Seed Technology

An International Journal Serving Seed Scientists and Technologists

Seed Germination Differences between Glyphosate-Resistant and -Susceptible Italian Ryegrass Populations

Vijay K. Nandula*, Daniel H. Poston and Krishna N. Reddy

Published Jointly by the Association of Official Seed Analysts
and the Society of Commercial Seed Technologists

Seed Germination Differences between Glyphosate-Resistant and -Susceptible Italian Ryegrass Populations

Vijay K. Nandula*, Daniel H. Poston and Krishna N. Reddy

ABSTRACT

Influence of environmental factors on germination and emergence of Italian ryegrass [*Lolium perenne* L. subsp. *multiflorum* (Lam.) Husn.], including glyphosate-resistant populations, is lacking. Such knowledge would help improve our understanding of the biology and ecology of this problem species, which could in turn, aid in its management. The objective of this study was to determine the effects of temperature, light, pH, salt and osmotic stress, shikimic acid and planting depth on germination of glyphosate-resistant (R) and susceptible (S) Italian ryegrass populations. Overall, germination of both populations of Italian ryegrass was highest at 13 °C and decreased when temperature increased to 20 or 27 °C under both light and dark conditions. Light stimulated germination (57%) compared to darkness (41%) at 13 °C, but light had no effect on germination at 20 and 27 °C. The resistant population had higher germination (69–87%) compared to the susceptible (37–57%) at a pH range of 4–7. Seed germination decreased as NaCl concentration increased from 20–160 mM and osmotic potential increased from 0 (distilled water) to −0.8 MPa in both populations. Germination of the R and S populations decreased from 76% to 25% and 67% to 12%, respectively, as shikimic acid concentration increased from 0–16 mM. Seedling emergence was highest from seed placed on the soil surface. Seedling emergence was less than 7% from seed planted at a 0.5 cm depth and no seedlings emerged from seed planted below 2.5 cm for both populations. Both populations germinated under a broad range of environmental conditions used in the study, however, the R population was higher than the S population.

INTRODUCTION

Glyphosate [N-(phosphonomethyl) glycine] is a non-selective, broad spectrum, systemic, post-emergence herbicide that has been used extensively throughout the world over the past three decades. With the commercialization of glyphosate-resistant crops in the mid 1990s, glyphosate is now widely used for weed control in glyphosate-resistant crops in addition to non crop lands. The intense use of glyphosate in resistant crops has increased the selection pressure on many weed species and several species have developed glyphosate resistance. To date, 16 weed species are reported to be resistant to glyphosate (Heap, 2009). We have reported a three-fold resistance to glyphosate in two

*V.K. Nandula, Mississippi State University, PO Box 197, Stoneville, MS 38776; D.H. Poston, Pioneer Hi-Bred International, Inc., PO Box 386, Stoneville, MS 38776; K.N. Reddy, USDA-ARS, PO Box 350, Stoneville, MS 38776. *Corresponding author (E-mail: vnandula@drec.msstate.edu.) Received 31 October 2008.

Italian ryegrass [*Lolium perenne* L. subsp. *multiflorum* (Lam.) Husn.] populations in Mississippi, USA (Nandula et al., 2007).

Italian ryegrass is an erect winter annual with a biennial-like growth habit. It germinates in the fall (September–November), grows vigorously in the winter (December–February) and early spring (March–June), and is highly competitive. The success of a weed is dependent on the ability of its seed to germinate and emerge under a wide range of environmental conditions. Various environmental factors such as temperature, light, pH, and soil moisture influence weed seed germination (Chachalis and Reddy, 2000; Koger et al., 2004; Taylorson, 1987). Chauhan et al. (2006) have examined the effects of several environmental factors on seed germination and seedling emergence of rigid ryegrass (*Lolium rigidum* Gaudin), a close relative of Italian ryegrass. However, very little information exists about the effect of environmental factors on Italian ryegrass germination. Furthermore, the effects of environmental factors on germination and emergence of glyphosate-resistant weed species is mostly lacking. Literature on side-by-side comparison of effects of environmental factors on glyphosate-resistant and -susceptible Italian ryegrass is non-existent. Information about glyphosate-resistant weed germination would help estimate the potential for their spread to new croplands and intensity of infestation. Therefore, the objective of this study was to determine the effects of temperature, light, pH, salt and osmotic stress, shikimic acid and planting depth on germination of glyphosate-resistant and -susceptible Italian ryegrass populations.

