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Article

Abiotic Factors Affecting Seed Germination and Early Seedling Emergence of Large Crabgrass (*Digitaria sanguinalis*)

*Fatores Abióticos que Afetam a Germinação e a Emergência Precoce de Plântulas de Milhã (**Digitaria sanguinalis**)*

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ABSTRACT - Large crabgrass is a worst exotic weed in tropical, subtropical, and temperate regions of the world. In this study, the abiotic factors affecting seed germination and early seedling emergence of large crabgrass were investigated under laboratory conditions. The optimum temperatures of germination occurred at the range from 25 to 35 °C under 12 h light/12 h dark condition. Some seeds could germinate in the dark, but light exposure significantly stimulated the germination. Large crabgrass seed was tolerant to salinity level range of 0 to 160 and low water potential (11% germination at -0.8 MPa). Medium pH had no significant effect on seed germination and more than 90% seeds germination was obtained over a broad pH range from 4.0 to 10.0. Seed germination was significantly influenced by heat-shock and completely inhibited at 140 °C for 5 min. The greatest seedling emergence rate was 96% when seeds were planted at a soil depth of 1 cm. Knowledge of germination biology obtained from this study will be useful in the development of the integrated weed management strategies for this species, and to avoid its establishment as a troublesome weed in economically important cropping regions.

Keywords: temperature, light, osmotic potential, heat-shock, burial depth.

RESUMO - A milhã é a pior planta daninha exótica em regiões tropicais, subtropicais e temperadas do mundo. Neste estudo, os fatores abióticos que afetam a germinação e a emergência inicial da plântula de milhã foram investigados em laboratório. As melhores temperaturas de germinação foram observadas entre 25 e 35 °C sob o regime de 12 horas de luz/12 horas de escuridão. Algumas sementes conseguem germinar no escuro, mas a exposição à luz estimulou a germinação consideravelmente. A semente de milhã apresentou tolerância à salinidade (entre 0 a 160) e baixo potencial hídrico (11% de germinação a -0,8 MPa). Não foi observado efeito significativo do pH médio sobre a germinação das sementes e mais de 90% da germinação das sementes ocorreu ao longo de uma grande faixa de variação de pH (entre 4,0 e 10,0). A germinação das sementes foi bastante influenciada pelo choque térmico, sendo completamente inibida a 140 °C durante 5 min. A maior taxa de emergência das plântulas foi de 96%, com as sementes que haviam sido plantadas a uma profundidade do solo de 1 cm. O conhecimento gerado a respeito da biologia de germinação neste estudo será útil no desenvolvimento de estratégias de manejo integrado para esta espécie, e também no combate ao estabelecimento da milhã como uma planta daninha problemática em regiões de cultivo economicamente importantes.

Palavras-chave: temperatura, luz, potencial osmótico, choque térmico, profundidade de enterrio.

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INTRODUCTION

Large crabgrass (*Digitaria sanguinalis*), a C_4 species, is an important grass weed in nonirrigated farmlands and widely distributed in tropical, subtropical, and temperate regions of the world. This plant is an annual weed, forms adventitious roots at the stem nodes, and is mainly propagated by seeds. According to previous reports, the plant can produce a large number of seeds, a fact which particularly improves the dispersal, competitive ability and fecundity of species in a competitive environment (Holm et al., 1979; Šerá and Šerý, 2004). It has also been found in cultivated fields, roadsides, or waste places (Šerá, 2008). Large crabgrass infests cultivated fields and reduces crop productivity, even at a low density (Aguyoh and Masiunas, 2003; Pereira et al., 2011).

