

Breaking of seed dormancy in three Dicotyledoneae weed species by plant growth regulators



MARTHA M. MANOTO, M.I. FERREIRA AND G.A. AGENBAG

Abstract

Seeds of the dicot weeds *Arctotheca calendula*, *Emex australis* and *Raphanus raphanistrum* were used to determine its germination response to three plant growth regulators in an incubator at 20 °C with a 12-hour day/night cycle. Cumulative germination of *A. calendula* seeds was not improved by any of the chemicals used. Germination of *E. australis* was significantly increased at 3-7 days by an application of 1 mg ℓ^{-1} gibberellic acid, with similar results for both 30 mg ℓ^{-1} hydroxylamine and 0.3 mg ℓ^{-1} kinetin at 0-3 days. The germination of *R. raphanistrum* was, apart from the 0-3 day incubation period, significantly improved at all other incubation periods when 100 mg ℓ^{-1} gibberellic acid was applied.

Introduction

Dormancy is a property of many weed seeds that enables them to survive conditions hazardous to plant growth and to germinate at some later time or in some other place. Seeds may persist in the soil for many years because of dormancy and germinate when conditions are favourable for seedling survival through to maturity (Duke, 1985). Bewley & Black (1982) stated that the ability of growth regulators, when applied to seeds, to release seeds from dormancy and promote germination is particularly interesting as it gives an indication to possible dormancy mechanisms.

Several chemicals, when applied to dormant seeds, might cause them to germinate. Although the seed of numerous species mostly respond to one or more of these chemicals, large differences are found between species (Corns, 1959; Bewley & Black, 1982; Metzger, 1983; Hurtt & Taylorson, 1986). Little is however known with regard to the response of some troublesome *Dicotyledoneae* (dicot) weed species in the Western Cape to these chemicals. Floral variation and other reproductive morphology provide the basis for dividing the flowering plants into two major classes: the class *Magnoliopsida* (dicots) and the class *Liliopsida* (monocots).

Povilaitis (1956), stated that the application of chemicals to the soil to stimulate weed seed germination might be an alternative method of weed control and could result in the weed population being destroyed in one season rather than by repeated annual applications of herbicides.

Generally, selective herbicides are registered for either broadleaf (dicot) or grass (monocot) weed control because of different physiological responses between the two classes. It is therefore botanically useful to distinguish anatomically and physiologically between dicot and monocot weeds.

The informal name dicot refers to the presence of two embryonic leaves (cotyledons) in the seed. The stomata are scattered in leaves of dicot plants. Most dicot leaves have netted venation, meaning they have one or a few prominent mid veins from which smaller minor veins branch into a meshed network (Moore et al., 1998). In dicots, flower parts usually occur in multiples of four or five. Pollen usually has three furrows or pores. The primary vascular bundles in the dicot stem are arranged in a ring. True secondary growth with vascular cambium is commonly present in dicots (Moore et al., 1998).

The objective of this study was to evaluate the effect of three growth regulators to break dormancy and enable simultaneous germination of the dicot weeds *Arctotheca calendula*, *Emex australis* and *Raphanus raphanistrum*.

Materials and Methods

Seeds of *A. calendula*, *E. australis* and *R. raphanistrum*, collected during the year 2000 at Langgewens Experimental Farm in the Swartland wheat producing area of South Africa and stored at room temperature (15-25 °C), were used in this germination study.

To determine the germination response of the above-mentioned weed species to three plant growth regulators, 100 seeds of each species were placed on Whatman's filter paper in 9.5 cm diameter petri dishes and

moistened with 5 ml of the test solutions gibberellic acid, hydroxylamine (auxin) or kinetin (cytokinin), respectively. Subsequently, the petri dishes were then sealed with parafilm and placed in an incubator at 20°C with a 12 hour day/night cycle.

The following test solutions were used:

Gibberellic acid

Gibberellic acid (purity > 90%) was tested at concentrations of 0 (control), 1 mg ℓ^{-1} , 10 mg ℓ^{-1} and 100 mg ℓ^{-1} . Each test solution also contained 2% (v/v) acetone and 0.1% (v/v) oxysorbic (Tween) which assisted with the dispersal of the test compounds (Metzger, 1983). Test solutions were adjusted to pH 4.8 using 6N KOH.

