

## Can desiccant application improve carrot seed quality?

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### Abstract

Though not a common practice, the desiccant diquat is applied to some carrot seed crops in Canterbury to facilitate seed harvest. In a laboratory experiment, diquat had reduced both mycelial growth and spore germination of the seed-borne carrot pathogen *Alternaria radicina*, the extent being application rate-dependent. In three field trials over two seasons, diquat was applied to carrot seed crops when 80-90% of the secondary umbels had turned brown (approximately four days before swathing) at a rate of 200, 400 or 600 ml active ingredient (ai)/ha. Seeds from five pre-tagged primary and secondary umbels per plot were hand harvested, cleaned and assessed for the presence of *A. radicina* and for germination. In control plots, seed-borne *A. radicina* ranged from 18 to 49%, differing with season and trial site, but seeds from secondary umbels always had higher infection. For all sites, diquat application significantly reduced the pathogen in seeds from both umbel positions, with infection decreasing as application rate increased. However, at 600 ml ai/ha, diquat negatively affected germination by killing the seeds. At the two lower rates, germination did not differ from the control, but the desiccant increased the number of dead seeds, whereas for the control the pathogen increased abnormal seedlings. While diquat does have fungicidal properties, further work will be required to determine whether its use can improve carrot seed quality.

**Additional keywords:** *Alternaria radicina*, diquat.

## Introduction

For Canterbury carrot seed crops, swathng when around 100% of the seeds on the secondary umbels have turned brown is most commonly the method used to facilitate seed harvest. However, occasionally the desiccant diquat (product name Reglone) is applied to the standing crop before swathng, particularly in wet seasons where plant tissues have not desiccated naturally. There is no published information on diquat use in carrot seed crops in New Zealand, but in Italy, Montanari and Lovato (1981) found that diquat applied at 600 ml ai/ha just prior to harvest had no effect on seed yield or quality, and in Russia, Mikheev *et al.* (2007) reported that applying diquat at 500 ml ai/ha six days prior to cutting increased seed yield and germination.

Diquat has previously been reported to have to have fungicidal properties (Wallnofer, 1968), and Abdel-Mallek and El-Shanawany (1994) found that diquat, when added to a sucrose agar medium, prevented sclerotium formation and mycelial growth of the pathogen *Sclerotium cepivorum*.

*Alternaria radicina* Meier, Drechsler, and Eddy, is a soil- and seed-borne pathogen of carrot which is currently causing quality problems in some New Zealand produced carrot seed lots (Trivedi, 2010). During germination, the pathogen attacks the developing seedling, either killing it or causing lesions to the extent that the seedling is classified as abnormal according to the Rules of the International Seed Testing Association (ISTA, 2010). Because of this problem, some seed lots fail to meet the germination standard required. In a laboratory experiment, Trivedi (2010) found that diquat at rates of active ingredient equivalent to 200, 400 and 600 ml ai/ha inhibited both mycelial growth and conidial germination of *A. radicina*, the inhibition increasing with application rate.

The objectives of the work presented in this paper were to determine the effects of diquat application to carrot seed crops on seed-borne *A. radicina* and on carrot seed germination.

## Materials and Methods

In the 2007/2008 seed production season a desiccation experiment using diquat was conducted at a mid-Canterbury farm (43°48'52.90"S 171°57'46.83"E) which had a hybrid carrot seed crop naturally infected with *A. radicina*. The male and female parent lines sown were MID C71 (♂) and MIDA5 (♀), although by the time of desiccant application, the male lines had been removed. The experimental area was situated within the commercial carrot seed crop. There were three blocks, with each treatment randomly distributed within each block. Diquat treatments were applied at 200, 400 and 600 ml ai/ha in 400 litres water/ha. Plot size was 5 × 3 m and included six rows of the female parent. In each plot, five primary and five secondary umbels were preselected for uniformity of maturity (80% brown seeds), and tagged before desiccant application.