MATERIALS AND METHODS

Seed source and general experimental procedure

Two Italian ryegrass populations, a glyphosate-resistant (Tribbett) and a glyphosate-susceptible (Elizabeth), hereafter referred to as R and S, respectively, were used in the studies. Detailed information about these populations, seed selection and storage procedures have been described by Nandula et al. (2007). Briefly, the Tribbett population was collected from a field planted to glyphosate-resistant cotton (*Gossypium hirsutum* L.) in 2001 to 2003 and glyphosate-resistant soybean [*Glycine max* (L.) Merr.] in 2004 and 2005. Reduced control of Italian ryegrass following application of glyphosate at 0.84 kg ai/ha has been observed since 2002 at the Tribbett population site. The Elizabeth population was collected from an abandoned home garden site with no history of herbicide applications including glyphosate. Both sites are located near Stoneville (N33° 22′ 30″ W90° 52′ 30″), Mississippi, USA. Italian ryegrass seed was collected in the summer of 2005 and air-dried in the greenhouse (28/20 °C day/night) for 48 h. Dry seed were shaken and stripped from panicles, dehulled, visually sorted to remove unwanted residues, broken/immature/discolored seed, and stored in a freezer at 0 °C until further use. Seed collection was two weeks after Italian ryegrass plants had reached maturity. Before setup of experiments including preliminary trials, seeds were warmed to room temperature and re-inspected for uniformity. Preliminary experiments with untreated seeds produced ≤ 20% germination; therefore, seeds were prechilled.

Prechilling (also called stratification) is described as the exposure of moist seeds to cold temperatures for a specific period of time (Bewley and Black, 1982).

Twenty five seeds were placed on a single layer of Whatman #1 filter paper (Fisher Scientific, Pittsburgh, PA, USA) moistened with 5 ml distilled water in a 9-cm plastic petri dish. Petri dishes were sealed with Parafilm (American National Can, Greenwich, CT, USA) and then placed in dark for 48 h at 4 ± 2 °C. Prechilled seeds were transferred to a new petri dish lined with a new filter paper moistened with 5 mL of distilled water or treatment solution. Dishes were sealed as described above and placed in a growth chamber set to 10.5 h light (300 μmol m^{-2} s^{-1} obtained from a combination of fluorescent and incandescent bulbs)/13.5 h dark cycle at a constant temperature of 13 °C, unless specified otherwise. Germination measurements were taken at 7 d after treatment (DAT) at which time experiments were terminated as there was no further increase in Italian ryegrass germination after 7 DAT. Seed was considered germinated when the radicle visibly protruded through the seed coat. Percent seed germination was calculated by multiplying the ratio of germinated seeds to total number of seeds in a single petri dish by 100.

Temperature and light

Germination was determined in growth chambers under constant 13, 20, and 27 °C temperatures and 10.5, 11.5, and 12.5 h photoperiods. Considering the growth chamber effect as random, the second run of the experiment was conducted in a growth chamber different from the one in which the first run was carried out. A set of petri dishes was wrapped in a layer of aluminum foil to study seed germination in the dark under all temperature regimes.

The above temperature and photoperiod measurements represent respective average soil temperature at 5–10-cm-depth and daylength in September, October, and November, based on 30-year average weather data at Stoneville (Boykin et al., 1995). We have noted that Italian ryegrass emergence occurs in the fall (September–November) in the Mississippi Delta. Furthermore, some type of fall tillage is commonly practiced; therefore, temperature treatments were based on average fall temperatures for tilled soil.

pH

The effect of pH on germination of Italian ryegrass was studied using buffer solutions ranging from pH 4–10 prepared as described previously (Chachalis and Reddy, 2000). A 2-mM KHP (potassium hydrogen phthalate) buffer solution was adjusted to pH 4 with 1 N HCl (hydrochloric acid). A 2-mM solution of MES [2-(*N*-morpholino) ethanesulfonic acid] was adjusted to pH 5 and 6 with 1 N NaOH (sodium hydroxide). A 2-mM solution of HEPES [*N*-(2-hydroxymethyl) piperazine-*N*-(2-ethanesulfonic acid)] was adjusted to pH 7 and 8 with 1 N NaOH. Buffer solutions of pH 9 and 10 were prepared with 2-mM tricine [*N*-tris(hydroxymethyl) methylglycine] and adjusted with 1 N NaOH. Unbuffered distilled water (pH 6.7) was used as a control. Petri dishes were incubated at 13 °C with 10.5 h light, the conditions that provided maximum germination in the temperature and light experiment.