Hydroxylamine (auxin)

Hydroxylamine concentrations of 0 (control), 3 mg ℓ^{-1} , 10 mg ℓ^{-1} and 30 mg ℓ^{-1} were used. Each concentration were adjusted to pH 7.3 using NaOH and made up to 1 ℓ with distilled water as described by Esashi et al., (1979).

Kinetin (cytokinin)

Kinetin concentrations of 0 (control), 0.2 mg ℓ^{-1} , 0.3 mg ℓ^{-1} , 0.5 mg ℓ^{-1} were used. Each quantity was dissolved in 10 ml of slightly heated 0.5N NaOH before diluting with distilled water and made up to 1 ℓ with distilled water (Igbinnosa & Okonkwo, 1992).

The germination was assessed by emergence of the radicle and determined after 3, 7, 10 and 14 days of incubation. With the tetrazolium test, the ugerminated seeds were categorized as either viable or dead (Wood et al., 1997). The germination percentage was based on the total number of seeds germinated as opposed to the total number of viable seeds tested. All treatments were replicated twice in a factorial design.

Statistical Analysis

Data were subjected to analysis of variance to assess the effect of different chemical concentrations on the germination of three weed species. The data were analysed using SAS (Statistical Analysis Systems, 1987).

Results

Arctotheca calendula

The germination rates of *Arctotheca calendula* were significantly affected by both the period of incubation and concentration of the chemicals used, but not by chemicals as a main factor (Table 1). None of the *A. calendula* seeds germinated during the first three days (0-3 day period) of incubation at any of the chemical treatments tested, while an average of 5.4%, 8.8% and 6.7% germinated during the 4-7 day, 8-10 day and 11-14 day periods of incubation, respectively. These results are in agreement with those reported previously at different temperature regimes (Manoto et al., 2004).

Results also showed a significant ($p \leq 0.05$) chemical x concentration x period interaction, indicating that the response of *A. calendula* to the

Table 1 Germination response (%) of *Arctotheca calendula* to three chemicals at four concentrations, over a period of 14 days at 20 °C.

Weed species	Chemical	Concentration	Periods				Mean
			0-3 days	4-7 days	8-10 days	11-14 days	
<i>Arctotheca calendula</i>	Gibberellic acid	0mg ℓ^{-1}	0 e	22.5 a	5 cde	5 cde	8.1 a
		1mg ℓ^{-1}	0 e	2.5 de	0 e	0 e	0.6 c
		10mg ℓ^{-1}	0 e	2.5 de	2.5 de	12.5 abcde	4.4 ab
		100mg ℓ^{-1}	0 e	0 e	5 cde	2.5 de	1.9 bc
		Mean	3.8				
	Hydroxylamine	0 mg ℓ^{-1}	0 e	7.5 bcde	20 ab	2.5 de	7.5 a
		3 mg ℓ^{-1}	0 e	0 e	0 e	7.5 bcde	1.9 bc
		10 mg ℓ^{-1}	0 e	2.5 de	20 ab	10 abcde	8.1 a
		30 mg ℓ^{-1}	0 e	0 e	7.5 bcde	17.5 abc	6.3 ab
		Mean	6.0				
	Kinetin	0 mg ℓ^{-1}	0 e	7.5 bcde	5 cde	7.5 bcde	5.0
		0.2 mg ℓ^{-1}	0 e	12.5	10 abcde	2.5 de	abc
		0.3 mg ℓ^{-1}	0 e	abcde	15 abcd	7.5 bcde	6.3 ab
		0.5 mg ℓ^{-1}	0 e	5 cde	15 abcd	5 cde	6.9 ab
		Mean	6.0				5.6
Mean		0 b	5.4 a	8.8 a	6.7 a		

LSD t' Main effect chemical ($P \leq 0.05$) = 2.7 N.S.
 LSD t' Main effect concentration ($P \leq 0.05$) = 3.1 *
 LSD t' Main effect periods ($P \leq 0.05$) = 3.7 *
 LSD t' chemical x concentration ($P \leq 0.05$) = 5.3 *
 LSD t' chemical x periods ($P \leq 0.05$) = 6.3 N.S.
 LSD t' concentration x periods ($P \leq 0.05$) = 7.3 N.S.
 LSD t' chemical x concentration x periods ($P \leq 0.05$) = 12.7 *
 * $P \leq 0.05$ = Significant, N.S. = Not Significant.

different concentrations of chemicals used, differed with time. This is due to significant differences found for different gibberellic acid concentrations during the 4-7 day period and hydroxylamine concentrations during the 8-10 and 11-14 day periods of incubation (Table 1). Results were very inconsistent and did not show any clear trend. Therefore, it is possible that the above-mentioned significant interactions are thus most likely due to experimental error.

