Low temperatures reduce glufosinate efficacy against *Raphanus raphanistrum* L. and *Sisymbrium orientale* L.

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Summary Glufosinate efficacy was examined in two major broad leaf weed species *Raphanus raphanistrum* L. and *Sisymbrium orientale* L. Dose response studies were conducted in controlled environmental chambers under cool (5/10°C) or warm (20/25°C) temperatures. These studies demonstrated that *R. raphanistrum* when grown under cool temperatures was poorly controlled with 1200 g ha^{-1} glufosinate, whereas half this rate was sufficient to cause 100% mortality under warm temperatures. A seven-fold enhancement of glufosinate efficacy occurred in *R. raphanistrum* under warm temperatures. In contrast, *S. orientale* was easily controlled with glufosinate under cool conditions and only a two-fold enhancement of efficacy under warm conditions occurred. Studies on absorption and translocation of ^{14}C-glufosinate in *R. raphanistrum* showed no differences in absorption, but basipetal translocation of glufosinate was greatly decreased at cool temperatures. Therefore, it is likely the poor control of *R. raphanistrum* at low temperatures is due to reduced accumulation of glufosinate in the meristematic region and newly expanding leaves.
Keywords Glufosinate, *Raphanus raphanistrum*, *Sisymbrium orientale*.

INTRODUCTION

Raphanus raphanistrum is a major broad leaf weed of cropping systems in southern Australia and due to its close relationship to canola, growers are unable to use herbicides to control this weed within conventional canola crops. The introduction of canola cultivars resistant to triazine and imidazolinone herbicides has made it possible to control this weed in the crop. However, *R. raphanistrum* populations have already evolved resistance to the triazines and ALS-inhibiting herbicides (Hashem *et al.* 2001a,b). The introduction of Liberty Link™ canola (resistant to glufosinate) will provide a new herbicide for weed control within the crop and a herbicide to which weeds have not yet evolved resistance. Glufosinate is a nonselective post emergence contact herbicide developed from the microbial phytotoxin alanyl-alanyl-phosphinothricin (Bayer *et al.* 1972). Although glufosinate is considered to be a non-selective herbicide, its efficiency can depend on various environmental conditions and other factors such as the weed species and application rates (Carlson and Burnside 1984). Anderson *et al.* (1993) demonstrated that both temperature and relative humidity have a considerable effect on the activity of glufosinate. Previous studies have also shown that glufosinate is not translocated extensively from the site of application (Haas and Muller 1987, Bromilow *et al.* 1993, Steckel *et al.* 1997). To date the reasons for this limited translocation are unknown (Beriault *et al.* 1999).

In trials conducted in Australia, control of *R. raphanistrum* by glufosinate was variable, however, acceptable control occurs in Europe (Mike Clarke, personal communication). As southern Australia has predominantly cool and wet winters, temperature may be an important factor in glufosinate efficacy against *R. raphanistrum*. This study investigated the role that temperature plays in glufosinate efficacy in *R. raphanistrum* in relation to *Sisymbrium orientale*, another species belonging to the Brassicaceae family.

MATERIALS AND METHODS

Dose response studies Experiments were performed in controlled growth chambers using populations of *R. raphanistrum* and *S. orientale* collected from South Australia and known to be susceptible to all herbicides used for the control of these species. Dehulled *R. raphanistrum* seeds were soaked in 50% (v/v) solution of sodium hypochlorite for 30 minutes and then the seeds were soaked in water for another 30 minutes. The seeds were germinated on 0.6% (w/v) agar in a germination cabinet with 12 h, 19°C day (30 µmol m^{-2} s^{-1}) and a 12 h, 19°C night after incubating at 4°C for 24 h. *S. orientale* seeds were germinated in moist potting soil in a growth room at temperatures ranging from 15 to 20°C. Germinated seedlings of both species were transplanted to 15 cm square pots filled with potting soil. Each pot contained four *R. raphanistrum* seedlings or six *S. orientale* seedlings. Plants were grown in controlled growth chambers where they were provided with cool (10°C days with a light intensity of 553 µmol m^{-2} s^{-1} and 5°C nights) or warm (25°C days with a light intensity of 553 µmol m^{-2} s^{-1} and 20°C nights) temperatures. Relative humidity was maintained between 60 and 70%. At the 2–4 leaf stage, seedlings

were sprayed with glufosinate (as Basta™). Herbicide was applied with a laboratory moving boom sprayer equipped with 110° flat fan nozzles at a speed of 1 m s^{-1}. The output of the sprayer was calibrated at 125 L ha^{-1} at a pressure of 250 kPa. Dose rates ranged from 0 to 1200 g a.i. ha^{-1}. Plants were returned to the growth room after the spray treatment and were assessed for survival 30 days after treatment. The lethal dose to kill 50% of the population (LD_{50}) was calculated by probit analysis.