Salt and osmotic stress and shikimic acid

Sodium chloride (NaCl) solutions of 0, 10, 20, 40, 80, and 160 mM were prepared with distilled water. Aqueous solutions with osmotic potentials of 0, −0.05, −0.10, −0.20, −0.40, and −0.80 MPa were prepared by dissolving appropriate amounts of polyethylene glycol (PEG) 8000 in distilled water (Steuter et al., 1981). Shikimic acid solutions of 0, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 mM were prepared using distilled water. Petri dishes were incubated as described in the pH study.

Planting depth

Twenty-five Italian ryegrass seeds were planted in soil (Bosket sandy loam, fine-loamy, mixed, thermic Mollic Hapludalfs) in 10-cm × 10-cm plastic pots (National Polymers Inc., Lakeville, MN, USA) at depths of 0, 0.5, 1.0, 2.5, 5.0, and 7.5 cm. Pots were placed in a greenhouse at 13 °C with 10.5 h light and sub-irrigated as needed to maintain adequate soil moisture. Seedlings emerged were counted 7, 14 and 21 d after planting (DAP). Seedlings were considered emerged when the coleoptiles could be visually discerned above the soil surface.

Statistical analysis

A completely randomized design with three replications was used in all experiments. Experiments were repeated and the data represent the average of the two experiments due to a nonsignificant experiment by treatment interaction and homogeneity of variance according to Bartlett's test, except for the pH experiments. The pattern of pH influence on Italian ryegrass germination was consistent between repeated experiments; hence, data from a single experiment are reported. In all experiments, percent germination data were transformed using the log(x + 1) transformation, where x is percent germination. Transformation of data did not improve homogeneity, hence, ANOVA and regression analysis was performed on non-transformed percent germination. Means were separated using Fisher's protected LSD test at "P = 0.05".

RESULTS AND DISCUSSION

Temperature and light

Temperature significantly affected germination at 7 DAT in both the R and S populations. Overall, germination of both Italian ryegrass populations was highest at 13 °C and decreased when temperature increased to 20 or 27 °C under both light and dark conditions (Table 1). The R population germinated significantly better than the S population at all temperature and light combinations, except at 27 °C under light conditions (Table 1). Light affected germination of the two populations differently. It stimulated germination (57%) compared to darkness (41%) at 13 °C, but it had no effect on germination at 20 and 27 °C for the S population. Light had no effect on germination at 13 °C while it inhibited germination at 20 and 27 °C for the R population. We suspect temperature rather than light played a greater role in influencing Italian ryegrass germination because the highest germination of either population occurred at the lowest temperature (13 °C) regardless of light conditions. Conversely, light enhancement of seed germination has been reported in other weed species, including goosegrass [*Eleusine indica* (L.) Gaertn., Nishimoto and McCarty, 1997], horseweed [*Conyza canadensis* (L.) Cronquist, Nandula et al., 2006], johnsongrass [*Sorghum halepense* (L.) Pers., Benech Arnold et al., 1990], and purple nutsedge (*Cyperus rotundus* L., Miles et al., 1996).

pH

Seed germination ranged from 37–87% over a pH range from 4–10 in both the R and S populations (Fig. 1). Seed germination of both populations tended

TABLE 1. Effect of temperature and light on germination of two Italian ryegrass (*L. perenne* subsp. *multiflorum*) populations.

	Germination 7 DAT†			
	Light (10.5 h photoperiod)		Dark (24 h)	
Temperature	R	S	R	S
°C	%			
13	85	57	88	41
20	51	24	67	26
27	32	22	47	19
LSD (0.05)	15			

†Abbreviations: **DAT**, days after treatment; **R**, Resistant and **S**, Susceptible to glyphosate.