Cumulative germination figures (Figure 1) also showed that the germination of *A. calendula* seeds was not improved by any of the chemicals used. Cumulative germination of below 40% after 14 days of incubation, as also found in the experiment with different temperature regimes, indicated that *A. calendula* needed either very specific conditions for germination or that germination is prevented by an impermeable seed-coat or immature embryo (Gardner *et al.*, 1985).

Emex australis

Germination rates of *E. australis* were significantly ($p \leq 0.05$) affected by period of incubation, chemicals used and chemical concentrations used as main factors, as well as chemical x concentration and chemical x concentration x period interactions (Table 2). Mean values of 23.5% and 43.1% germination during the 0-3 and 4-7 day incubation periods, clearly indicated that seeds of *E. australis* used in this experiment were not dormant.

Although mean chemical x concentration values showed significant differences ($p \leq 0.05$) between concentrations for all chemicals tested, mean germination was not significantly increased by any of the chemicals used. During individual periods of incubation, germination of *E. australis* was significantly increased due to the application of 1 mg ℓ^{-1} of gibberellic acid at 3-7 days, 30 mg ℓ^{-1} hydroxylamine at 0-3 days and 0.3 mg ℓ^{-1} kinetin at 0-3 days.

This resulted in slightly higher cumulative germination values for *E. australis* if treated with low concentrations (1 and 10 mg ℓ^{-1}) gibberellic acid

Figure 1: Cumulative germination of *A. calendula* due to (a) gibberellic acid, (b) hydroxylamine and (c) kinetin treatments.

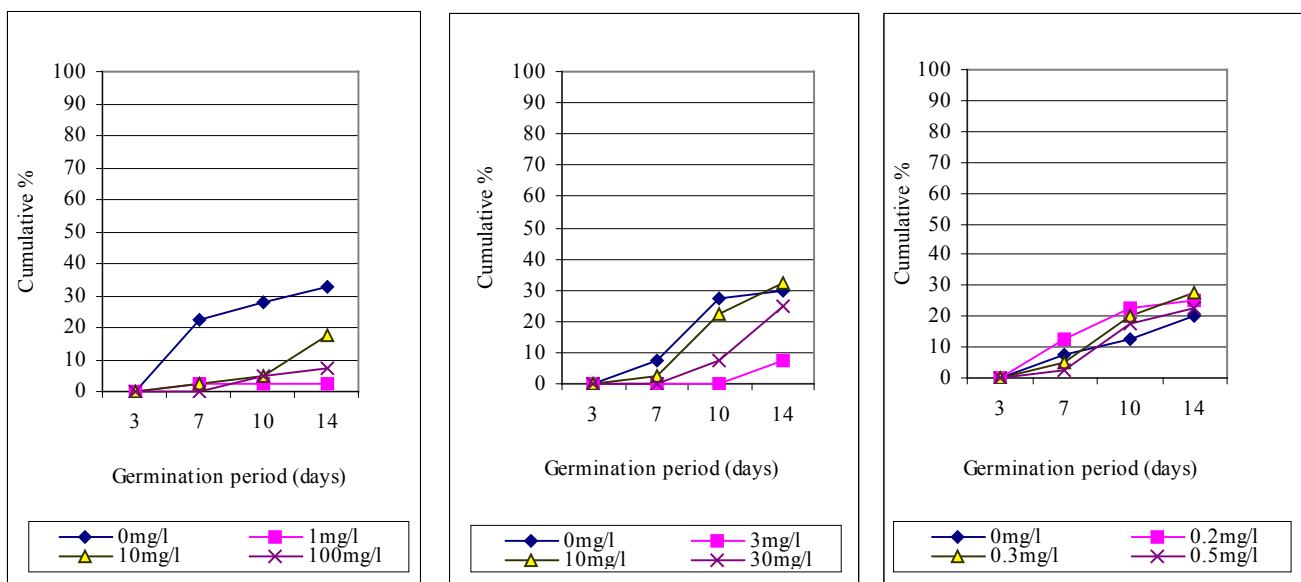


Table 2: Germination response (%) of *Emex australis* to three chemicals at four concentrations, over a period of 14 days at 20 °C.