Absorption and translocation studies Experiments on absorption and translocation of glufosinate were performed using seedlings of *R. raphanistrum*. These were germinated and grown as described above at either 5/10°C or 20/25°C, except five seedlings were grown in each pot. When the plants had just reached the 3-leaf stage they were sprayed with glufosinate (300 g a.i. ha^{-1}) and immediately afterwards 2 µL of ^{14}C glufosinate (450 Bq μL^{-1} made up in a commercial formulation of glufosinate at 2.4 g L^{-1}) was applied to the centre of a fully expanded leaf on either side of the mid vein and the area of application clearly marked. The pots were kept in the same growth room after treatment. Plants were harvested 72 hours after treatment (HAT). Each plant was divided into five segments, the treated area (treated zone), area above the treated section (leaf tip), basal portion of the treated leaf (leaf base), apical meristem and untreated leaves. The treated zone was washed in 5 mL of 0.1% (v/v) Triton X-100 in water. The amount of radioactivity present in the wash solution was determined by liquid scintillation spectroscopy (LSS) following the addition of scintillation cocktail. Harvested shoots were dried in an oven at 60°C for 24 hours and combusted in a biological sample oxidiser. Radioactive CO_2 released was trapped in a 1:1 ratio of carbon trap and scintillation cocktail mix. The amount of radioactivity per sample was analysed by LSS. Absorption of ^{14}C-glufosinate was calculated as a percentage of the radiolabel applied. Translocation of ^{14}C-glufosinate was calculated as a percentage of radiolabel absorbed.

RESULTS

Dose response studies Application of glufosinate to *R. raphanistrum* plants under cool conditions provided poor control of this species. The highest rate of glufosinate (1200 g ha^{-1}) controlled only 47% of plants (Figure 1A). In contrast, the same population grown under warm temperatures had 100% mortality at half this rate (600 g ha^{-1}). *R. raphanistrum* seedlings grown under cold temperatures showed signs of damage after application of 300 g ha^{-1} glufosinate, particularly tip burn, tip curling and bleaching of leaves. However, 92% of the treated population regenerated following this treatment. In contrast, *R. raphanistrum* seedlings grown under warm temperatures were severely affected by this rate of glufosinate and only 8% regenerated. Based on LD_{50} values *R. raphanistrum* grown under warm temperatures was seven fold more susceptible to glufosinate when compared to plants grown under cold temperatures (Table 1).

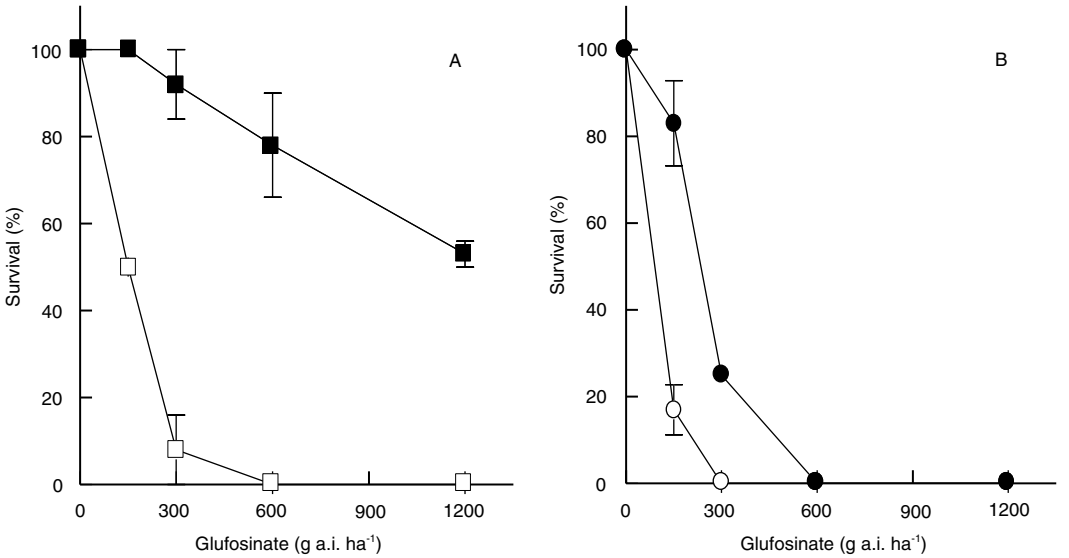

Figure 1. Survival of *R. raphanistrum* (A) and *S. orientale* (B) treated with glufosinate under cool temperatures 5/10°C (■,●) or warm temperatures 20/25°C (□,○).

In contrast to *R. raphanistrum*, *S. orientale* was better controlled by glufosinate under cool temperatures. With 300 g ha^{-1} glufosinate, severe bleaching occurred in leaves with prominent tip burn and curling. Under cool conditions only 25% of plants regenerated at this herbicide rate. Under warm conditions 100% mortality was achieved (Figure 1B). Based on LD_{50} values, *S. orientale* was about five times more sensitive to glufosinate under cool conditions than *R. raphanistrum* (Table 1). Under warm temperatures the efficacy of glufosinate on *S. orientale* was further enhanced.