FIGURE 1. Effect of buffered pH solutions on germination of Italian ryegrass (*L. perenne* subsp. *multiflorum*) seed from two populations (susceptible or resistant to glyphosate) incubated at 13 °C with a 10.5-h photoperiod for 1 week. Unbuffered distilled water (DW, pH 6.7) was also included. Vertical bars represent standard error of means.

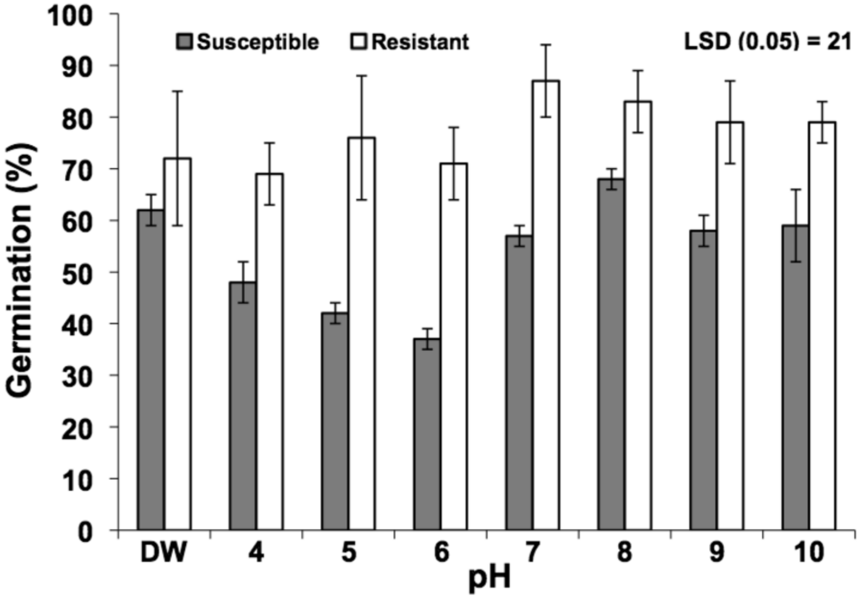
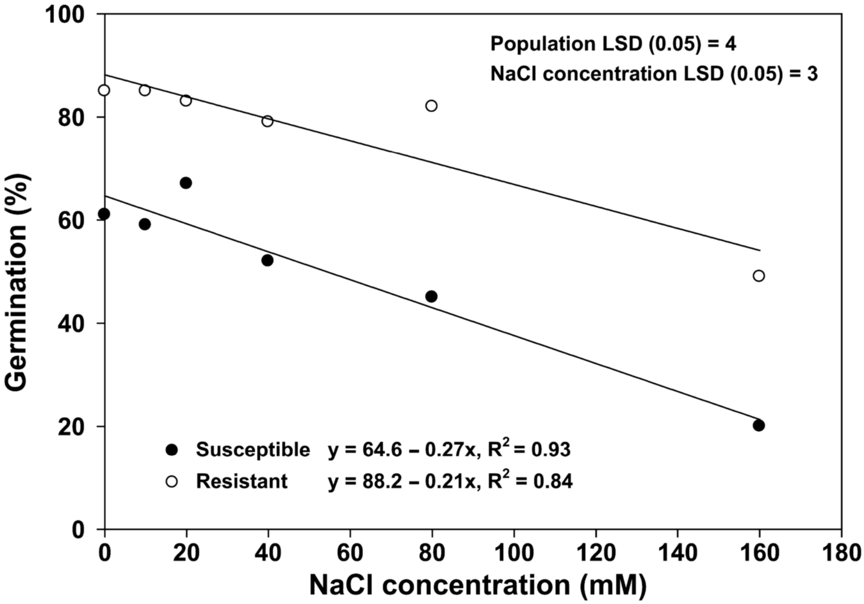
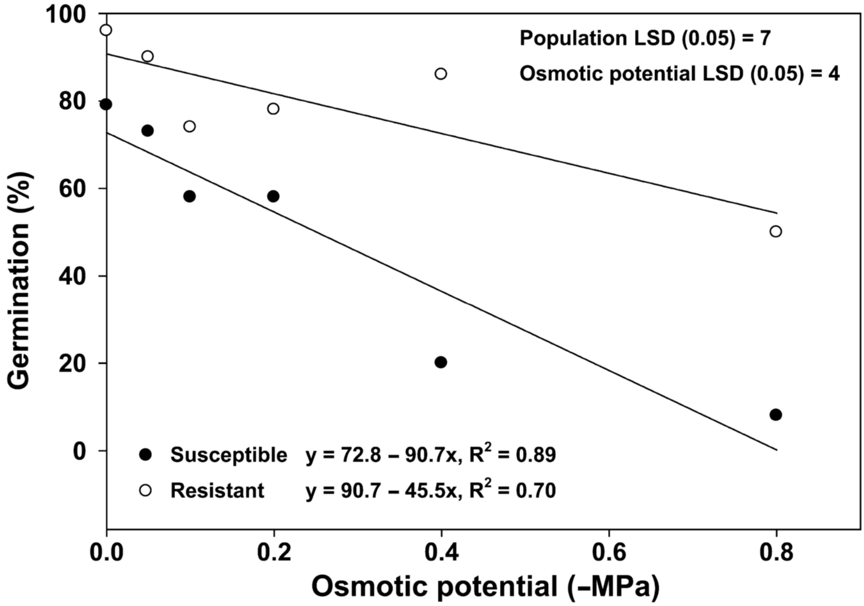