Weed species	Chemical	Concentration	Periods				Mean
			0-3 days	4-7 days	8-10 days	11-14 days	
<i>Emex australis</i>	Gibberellic acid	0 mg l ⁻¹	22.5 defgh	37.5 bcde	5 gh	2.5 h	16.8 abc
		1 mg l ⁻¹	12.5 fgh	60 ab	5 gh	0 h	19.4 a
		10 mg l ⁻¹	12.5 fgh	37.5 bcde	10 fgh	15 efgh	18.7 abc
		100 mg l ⁻¹	2.5 h	22.5 defgh	10 fgh	15 efgh	12.5 bc
		Mean	16.9 b				
	Hydroxylamine	0 mg l ⁻¹	15 efgh	57.5 ab	2.5 h	2.5 h	19.4 abc
		3 mg l ⁻¹	7.5 gh	12.5 fgh	17.5 efgh	10 fgh	11.9 c
		10 mg l ⁻¹	17.5 efgh	67.5 a	2.5 h	5 gh	23.1 a
		30 mg l ⁻¹	50 abc	45 abcd	0 h	2.5 h	24.4 a
		Mean	19.7 a				
	Kinetin	0 mg l ⁻¹	17.5 efgh	50 abc	5 gh	0 h	18.1 abc
		0.2 mg l ⁻¹	27.5 cdefg	62.5 a	7.5 gh	0 h	24.3 ab
		0.3 mg l ⁻¹	60 ab	32.5 cdef	7.5 gh	0 h	25.0 a
		0.5 mg l ⁻¹	37.5 bcde	32.5 cdef	0 h	10 fgh	20.0 abc
		Mean	21.9 a				
Mean			23.5 b	43.1 a	6.0 c	5.8 c	

LSD 't' Main effect chemical (P ≤ 0.05) = 4.7 *
 LSD 't' Main effect concentration (P ≤ 0.05) = 5.4 *
 LSD 't' Main effect periods (P ≤ 0.05) = 7.1 *
 LSD 't' chemical x concentration (P ≤ 0.05) = 9.3 *
 LSD 't' chemical x periods (P ≤ 0.05) = 12.3 *
 LSD 't' concentration x periods (P ≤ 0.05) = 14.2 N.S.
 LSD 't' chemical x concentration x periods (P ≤ 0.05) = 24.6 *
 * P ≤ 0.05 = Significant, N.S. = Not Significant

or medium concentrations of hydroxylamine (3 and 10 mg l⁻¹) and kinetin (0.2 and 0.3 mg l⁻¹) (Figure 2). These treatments with hydroxylamine and kinetin resulted in cumulative values of more than 90% germination of *E. australis* after 14 days of incubation.

Raphanus raphanistrum

Germination of *R. raphanistrum* seeds were also significantly (p ≤ 0.05) affected by chemicals used, concentration rates and period of incubation as main factors (Table 3).

Although mean germination percentages for different incubation periods were generally low (less than 20%), significant interactions between concentration rates and chemicals used, resulted in high germination rates during specific incubation periods.

The application of gibberellic acid did not affect the germination of *R.*

raphanistrum

during the 0-3 day incubation period (Table 3 and Figure 3). At all other incubation periods, germination percentage were significantly (p ≤ 0.05) improved if 100 mg l⁻¹ gibberellic acid was applied. Hydroxylamine and kinetin did not have any effect. The germination of *R. raphanistrum* at all kinetin treatments increased with an increase in incubation period.

Cumulative values (Figure 3) confirmed the stimulating effects of gibberellic acid on the germination of *R. raphanistrum* seeds since the application of 100 mg l⁻¹ gibberellic acid caused more than 90% of the seed to germinate within 14 days of incubation, while kinetin also showed a stimulating effect at the highest concentration.