To improve desiccant coverage, penetration and uptake, an organo-silicone surfactant (Highway at 25 ml/100 l water) was added to the spray mixture (Anonymous, 2010). Treatments were applied on 30 March 2008 when approximately 80% of the seeds on the secondary umbels had turned brown (four days before swathing). The two person hand-held spray rig was operated approximately 45 cm above the carrot plants at 30 psi and delivered 400 l of spray mixture per ha from flat fan 11004 DG nozzles spaced 50 cm apart along the spray boom. On 3 April the tagged primary and secondary umbels were hand harvested (stalk cut approximately 30 cm below the umbel) and placed into paper bags, which were kept open and placed in a glasshouse at Lincoln University for 5 days. Thereafter the stalks were removed and umbels were dried for 24 h at 30 °C to approximately 8% seed moisture, as determined using a portable seed moisture meter (Precisa XM60, Switzerland). Seeds were then hand removed from the dried umbels and were cleaned by rubbing between the hands to remove spines, dirt and other plant material, then thoroughly mixed and placed in a paper bag. The hands were washed in 70% ethanol between cleaning each sample to reduce the chances of contamination. Bags were placed into a sealed plastic container and stored at 8 °C for two months.

From the stored seeds from each plot, two sets of 50 seeds from each plot were selected at random and a total of 100 seeds were each tested for the presence of *A. radicina* and for germination.

For seed infection, seeds were placed using sterilised forceps onto a semi-selective agar (ARSA) (Pryor *et al.*, 1994) in a Petri dish, and incubated in the dark at 27 °C for 14 days. On ARSA, black hyphae of *A. radicina* grow downwards into the agar, and this distinguishes them from any other fungi growing (Pryor *et al.*, 1994). Seeds which produced these black hyphae were counted as infected seeds and those that did not as non-infected seeds (Pryor *et al.*, 1994).

For germination, seeds were spread equidistantly along a straight line in the centre of a folded germination paper towel (31 × 45.5 cm) (Anchor Paper Company, MN, USA) and moistened with sterile distilled water. The paper towel was folded with the seeds at the lower fold, then rolled and placed in a sealed plastic bag to prevent the roll from drying out; the bags were kept in an upright position in an incubator at 20°C for 14 days. After incubation, seedlings were evaluated as normal or abnormal seedlings (ISTA, 2010). Seedlings that had a strongly developed radicle and plumule or with slight defects or with a secondary infection were considered as normal, while those with deformities such as stunted roots or shoots, missing or fractured integral parts or decay as a result of primary infection caused by *A. radicina* were counted as abnormal. Non-germinated seeds which were soft, discoloured, and frequently mouldy were classed as dead (ISTA, 2010).

In the 2008/2009 seed production season the previous year's experiment was repeated at two mid-Canterbury farms (Farm 1 at 43°48'52.90"S 171°57'46.83"E and Farm 2 at 43°51'28.85"S 171°54'54.76"E) which had hybrid carrot seed crops naturally infected with the pathogen. At Farm 1 the male and female parent lines sown were MID C74 (♂) and MID A5 (♀); at Farm 2, the same female parent was used, but the male line was MID C75. The plot size, application rates and application method were the same as the previous season,

and five primary and five secondary umbels were again pre-selected for later harvest.

Treatments were applied at both farms on 13 March 2009 and umbels were hand harvested on 17 March. Seed processing and quality determination were as described for the previous year.

### **Statistical analysis**

Seed infection, normal and abnormal germination and dead seed percentages for each experiment were subjected to one-way ANOVA suitable for the randomised block design to compare the effects of desiccant application rate on seeds from each umbel order. Data for each umbel type and farm were analysed separately because there was substantial variation between seed maturity of the primary and secondary umbels, and different hybrids were produced at each farm in differing environment and management. As the data were normally distributed no transformation of the data was required. When the ANOVA produced a significant effect ( $P \leq 0.05$ ) then only the treatment mean values were further compared using Fisher's protected  $LSD_{0.05}$ . Since primary and secondary umbel data were separately analysed, to compare them the mean values are presented with their standard errors. Data analysis was performed using Genstat Edition 12.

### **Results**

#### 1. 2007/08 Season

##### (a) Seed infection

Seeds from primary and secondary umbels from the control plots did not differ in *A. radicina* infection (22% ( $\pm 1.1$ ) and 23% ( $\pm 3.1$ ) respectively). All three diquat application rates significantly reduced seed infection in seeds from both primary ( $P=0.05$ ) and secondary ( $P=0.05$ ) umbels. For seeds from primary umbels, seed infection decreased with increasing application rate, but for seeds from secondary umbels, infection did not differ among the application rates (Figure 1).

(b) Germination

Higher germination was recorded from seeds from the primary umbels than from the secondary umbels (Figure 2), and for the control the germinations were 60% ( $\pm 1.9$ ) and 33% ( $\pm 2.8$ ) respectively. The lower germination from secondary umbel seeds was associated with slightly more abnormal seedlings (28%  $\pm 1.9$  cf 22%  $\pm 1.3$ ) and considerably more dead seeds (39%  $\pm 2.7$  cf 17%  $\pm 2.5$ ) than for primary umbel seeds.