It was recognized as a kind of problem weed because it is difficult to control in some subtropical crops, mainly sugarcane, corn, and soya (Šerá et al., 2011). According to our survey, it is difficult to control this weed in sugarcane producing areas of the province of Guangxi, Southern China. It has tolerance or resistance to multiple herbicides such as atrazine, nicosulfuron, quizalofop-P-ethyl (Heap, 2016). Thus, integrated systems for weed control were one of the most important strategies in further attempts of weed control. Because large crabgrass is mainly propagated by seeds, germination is a critical step in its lifecycle. Several factors are known to affect seed germination and weed emergence. Optimum temperature, light, and pH conditions vary considerably depending on the species. To date, there has been relatively little detailed research on the biology and ecology of large crabgrass. Zhang et al. (2011) studied the effect of salinity and temperature on the germination of *Chloris virgata*, and *Digitaria sanguinali*. Hoyle and Scott McElroy (2012) reported the relationship between temperature and heat duration on seed mortality of large crabgrass. However, other environmental factors such as light, pH, seed burial depth, and crop residue can also affect seed germination (Chachalis and Reddy, 2000; Koger and Reddy, 2004; Chauhan and Johnson, 2008; Chachalis et al., 2008; Wang et al., 2015; Fernando et al., 2016). Hence, it is essential to understand how environmental factors affect germination and emergence, which will help predict the species potential to spread and make effective control measures.

The objectives of this study were to determine the effect of (1) light and temperature, salt and osmotic stress, and pH on seed germination of large crabgrass, (2) heat-shock on seed germination of large crabgrass, and (3) sugarcane leaf residue on seedling emergence of large crabgrass. This information could help predict the potential of this species for spreading into new areas and provide a strategy for effective management and control of this weed.

MATERIALS AND METHODS

Seed description and general experimental procedure

Large crabgrass seeds were collected from a sugarcane field in Binyang (23°18'22.9716"; 108°50'50.6364"), Nanning, Guangxi province, Southern China during their maturity season in 2015. Mature seeds were gently shaken into a labeled envelope. The seeds were air dried for 7 d and then stored at 10 °C and 50% relative humidity (RH) prior to use (Hoyle and Scott McElroy, 2012). The 100-seed weight value was 52.20±0.99 mg.

All experiments were conducted in a growth chamber fitted with cool white fluorescent lamps which produced light intensity of 12,000 lux. Seed germination was evaluated by evenly spacing a 25 seed sample in a 9 cm Petri dish containing two layers of sterilized filter paper moistened with 5 mL reverse osmosis (RO) water or a treatment solution. Petri dish was wrapped with parafilm and placed in an incubator at 30 °C with a 12 h light and 12 h dark cycle, unless otherwise stated. Germination was surveyed every other day for a period of 14 d. Seeds were considered to have germinated when the radicles were approximately 2 mm long and cotyledons had emerged from the seed coat (Zhang et al., 2012). Germination data were analyzed as a percentage of germinated seeds from the total number of seeds for each replicate.

Effect of temperature and light on seed germination

The influence of temperature and light on seed germination was determined by incubating seeds in the growth chamber under constant temperatures (15, 20, 25, 30, 35, 40 and 45 ^{o}C) in light/dark and dark regimes. Other environmental conditions were described in the general germination test.

Effect of osmotic stress and salt stress on seed germination

To test the effect of water stress on seed germination, aqueous solutions with osmotic potentials of 0, -0.05, -0.1, -0.2, -0.4, -0.8, -1.0, and -1.2 MPa were prepared with polyethylene glycol 6000 as described by Michel and Kaufaman (1973). The effect of salt stress on seed germination was measured in sodium chloride (NaCl) solutions with the concentration of 0, 20, 40, 80, and 160 mM.

Effect of pH value on seed germination

To determine the effect of pH value on seed germination, seeds were placed onto the filter paper moistened using buffer solutions with pH values ranging from 4.0 to 10.0 (Chachalis and Reddy, 2000). Unbuffered distilled water (pH 6.5) was used as control. Buffer solutions with pH values from 4.0 to 6.0 were prepared with 2 mM MES [2-(N-morpholino) ethanesulfonic acid] solution and adjusted with 0.1 M hydrogen chloride (HCl) or sodium hydroxide (NaOH). Buffer solutions of pH 7.0 and 8.0 were prepared with 2 mM HEPES [N-(2-hydroxymethyl) piperaziine-N-(2-ethane sulfonic acid)] solution and adjusted with 0.1 M NaOH. Buffer solutions of pH 9.0 and 10.0 were prepared with 2 mM tricine [N-Tris (hydroxymethyl) methyl glycine] solution and adjusted with 0.1 M NaOH.