Figure 2 Cumulative germination of *E. australis* due to (a) gibberellic acid, (b) hydroxylamine and (c) kinetin treatments.

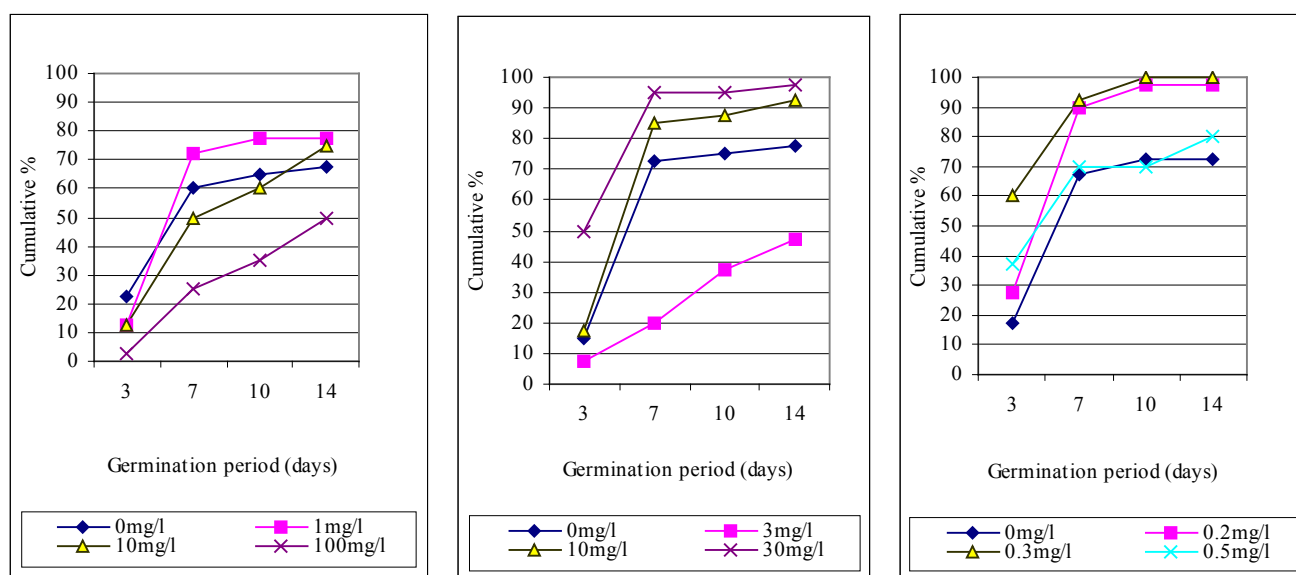


Table 3 Germination response (%) of *Raphanus raphanistrum* to three chemicals at four concentrations, over a period of 14 days at 20 °C.

Weed species	Chemical	Concentration	Periods				Mean
			0-3 days	4-7 days	8-10 days	11-14 days	
<i>Raphanus raphanistrum</i>	Gibberellic acid	0 mg l ⁻¹	7.5 fgh	0 h	0 h	0 h	1.9 e
		1 mg l ⁻¹	2.5 h	0 h	0 h	0 h	0.6 e
		10 mg l ⁻¹	12.5 efgh	7.5 fgh	5 gh	10 efgh	8.8 d
		100 mg l ⁻¹	2.5 h	22.5 cdefg	40 bc	25 cdef	22.5 ab
		Mean	8.4 a				
	Hydroxylamine	0 mg l ⁻¹	7.5 fgh	0 h	0 h	0 h	1.9 e
		3 mg l ⁻¹	2.5 h	0 h	0 h	0 h	0.6 e
		10 mg l ⁻¹	0 h	5 gh	2.5 h	2.5 h	2.5 e
		30 mg l ⁻¹	5 gh	7.5 fgh	0 h	0 h	3.1 e
		Mean	2.0 b				
	Kinetin	0 mg l ⁻¹	2.6 h	2.6 h	10.5 efgh	13.2 efgh	7.2 d
		0.2 mg l ⁻¹	2.6 h	5.3 gh	15.8 efgh	13.2 efgh	9.2 d
		0.3 mg l ⁻¹	0 h	0 h	16.7 efgh	19.4 cdefg	9.0 d
		0.5 mg l ⁻¹	0 h	0 h	25 cdef	16.7 efgh	10.4 d
		Mean	9.0 a				
Mean			3.8 b	4.2 b	10.0 a	8.3 a	