Diquat at the two lowest application rates significantly ( $P=0.05$ ) increased the germination of seeds from the primary umbels, but only the lowest rate increased germination of seeds from the secondary umbels. At the highest application rate, diquat increased the percentage of dead seeds from both umbel positions (Figure 2).

2. 2008/09 Season

(a) Seed infection

Seeds from the primary and secondary umbels at Farm 1 carried more *A. radicina* (33% ( $\pm 1.5$ ) and 49% ( $\pm 1.4$ ) respectively) than did those from Farm 2 (18% ( $\pm 1.2$ ) and 26% ( $\pm 1.0$ ) respectively). However, at both sites and for both umbel types, diquat application significantly ( $P=0.05$ ) reduced seed-borne *A. radicina*, with the decrease tending to increase with increasing application rate, the exception being at Farm 2 where infection of the primary umbel seeds at the lowest rate did not differ from that of the control (Figure 3).

(b) Germination

As in the previous year, germination of seed from primary umbels was greater than that from secondary umbels (Figure 4; Figure 5), and the germination of seed from both umbel positions was greater at Farm 1 (80% ( $\pm 1.5$ ) and 64% ( $\pm 3.0$ ) respectively for the control) than at Farm 2 (75% ( $\pm 1.6$ ) and 55% ( $\pm 1.7$ ) respectively for the control). At Farm 1, seeds from the primary umbel had a small increase ( $P=0.05$ ) in germination at the two lowest diquat application rates

(Figure 4), but there was no increase at Farm 2 (Figure 5). The intermediate rate (400 ml ai/ha) of diquat provided a small increase (by 6.5%;  $P=0.05$ ) in germination of seeds from the secondary umbel at Farm 1 (Figure 4), whereas at Farm 2 the lowest rate (200 ml ai/ha) provided a small increase (by 4%;  $P=0.05$ ) in germination as compared to the control (Figure 5).

Diquat at all three application rates decreased ( $P=0.05$ ) the percentage of abnormal seedlings from seeds from both umbel orders at both farms (Figure 4; Figure 5), but at both sites, diquat at the highest rate significantly ( $P=0.05$ ) increased dead seeds (Figure 4; Figure 5).

## **Discussion**

The results presented in this paper from two seasons of field trials support the previous laboratory testing (Trivedi, 2010) and have demonstrated that diquat can offer some control of seed-borne *A. radicina* when applied to the umbels shortly before harvest. As in the laboratory testing control was dependent on the application rate. At 600 ml ai/ha, from 40% to 70% (based on the 2007/2008 secondary umbel infection decreasing from 23% to around 7%) fewer harvested seeds carried the pathogen. This is the first report of diquat activity against *A. radicina*.

There are very few previous reports of fungicidal activity of diquat following field application. Ivaniuk and Brukish (1999) reported a reduction in the potato diseases caused by *Phytophthora infestans* and *Rhizoctonia solani* in Russia while Jordan and Allen (1984) found that a mixture of diquat and paraquat heavily reduced sporulation of *Pyrenophora teres* on barley stubble. A mixture of diquat and paraquat has also been reported to reduce soil inoculum levels and cereal stubble colonisation of the take-all fungus, *Gaeumannomyces graminis* in Western Australia (Sivasithamparam and Bolland, 1985; Mekwatanakarn and Sivasithamparam, 1987). Since, *A. radicina* can saprophytically colonise tissues, it may be possible that diquat produces the same kind of effect on the pathogen in carrots.

During their development, seeds undergo a natural transition from desiccation intolerant to desiccation tolerant (Kermode *et al.*, 1986). When using a desiccant, care must be taken to ensure that seeds are dry enough to prevent the rupture of cells and subsequent release of destructive hydrolytic enzymes (Bewley and Black, 1994) resulting in germination loss. Several authors (Austin and Longden, 1968; Gubbels and Kenaschuk, 1981; Miller, 2002) have reported germination reductions following diquat application, but these effects were mostly related to time of application (i.e. seeds were still desiccation intolerant). In Canterbury, desiccation of large seeded legumes including pea, bean and soybean at seed moisture contents of less than 40% has not affected germination (Taweekul, 1999; Greven *et al.*, 2001; Rahman *et al.*, 2004).

