increasing alachlor rates, while the cultivar PHB 65A02 showed decreasing dry mass with increasing alachlor rates (Figure 2). Following a similar procedure to that used on plant height, it was found that the top two cultivars (Mono Sun 150 and CRN 1414) were both significantly heavier than the bottom cultivas (PHB 65A02). As a result these cultivars were classified as being either tolerant or sensitive to the herbicide. The remainder of the cultivars in the Table did not differ significantly from either these "tolerant" or "sensitive" types, and were then classified as being intermediate in tolerance to alachlor.

The lowest rate of alachlor application (0.96 kg ai ha^{-1}) did not result in a significant reduction in either plant height or dry mass of the top growth of sunflower seedlings. Increasing alachlor to 1.92 kg ai ha^{-1} significantly reduced plant height (by 13.3%) over that of the 0.96 kg ai ha^{-1} treatment, and further increases in herbicide rate each resulted in a significant increase in seedling injury. Although there was a significant difference in seedling top dry mass between the control and the recommended rate of alachlor application (1.92 kg ai ha^{-1}), the first significant decrease with increasing application rate was noted as the rate increased to 3.84 kg ai ha^{-1} (12.65% reduction). Increasing the application rate to 3.84 kg ai ha^{-1} resulted in a further significant decrease of 13.59% in the top dry mass of seedlings.

The greater effect of the herbicide on plant height than on the seedling dry mass could be explained by the thickening of stems and leaves caused by alachlor treatment, which resulted in squat thickset plants. These findings substantiate those of Allemann & Reinhardt (1995).

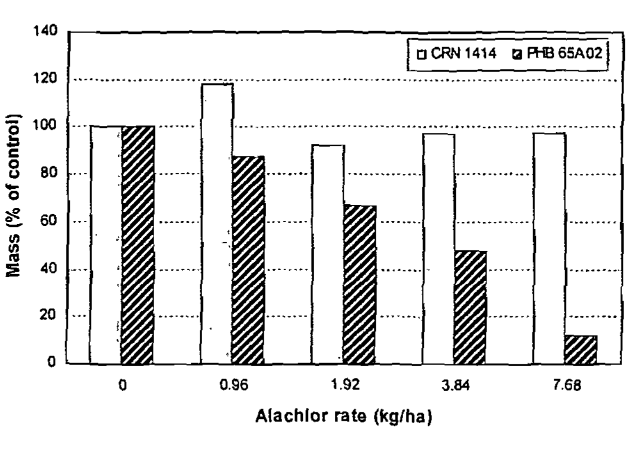

Figure 2 Effect of increasing alachlor rate on the dry mass of sunflower seedlings (LSD_C = 45.1; LSD_A = 13.75)

Differences with respect to alachlor tolerance were noted between the various sunflower cultivars used in this trial, and these differences became more apparent as the alachlor concentration increased, a result that was also noted by Chang *et al.* (1975) on *Avena*, as well as Deal & Hess (1980) on both peas and oats. However, the reduction in plant growth at 0.96 kg ai ha^{-1} (half the recommended application rate) was not significantly different from that observed in the control treatments for either plant height or dry mass. This finding concurs with that of de Prado, Romero & Jorrin (1993), who found that sunflower exhibited good tolerance to alachlor at rates up to 1 kg ai ha^{-1}. A spectrum of response to alachlor was observed from the sunflower cultivars that were evaluated in this trial. The diversity of alachlor injury that has been observed in the field could, therefore, be explained by the selection of a sensitive cultivar.

Using the data from Tables 2 and 3 cultivars were ranked according to their sensitivity to the herbicide. Those cultivars placed at the top of the list were classified as tolerant (e.g. Mono Sun 150), while those that were placed at the bottom of the list, such as PHB 65A02, were classified as sensitive. Cultivars were divided into three tolerance classes, *viz.* tolerant, intermediate and sensitive. The criteria that were used to rank the cultivars were based on the percentage reduction in plant height and dry mass over all application rates of alachlor, and were as follows:

Tolerant: plant height <20% and dry mass 5%

Intermediate: plant height >20% but <40%, and dry mass >5% but <40%

Sensitive: plant height and dry mass >40%

The resultant divisions are shown in Table 4. Cultivars in the intermediate group were not overly sensitive, nor overly tolerant to the herbicide. From Table 4 it can be seen that the majority of cultivars on the market in South Africa were not negatively influenced by alachlor at the rates used in this study, but there were exceptions.

The differences in alachlor tolerance found between the cultivars would appear to be in agreement with the findings of other researchers on a variety of crops. In maize it has been shown that tolerance to the chloroacetanilide group of herbicides is genetically controlled (Niccum, 1970; Rowe & Penner, 1990). It is probable that the tolerance of sunflower to alachlor is also genetically controlled; suggesting that resistance to the herbicide might be attainable through plant breeding. It also has immediate practical implications in that some sunflower cultivars may be damaged by recommended application rates of the herbicide if the climatic conditions are suitable for the absorption of sufficient qualtities of herbicide to cause phytotoxicity. The diversity of alachlor injury that has been observed in the field could, therefore, be explained by the selection of a sensitive cultivar, although it is possible that injury could also result from the use of an intermediate cultivar from a seed lot with reduced vigour, resulting in slow germination and growth through treated soil.

At this stage it is not known if the plants would outgrow the injuries sustained in the seedling stage. In potatoes it was found that while some cultivars were able to outgrow the injury, if this took place in a late planting it delayed maturity to such an extent that yield losses resulted (Belote & Monaco, 1977). Previous research carried out on sunflower cultivars that are no longer commercially available in South Africa indicated that some varieties were so sensitive to the herbicide that even the recommended rate of alachlor application (1.92 kg ai ha^{-1}) resulted in yield losses, while others were unaffected even at double that rate (Allemann, 1993). It is, therefore, imperative that a field trial be undertaken in order to see if the sunflower plants are able to outgrow the alachlor injury, or if yield reductions would result.

Table 4 Sensitivity of South African sunflower cultivars to alachlor application in pots

Class	Cultivar
Tolerant	Mono Sun 150
Intermediate	Hysun 350[a]
	Nonyana[a]
	PAN 7351
	CRN 1414[b]
	Hysun 334
	PAN 7371
	PAN 7010
	PAN 7355
	Sunstripe
	DK 4040
	SNK 28
	Hysun 333
	PHB 6488
	HV 3037
	Agsun 8751
	Agsun 8251
	Agsun 5551
	DKF 68-27
	Hysun 338[c]
	DKF 68-22[c]
Sensitive	PHB 65A02

[a] - On a height basis these two cultivars could be classified as tolerant

[b] - On a dry matter basis this cultivar could be classified as tolerant

[c] - On a height basis these two cultivars could be classified as sensitive

Conclusions

Plant height was found to be the best predictor of alachlor activity in sunflower seedlings in pot trials. South African sunflower cultivars differ with respect to their sensitivity to alachlor at the seedling stage. At this juncture it is unknown if this sensitivity could affect the ultimate yield obtained from the sunflower crop. This will need to be tested in a field trial. It is apparent that some unexplained early alachlor injury to sunflower in the field could probably be explained by the use of a sensitive cultivar.

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