Figure 2. Effect of NaCl concentration on germination of Italian ryegrass (*L. perenne* subsp. *multiflorum*) seed from two populations (susceptible or resistant to glyphosate) incubated at 13 °C with a 10.5-h photoperiod for 1 week.



to be highest (57–87%) when pH was 7 or higher compared to germination at pH 4–6. The R population had higher germination (69–87%) compared to the S population (37–57%) at a pH 4–7 range. Similar to Italian ryegrass, other weed species including common milkweed (*Asclepias syriaca* L., Evetts and Burnside, 1972), horseweed (Nandula et al., 2006), redvine [*Brunnichia ovata* (Walt.) Shinners, Shaw et al., 1991], *Scoparia dulcis* L. (Jain and Singh, 1989), texasweed [*Caperonia palustris* (L.) A. St.-Hil., Koger et al., 2004], and trumpetcreeper [*Campsis radicans* (L.) Seem. ex Bureau, Chachalis and Reddy, 2000] germinated over a wide range of pH. In contrast, seeds of Canada thistle [*Cirsium arvense* L. (Scop.), Wilson, 1979] and stranglervine [*Morrenia odorata* (Hook & Arn.) Lindl., Singh and Achhireddy, 1984] germinated best between pH 6–7. Knowledge about seed germination of Italian ryegrass over a broad pH range will help manage this troublesome weed through soil fertility amendments that can alter soil pH to conditions that are not conducive to Italian ryegrass germination. These results suggest that both R and S populations may germinate better in neutral to alkaline soils compared to acidic soils. Overall, the R population had a germination advantage over the S population especially at the 4–7 pH range. Most Mississippi soils are acidic to neutral, while some soils have pH values greater than 7 (Oldham, 2008). In certain situations, soil pH could be increased from an acidic-neutral scale to a neutral-alkaline range by selected soil amendments such as addition of nitrogen, lime, and micronutrients or limited flooding. This could encourage germination of

FIGURE 3. Effect of osmotic potential on germination of Italian ryegrass (*L. perenne* subsp. *multiflorum*) seed from two populations (susceptible or resistant to glyphosate) incubated at 13 °C with a 10.5-h photoperiod for 1 week.



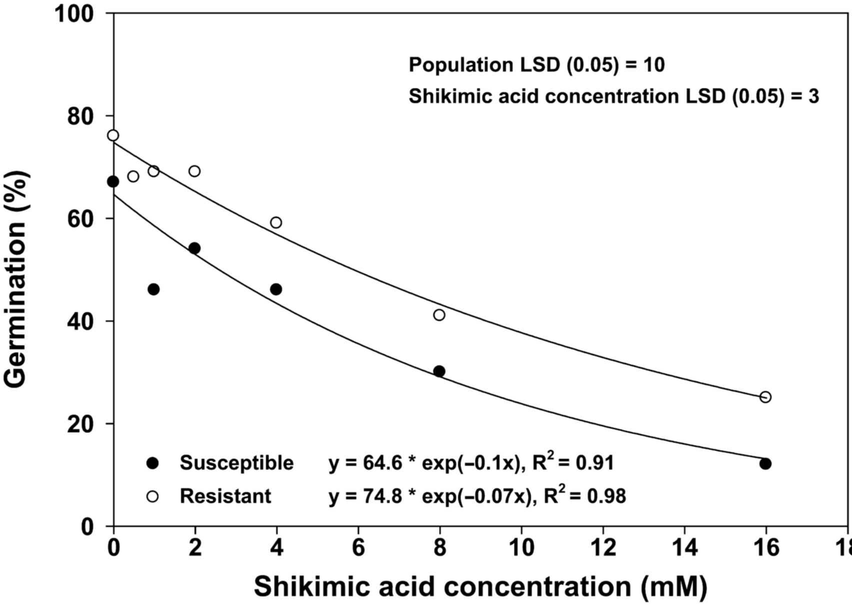
Italian ryegrass, thereby reducing its soil seedbank and facilitating postemergence management.