The germination responses in both 2008 and 2009 were affected by both *A. radicina* and diquat. With the exception of seeds from the secondary umbel in 2008 physiological germination (i.e. normal seedlings plus abnormal seedlings) was more than 80%. However, when assessed according to internationally standardised rules (ISTA, 2010), the presence of abnormal seedlings resulting from *A. radicina* infection reduced germination (i.e. percentage normal seedlings) to between 60-80%. In 2008 diquat application at 200 and 400 ml ai/ha increased germination of seeds from the primary umbels by decreasing abnormal seedlings, and this also occurred for the lower rate for seeds from the secondary umbels. A similar response was recorded from Farm 1 in 2009 for seeds from both umbel positions, but only for seeds from secondary umbels at Farm 2. However, the 600 ml ai/ha application rate consistently reduced germination by increasing the percentage of dead seeds at all three sites. Miller (2002) reported that in spinach and coriander, the effect of diquat application on germination was rate dependent, but Mikheev *et al.* (2007) considered that for carrot, 500 ml ai/ha at 6 days prior to seed harvesting was the optimum diquat application rate to increase germination and yield.

Neither Montanari and Lovato (1981) or Mikheev *et al.* (2007) reported carrot seed germination loss following diquat application at 600 and 500 ml ai/ha, respectively. Their results therefore contrast with those reported in this paper.



Application time can not explain the differing germination results. However one possible difference between the present work and that reported from Europe is the presence of *Alternaria*. Fungal damage to carrot seed coats may have allowed direct diquat access to the embryo, but whether this did occur is unknown.

## **Conclusion**

This study has demonstrated that diquat has fungicidal activity against the seed-borne carrot pathogen *A. radicina*, and that the reduction in inoculum obtained is application rate dependent. However, at the highest rate (600 ml ai/ha) germination was reduced because seeds were killed, and the 400 ml ai/ha application rate effects on germination may be marginal. Further work on application rate and timing will be required before any recommendation could be made for the use of diquat to control *A. radicina*.

## **Acknowledgements**

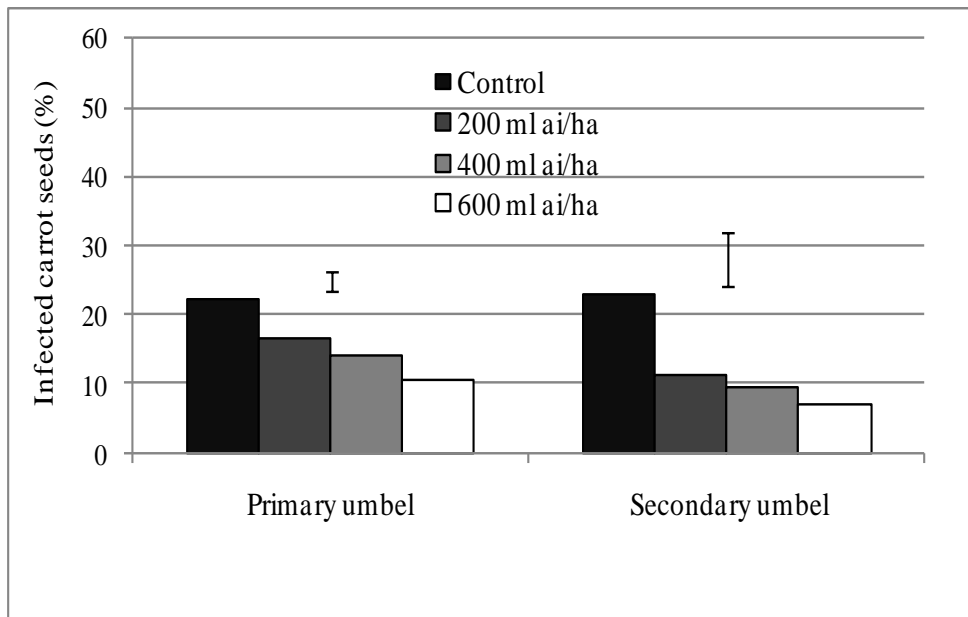
We thank the Foundation for Research, Science and Technology and Midlands Seed Ltd for financial support, and Messrs Lowe, Watson and Hewson for providing trial sites.