Effect of heat-shock on seed germination

To evaluate the effect of heat-shock on seed germination, seeds were pretreated in an oven for 5 min at 40, 60, 80, 100, 120 and 140 ^{o}C, and then placed in Petri dishes with filter papers dampened with 5 mL sterilized water, and finally incubated at 30 ^{o}C under a 12 h photoperiod.

Seedling emergence response to burial depth

Twenty five seeds were planted in 12 cm diameter x 10.5 cm deep plastic pots and covered to attain burial depths of 0 (soil surface), 1, 3, 5, and 7 cm. The soil was characterized with sand (33.46%), silt (21.25%), clay (43.29%) and organic matter fraction (4.33%). Soil was ground to powder after air drying and passed through a 2 mm sieve, then stored at 4 ^{o}C until use. Pots were incubated at 30 ^{o}C with a 12 h photoperiod and were watered with subirrigation. Emergence was defined as the appearance of coleoptile. The experiment was terminated when no further emergence was recorded 14 d after planting.

Seedling emergence response to sugarcane residue

Twenty five seeds were planted in the soil at the depth of 1 cm and the chopped air-dried sugarcane leaves (Taitang 22) were spread on the surface at rates equivalent to 0, 0.25, 0.5, 1, 1.5, and 2 ton ha^{-1}. The soil used in this experiment was the same as in the seed burial experiment. Emerged seedlings were recorded 14 d after sowing and expressed as a percentage of the seeds sown.

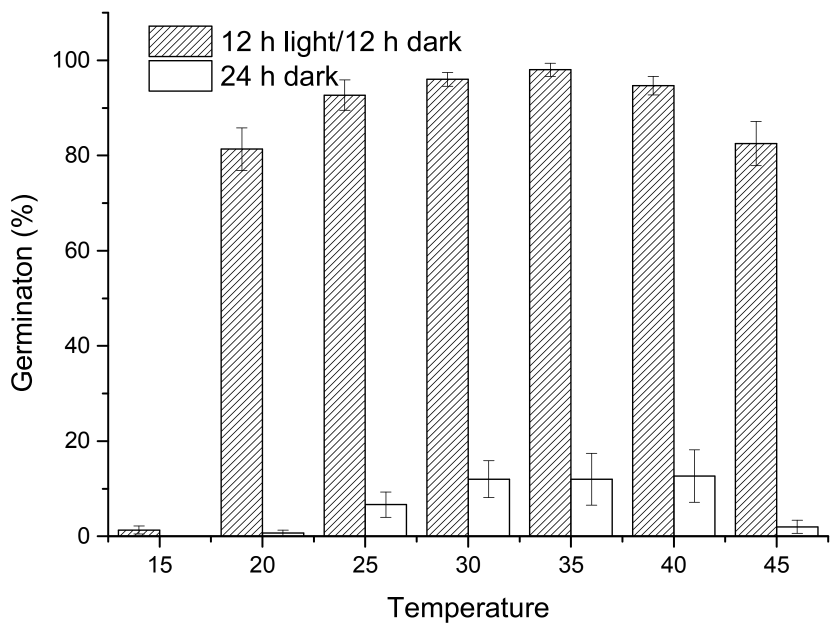
Data analysis

All experiments were carried out twice in a completely randomized design with three replications. There was no significant difference between two experimental runs, hence the data were pooled for analysis. Data were subjected to one-way ANOVA to assess all main effects with the Origin (version 9.0) software. Treatment differences were evaluated using Fisher's test at a significance level of 0.05. Regression models were used to determine the effect of osmotic potential and heat-shock on seed germination.