LSD 't' Main effect chemical (P ≤ 0.05) = 2.4 *
 LSD 't' Main effect concentration (P ≤ 0.05) = 2.8 *
 LSD 't' Main effect periods (P ≤ 0.05) = 3.1 *
 LSD 't' chemical x concentration (P ≤ 0.05) = 4.9 N.S.
 LSD 't' chemical x periods (P ≤ 0.05) = 5.3 *
 LSD 't' concentration x periods (P ≤ 0.05) = 6.2 N.S.
 LSD 't' chemical x concentration x periods (P ≤ 0.05) = 10.7 *
 * P ≤ 0.05 = Significant, N.S. = Not Significant

Discussion

No chemical proved to be successful in stimulating the germination of all species tested, but individual weed species did respond to specific concentrations of the chemicals tested. This corresponds with earlier findings by Murdoch & Carmona (1993), who investigated ways to deplete seed banks by stimulating seeds to germinate.

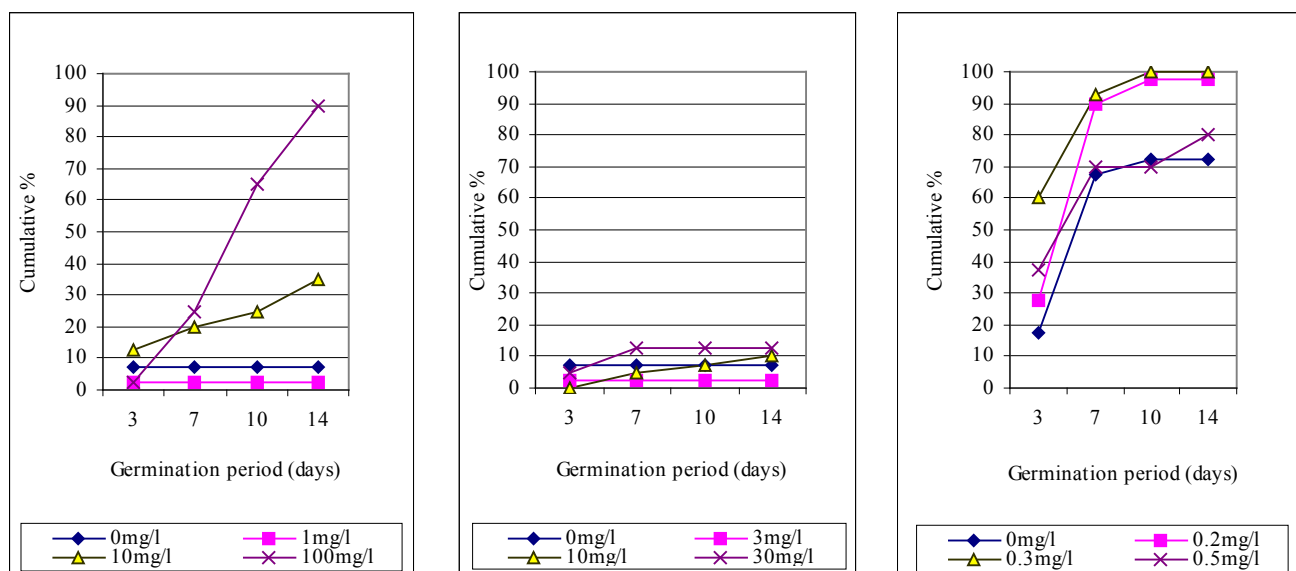
Germination values of below 40% for *A. calendula* for all treatments tested after the 14 day incubation period, indicated that seeds of this species were in a dormant state and that this dormancy was most probably not due to the shortage of growth stimulators such as gibberellic acid, kinetin or hydroxylamine. These results support earlier findings by Mayer & Poljakoff-Mayber (1982). Ellery & Chapman (2000) showed that light may reduce dormancy in *A. calendula* seeds, while Chaharsoghi & Jacobs (1998) found that germination of this species can be enhanced

by scarification.