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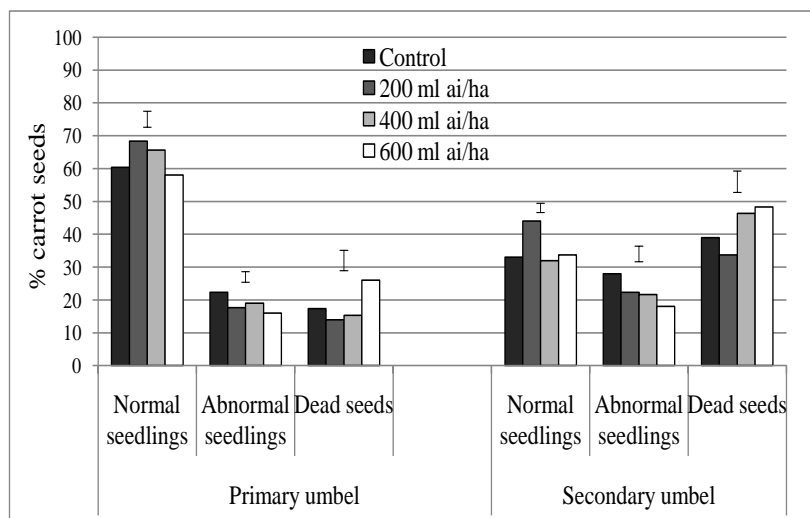
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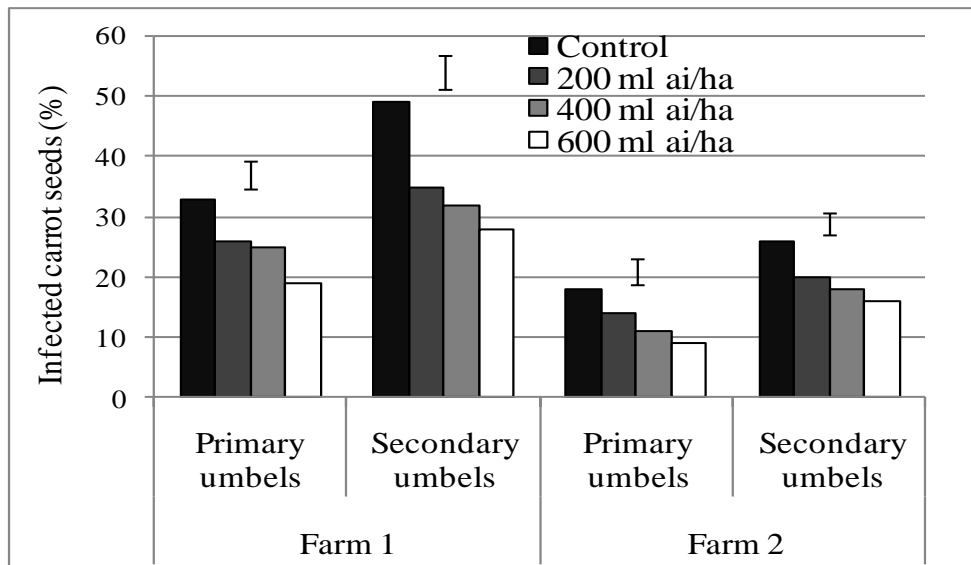
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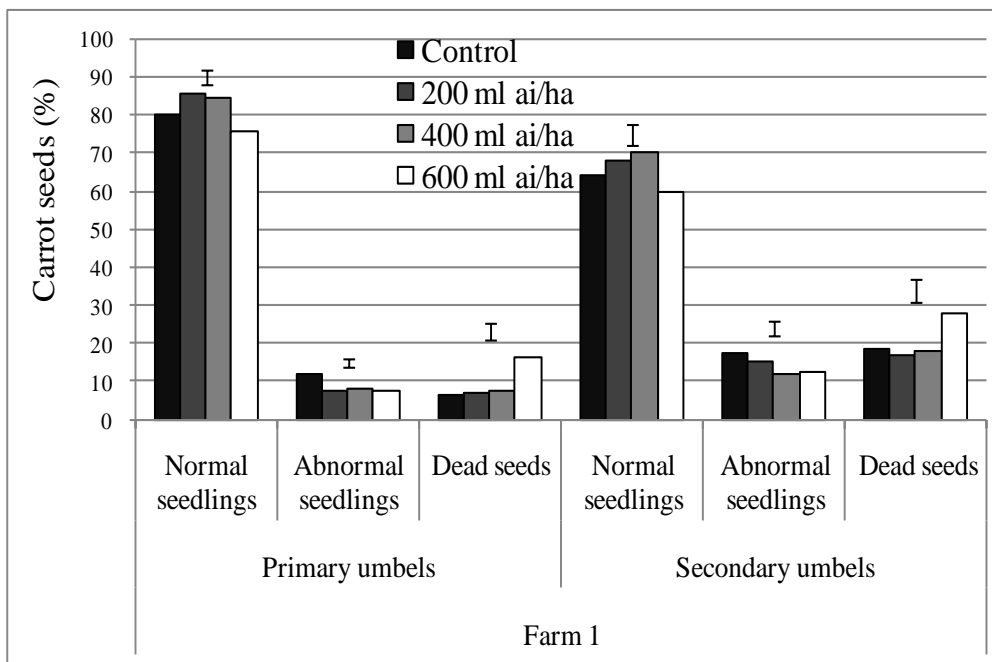
**Figure 1:** Effect of diquat on *Alternaria radicina* in carrot seeds from primary and secondary umbels harvested in 2008. Seeds from primary and secondary umbel were analysed separately. Bars are the least significant differences (P=0.05).