Salt and osmotic stress and shikimic acid

Germination of Italian ryegrass in the presence of NaCl could best be described by a linear equation of the form: $y = y_0 + ax$, where 'y' is the germination percentage, 'y_0' the intercept, 'a' the slope, and 'x' the concentration of NaCl (Fig. 2). Seed germination decreased as NaCl concentration increased from 20–160 mM in both the R and S populations, from 61 to 20% and 85 to 49%, respectively. These data suggest that Italian ryegrass has the ability to germinate under high soil salinity conditions and that the R population had a germination advantage over the S population. Similar results were reported for trumpetcreeper (Chachalis and Reddy, 2000), texasweed (Koger et al., 2004), and horseweed (Nandula et al., 2006).

A linear equation of the form: $y = y_0 + ax$, where 'y' is the germination percentage, 'y_0' the intercept, 'a' the slope, and 'x' the osmotic potential, best described germination of Italian ryegrass response to increasing osmotic potential (Fig. 3). Germination decreased from 96% to 50% and 79% to 8% in the R and S populations, respectively, as osmotic potential increased from 0 (distilled water) to −0.8 MPa. The magnitude of decline in germination in response to increasing osmotic potential was higher in the S population compared to the R population. Germination of Italian ryegrass under moderate water stress

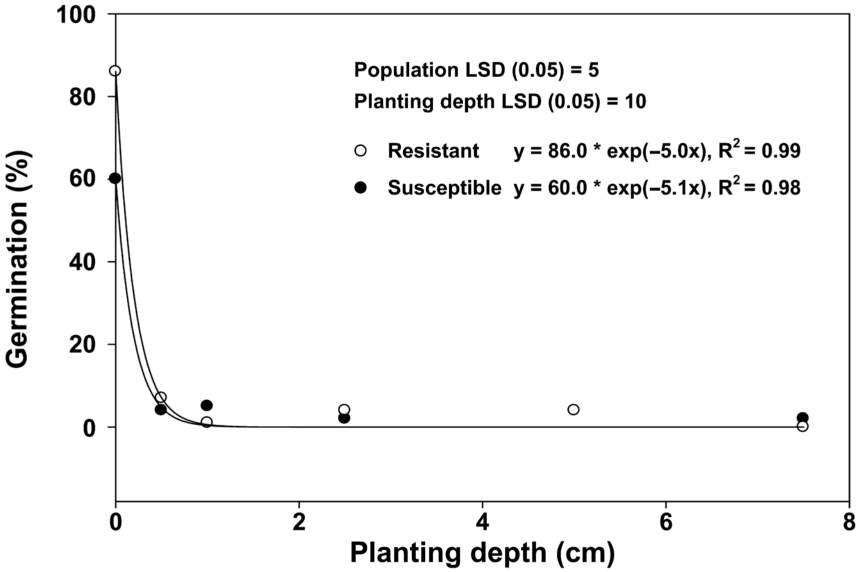
Figure 4. Effect of shikimic acid on germination of Italian ryegrass (*L. perenne* subsp. *multiflorum*) seed from two populations (susceptible or resistant to glyphosate) incubated at 13 °C with a 10.5-h photoperiod for 1 week.



conditions demonstrated in this study is similar to horseweed reported previously (Nandula et al., 2006). Water stress conditions are not common in the fall, but such conditions are typical during summer in the lower Mississippi River Delta region (Koger et al., 2004). In contrast, germination of two other weed species commonly encountered in the southern U.S. such as redvine (Shaw et al., 1991) and trumpercreeper (Chachalis and Reddy, 2000) were highly sensitive to low osmotic potential (less than −0.2 MPa). Germination over a broad range of osmotic potential indicates that Italian ryegrass could pose a weed threat under both adequate as well as moisture stress soil conditions.