Although low germination percentages for the control treatments of *R. raphanistrum* indicated that seeds of this species were also dormant, germination of this species was significantly (p ≤ 0.05) improved (or inhibited) by specific concentrations of one or more of the chemicals tested. Dormancy in this species, as also found by Harradine (1986), were therefore most probably the result of some chemical obstruction.

In contrast to this, high cumulative germination values of the control treatments of *E. australis* clearly indicated that seeds of this species used in the experiment were not dormant. Germination of *E. australis* seed were increased to nearly 100% after 14 days of incubation by high concentrations of hydroxylamine as well as low and medium concentrations of kinetin.

Figure 3 Cumulative germination of *R. raphanistrum* due to (a) gibberellic acid, (b) hydroxylamine and (c) kinetin treatments.



Conclusions

This study showed that different chemical concentrations might have an effect on breaking dormancy of different weed seeds. This is due to differing weed seeds that differ according to the state and longevity of dormancy as well as the physiological basis of dormancy.

References

1. BEWLEY, J. D. & BLACK, M., 1982. Physiology and biochemistry of seeds in relation to germination – Viability, dormancy and environmental control. Springer-Verlag. New York.
2. CHAHARSOGHI, A. T. & JACOBS., B., 1998. Manipulating dormancy of capeweed (*Arctotheca calendula* L.) seed. *Seed Science Research* 8, 139 – 146.
3. CORNS, W.M. G., 1959. Effects of gibberellin treatments on germination of various species of weed seeds. *Canadian Journal of Plant Science* 40, 47 – 51.
4. DUKE, O. S., 1985. Weed physiology: Production and Ecophysiology. Volume 1. CRC Press, Inc. Florida.
5. ELLERY, A. J. & CHAPMAN, R., 2000. Embryo and seed coat factors produce seed dormancy in capeweed (*Arctotheca calendula*). *Australian Journal of Agricultural Research* 51, 849 – 854.
6. ESASHI, Y., OHHARA, Y., OKAZAKI, M. & HISHINUMA, K., 1979. Control of cocklebur seed germination by nitrogenous compounds: Nitrite, nitrate, hydroxylamine, thiourea, azide and cyanide. *Plant and Cell Physiology* 20 (2), 349 – 361.
7. GARDNER, F. P., PEARCE, R.B. & MITCHELL, R.L. (eds.), 1985. Physiology of crop plants. Iowa State University Press, Ames, USA.
8. HARRADINE, A. R., 1986. Seed longevity and seedling establishment of *Bromus diandrus* Roth. *Weed Research* 26, 173 – 180.
9. HURTT, W. & TAYLORSON, R. B., 1986. Chemical manipulation of weed emergence. *Weed Research* 26, 259 – 267.
10. IGBINNOSA, I. & OKONKWO, S. N. C., 1992. Stimulation of germination of seeds of cowpea witchweed (*Striga gesnerioides*) by Sodium Hypochlorite and some growth regulators. *Weed Science* 40, 25 – 28.
11. MANOTO, M.M., FERREIRA, M.I. & AGENBAG, G. A., 2004. The effect of temperature on the germination of six selected weed species. *South African Journal of Plant and Soil* 21 (4), 214 –219.
12. MAYER, A. M. & POLJAKOFF-MAYBER, A. 1982. The germination of seeds. Third edition. Pergamon Press Ltd. New York.
13. METZGER, J. D., 1983. Promotion of germination of dormant weed seeds by substituted phthalimides and gibberellic acid. *Weed Science* 31, 285 –289.
14. MOORE, R., CLARK, W.D. & VODOPICH, D.S., 1998. Botany. Second edition. McGraw-Hill. Boston.
15. MURDOCH, A.J. & CARMONA, R., 1993. The implications of the annual dormancy cycle of buried weed seeds for novel methods of weed control. *Brighton crop protection conference - Weeds*, 329 – 334.
16. POVILAITIS, B., 1956. Dormancy studies with seed of various weed species. Proceedings of the International Seed Testing Association 21, 99 – 101.
17. [SAS] Statistical Analysis Systems. 1987. SAS/STAT Guide for personal Computers. Version 6. Cary, NC: Statistical Analysis Systems Institute.
18. WOOD, B.L., McDaniel, K. C. & CLASON, D., 1997. Broom snake weed (*Gutierrezia sarothrae*) dispersal, viability and germination. *Weed Science* 45, 77 – 84.