Cool temperatures decreased the efficacy of glufosinate in both species. However, there were marked differences between the species. While *S. orientale* was still relatively easy to control with glufosinate under cool conditions, control of *R. raphanistrum* was lost.

Absorption and translocation studies ^{14}C-glufosinate was readily absorbed by leaves of *R. raphanistrum* with 95% of the herbicide absorbed within 72 HAT under both temperature regimes (not shown). There were no differences in the total amount of glufosinate absorbed at the two temperatures. Considerably more glufosinate was translocated in the xylem to the tip of the treated leaf under cool temperatures when compared to warm temperatures (Table 2). In contrast, basipital translocation of glufosinate was significantly greater under warm temperatures compared to cool temperatures.

There was limited phloem mobility of glufosinate under cool temperatures and much greater mobility under warm temperatures. In particular, the amount of glufosinate found in the meristematic region and in the untreated leaves was much lower under cool temperatures. For mortality to occur following glufosinate application, the herbicide needs to be translocated to new leaves emerging from the meristem, otherwise the plants will regenerate. Under cool temperatures only a minute amount of herbicide was translocated to the meristem or to untreated leaves (Table 2).

Table 1. LD_{50} values for glufosinate of *R. raphanistrum* and *S. orientale* under cool or warm temperatures.

Species	Cool (5/10°C)	Warm (20/25°C)
	LD_{50} (g ha^{-1})	
R. raphanistrum	1160	165
S. orientale	237	125

Table 2. Distribution of ^{14}C-glufosinate in *R. raphanistrum* plants 72 HAT under cool or warm temperatures.

Plant part	Cool (5/10°C)	Warm (20/25°C)
	Glufosinate detected (% absorbed)	
Treated zone	43.10 ± 3.70	35.80 ± 6.11
Leaf tip	53.44 ± 5.43	34.13 ± 9.20
Leaf base	3.12 ± 3.12	20.43 ± 10.75
Meristematic zone	0.02 ± 0.02	4.37 ± 2.15
Untreated leaves	0.34 ± 0.14	5.30 ± 3.90

DISCUSSION

This study has demonstrated that glufosinate efficacy on two Brassicaceae weeds is reduced at low temperatures (Figure 1, Table 1). Previous studies have also indicated that although glufosinate is considered to be a non-selective herbicide, its efficiency depends on various environmental conditions and other factors such as the weed species and application rates (Carlson and Burnside 1984). Ridley and McNally (1985) reported differences of up to 70 fold in tolerance of seven annual weed species to glufosinate. Similarly, Mersey *et al.* (1990), Steckel *et al.* (1997) and Pline *et al.* (1999) observed variability in glufosinate efficacy among different weed species. Anderson *et al.* (1993) concluded that low temperatures prior to glufosinate application reduced efficacy in *Hordeum vulgare* L. and *Setaria viridis* (L.) P.Beauv. The same study also demonstrated that as the temperature was decreased less ammonia was produced in treated plants.

Environmental conditions are also known to influence efficacy of other herbicides. For example, Purba *et al.* (1995) demonstrated that paraquat resistant populations of both *Hordeum leporinum* Link. and *H. glaucum* Steud. were highly resistant when grown and treated under cool conditions. However, the same populations were more susceptible to paraquat when grown and treated under warm conditions. McWhorter *et al.* (1980) reported that in *Sorghum halepense* (L.) Pers. absorption of glyphosate was approximately doubled and the translocation slightly increased as the air temperature was raised from 24° to 35°C.

It is likely that the reason for poor efficacy of glufosinate on *R. raphanistrum* under cool temperatures is the greatly reduced translocation of glufosinate to the meristem and newly emerging leaves (Table 2). Temperature is known to influence the basipetal translocation of herbicides, for example Purba *et al.* (1995) in their study with paraquat resistant *Hordeum leporinum* showed greater basipital translocation under warm temperatures. Similarly, Xie *et al.* (1996) showed that basipital translocation of imazamethabenz-methyl was increased in *Avena fatua* L. by high temperatures. Duke and Hunt (1977) reported that cool conditions were responsible for the lack of glyphosate translocation in *Agropyron repens* (L.) P.Beauv. at temperatures

below 7°C. Harker and Dekker (1988) showed strong evidence indicating higher temperatures increased basipital translocation of a range of herbicides including glyphosate, sethoxydim, cloproxydim and fluazifop-butyl in *A. repens*.

The current study also demonstrated that *S. orientale* was more easily controlled when grown under cool temperatures than *R. raphanistrum* (Figure 1, Table 1), suggesting that while temperature can play an important role in glufosinate efficacy, efficacy is also greatly influenced by the plant species involved. The studies with ^{14}C-glufosinate demonstrated that translocation of this herbicide to the meristematic zone in *R. raphanistrum* is vital to efficacy, such that increased translocation to the meristematic zone results in increase herbicide efficacy (Table 2, Figure 1). Where environmental variables, such as temperature, significantly reduce translocation a reduction in efficacy can be expected. Such is the case with *R. raphanistrum* where poor efficacy of glufosinate is achieved under the cool, wet conditions prevalent during winter in Southern Australia.

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