Seed germination of the R and S populations in the presence of shikimic acid followed an exponential decay pattern defined by the equation of the form: $y = ae^{-bx}$, where 'y' is the germination percentage, 'a' an asymptote, 'b' the slope, and 'x' the shikimic acid concentration (Fig. 4). Germination of R and S populations declined from 76% to 25% and 67% to 12%, respectively, as shikimic acid concentration increased from 0–16 mM. Following glyphosate treatment, shikimic acid accumulation at higher levels in susceptible biotypes compared to their resistant counterparts has been reported in whole plants of Italian ryegrass (Perez-Jones et al., 2005) and rigid ryegrass (Simarmata et al., 2003). Furthermore, it was hypothesized that shikimic acid accumulation after glyphosate application caused injury to susceptible rigid ryegrass plants. Shikimic acid at 2–5 mM inhibited germination of susceptible rigid ryegrass seed, but not seed from a resistant biotype. Conversely, our results indicate

Figure 5. Effect of planting depth on germination of Italian ryegrass (*L. perenne* subsp. *multiflorum*) seed from two populations (susceptible or resistant to glyphosate) kept in a greenhouse at 13 °C with a 10.5-h photoperiod for 3 weeks after planting.



similar level of inhibition of seed germination in both R (10%) and S (8%) populations by shikimic acid at comparable concentration levels (2–4 mM).

Planting depth

Italian ryegrass emergence decreased dramatically with increasing planting depth (Fig. 5). A regression equation of the form: $y = ae^{-bx}$, where 'y' is the germination percentage, 'a' an asymptote, 'b' the slope, and 'x' the planting depth, was fitted to the data that exhibited an exponential decay pattern. Seedling emergence was highest from seeds placed on the soil surface. Seedling emergence was less than 7% from seeds planted at 0.5 cm depth and no seedlings emerged from seeds planted below 2.5 cm in both populations. Although both R and S populations can germinate under darkness (Table 1), not many seedlings emerged from seeds planted at a shallow depth (0.5 cm). These results are in contrast to the results reported for rigid ryegrass by Chauhan et al. (2006). Seedling emergence in their study was higher for seeds buried at 1 cm depth than seeds placed on the soil surface. We suspect the soil type could have had some influence on Italian ryegrass germination. Soil-to-seed contact and water availability may have affected germination of the seed on the soil surface. Seeds on the soil surface in our study had adequate moisture for germination. Reduced emergence from increased planting depths for Italian ryegrass seen in this study was also reported in hairy beggarticks (*Bidens pilosa* L., Reddy and Singh, 1992), horse purslane (*Trianthema portulacastrum* L., Balyan and Bhan, 1986), horseweed (Nandula et al., 2006), and stranglervine (Singh and Achhireddy, 1984). Since Italian ryegrass germinated only on the soil surface for the most part, it can be expected to thrive under no-tillage or minimal-tillage practices that are common in glyphosate-resistant crop production systems. Agricultural practices such as conventional tillage and deep tillage would be beneficial in fields infested with Italian ryegrass, whereby, germination by deeply buried Italian ryegrass seed would be minimal.

In summary, these results suggest that both the resistant and susceptible Italian ryegrass populations have the ability to germinate under a broad range of environmental conditions. In general, these populations could germinate with and without light. Warm temperatures (20 and 27 °C), high salt concentrations, high osmotic potential, and planting seeds below the soil surface reduced seed germination and emergence. Overall, the R population germinated better than the S population at all the environmental factors studied. In the future, prior to setup of germination studies, seed moisture measurements will be recorded and seeds will be cleaned under conditions of specific air velocity to avoid skewing of results due to partially developed seeds.

REFERENCES

Balyan, R.S. and V.M. Bhan. 1986. Germination of horse purslane (*Trianthema portulacastrum*) in relation to temperature, storage conditions, and seedling depths. Weed Sci. 34:513–515.