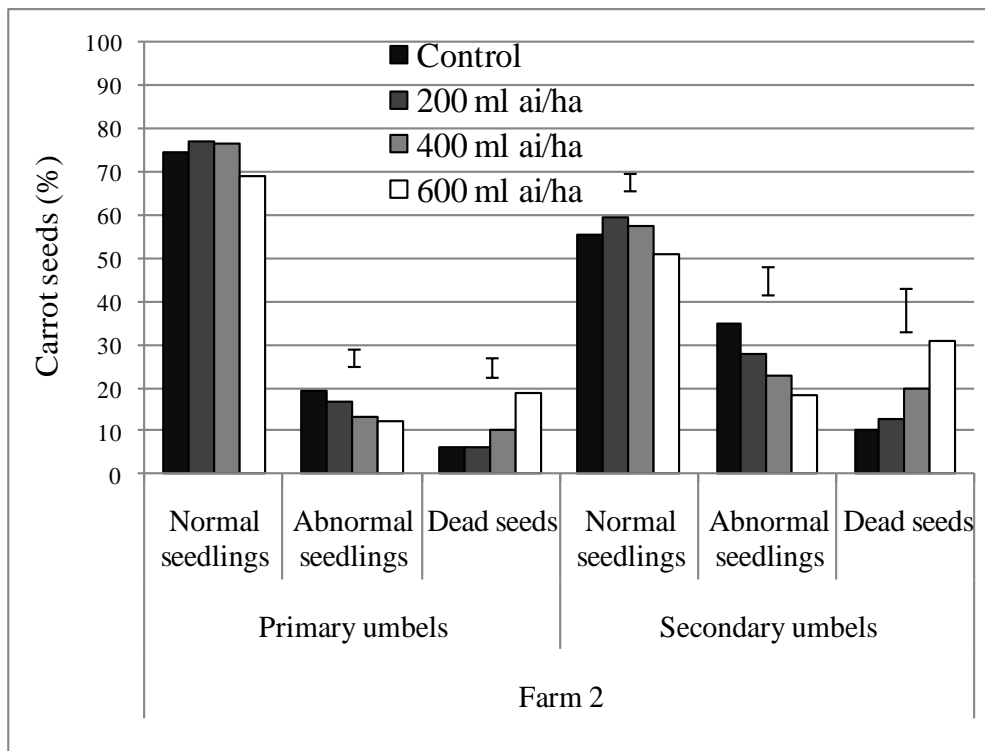
**Figure 2:** Effect of diquat on germination of carrot seeds from the primary and secondary umbels harvested in 2008. Germination data from primary and secondary umbel were analysed separately. Bars are the least significant differences (P=0.05).



**Figure 3:** Effect of diquat on *Alternaria radicina* in carrot seeds from primary and secondary umbels harvested from two farms in 2009. Seeds from primary and secondary umbel from each farm were analysed separately. Bars are the least significant differences (P=0.05).



**Figure 4:** Effect of diquat on germination of carrot seeds from primary and secondary umbels harvested at Farm 1 in 2009. Germination data from primary and secondary umbel were analysed separately. Bars are the least significant differences (P=0.05).



**Figure 5:** Effect of diquat on germination of carrot seeds from primary and secondary umbels harvested at Farm 2 in 2009. Germination data from primary and secondary umbel were analysed separately. Bars are the least significant differences ( $P=0.05$ ). Where no bars are shown treatment means were not significantly different.