Benech Arnold, R.L., C.M. Ghersa, R.A. Sanchez and P. Insausti. 1990. Temperature effects on dormancy release and germination rate in *Sorghum halepense* (L.) Pers. seeds: a quantitative analysis. Weed Res. 30:81–89.

Bewley, J.D. and M. Black. 1982. The release from dormancy. p. 127–198. *In* Physiology and biochemistry of seeds. J.D. Bewley and M. Black (eds.). Springer-Verlag, Berlin.

Boykin, D.L., R.R. Carle, C.D. Ranney and R. Shanklin. 1995. Weather Data Summary for 1964–1993 for Stoneville, MS. MS Agric. For. Exp. Stn. Tech. Bull. 201, Mississippi State University, Mississippi State.

Chachalis, D. and K.N. Reddy. 2000. Factors affecting *Campsis radicans* seed germination and seedling emergence. Weed Sci. 48:212–216.

Chauhan, B.S., G. Gill and C. Preston. 2006. Influence of environmental factors on seed germination and seedling emergence of rigid ryegrass (*Lolium rigidum*). Weed Sci. 54:1004–1012.

Evetts, L.L. and O.C. Burnside. 1972. Germination and seedling development of common milkweed and other species. Weed Sci. 20:371–378.

Heap, I. 2009. International Survey of Herbicide Resistant Weeds. Weed Science Society of America: Web page: http://www.weedscience.org. Accessed: April 21, 2009.

Jain, R. and M. Singh. 1989. Factors affecting goatweed (*Scoparia dulcis*) seed germination. Weed Sci. 37:766–770.

Koger, C.H., K.N. Reddy and D.H. Poston. 2004. Factors affecting seed germination, seedling emergence, and survival of texasweed (*Caperonia palustris*). Weed Sci. 52: 989–995.

Miles J.E., R.K. Nishimoto and O. Kawabata. 1996. Diurnally alternating temperatures stimulate sprouting of purple nutsedge (*Cyperus rotundus*) tubers. Weed Sci. 44: 122–125.

Nandula, V.K., T.W. Eubank, D.H. Poston, C.H. Koger and K.N. Reddy. 2006. Factors affecting germination of horseweed (*Conyza canadensis*). Weed Sci. 54:898–902.

Nandula, V.K., D.H. Poston, T.W. Eubank, C.H. Koger and K.N. Reddy. 2007. Differential response to glyphosate in Italian ryegrass (*Lolium multiflorum*) populations from Mississippi. Weed Technol. 21:477–482.

Nishimoto, R.K. and L.B. McCarty. 1997. Fluctuating temperatures and light influence seed germination of goosegrass (*Eleusine indica*). Weed Sci. 45:426–429.

Oldham, L. 2008. Phosphorus in Mississippi soils. Information sheet 871, Mississippi State University Extension Service, Stoneville, MS. Online: http://msucares.com/pubs/infosheets/is0871.pdf. Accessed 21 April 2009.

Perez-Jones, A., K.W. Park, J. Colquhoun, C. Mallory-Smith and D. L. Shaner. 2005. Identification of glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in Oregon. Weed Sci. 53:775–779.

Reddy, K.N. and M. Singh. 1992. Germination and emergence of hairy beggarticks (*Bidens pilosa*). Weed Sci. 40:195–199.

Shaw, D.R., R.E. Mack and C.A. Smith. 1991. Redvine (*Brunnichia ovata*) germination and emergence. Weed Sci. 39:33–36.

Simarmata, M., J.E. Kaufmann and D. Penner. 2003. Potential basis of glyphosate resistance in California rigid ryegrass (*Lolium rigidum*). Weed Sci. 51:678–682.

Singh, M. and N.R. Achhireddy. 1984. Germination and ecology of milkweedvine (*Morrenia odorata*). Weed Sci. 32:781–785.

Steuter, A.A., A. Mozafar and J.R. Goodin. 1981. Water potential of aqueous polyethylene glycol. Plant. Physiol. 67:64–67.

Taylorson, R.B. 1987. Environmental and chemical manipulation of weed seed dormancy. Rev. Weed Sci. 3:135–154.

Wilson, Jr., R.G. 1979. Germination and seedling development of Canada thistle (*Cirsium arvense*). Weed Sci. 27:146–151.