RESULTS AND DISCUSSION

Effect of temperature and light on seed germination

Temperature and light had significantly synergistic effect on seed germination. Germination was 81, 92, 96, 98, 95, and 83% at 20, 25, 30, 35, 40, and 45 ^{o}C in the light/dark regime, respectively (Figure 1). Lowest seed germination (1%) was found at 15 ^{o}C under 12 h light/12 h dark conditions. Seed germination was significantly lower in dark than in the light/dark regime. Our results showed that germination was strongly stimulated by light at all tested temperatures. Under the dark condition, seed germination was completely inhibited at all experimental temperatures. Light is especially necessary for germination of most small seeds, based on a previous investigation (Grime, 1981). Batlla and Luis (2014) also reported that light influenced seed germination by regulating dormancy termination in many weed species. The stimulation of seed germination by light has been reported in many weed species, including southern crabgrass (*Digitaria ciliaris*), India crabgrass (*Digitaria longiflora*) (Chauhan and Johnson, 2008), Chinese sprangletop [*Leptochloa chinensis* (L.) Nees] (Benvenuti et al., 2004) and feather fingergrass (*Chloris virgata*) (Fernando et al., 2016). These data indicated that large crabgrass may tolerate high temperatures at the presence of light.

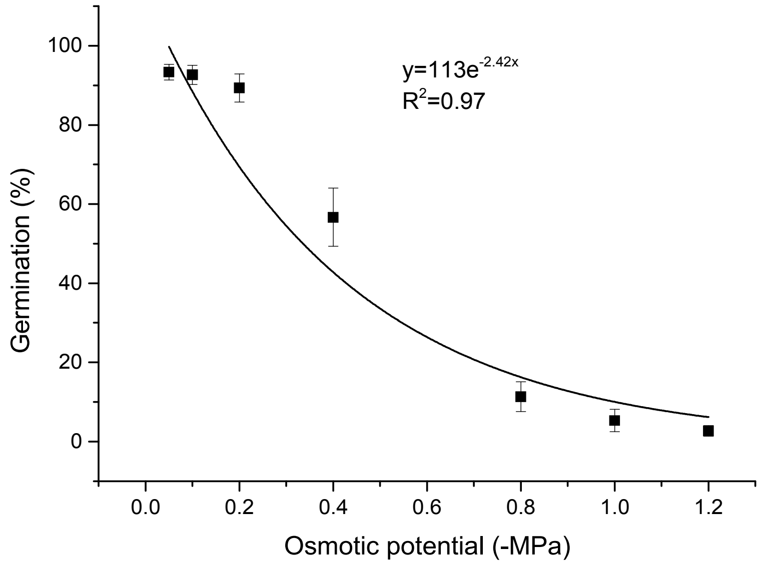


The data represent the mean ± standard errors (n=6).

Figure 1 - Effect of constant temperatures on germination of large crabgrass seeds under 12 h light/12 h dark and complete dark (24 h dark) conditions for 14 d.

Effect of osmotic stress and salt stress on seed germination

The relationship between germination and osmotic stress can be described by an exponential curve model ($y=113e^{2.42x}$, $R^2=0.97$). Seed germination was 93, 57, and 11% at osmotic potentials of -0.05, -0.4 and -0.8 MPa, respectively (Figure 2). Optimum germination was found at osmotic potentials from 0 to -0.2 MPa, where germination was more than 89%. Germination rate was



The line represents the exponential curve model *Y (%)=ae^{bx}* fitted to the data. The data represent the mean ± standard errors (n=6).

Figure 2 **-** Effect of osmotic potential on germination of large crabgrass seeds incubated at 30 °C under a 12 h light/12 h dark condition for 14 d.

gradually decreased to 11% when osmotic potential was reduced to -0.8 MPa. These results implied that large crabgrass seeds are rather tolerant to low water potential. Similar results were reported in buffalobur (*Solanum rostratum*) (Wei et al., 2009), and Venice mallow (*Hibiscus trionum*) (Chachalis et al., 2008). In contrast, other weed species were sensitive to low osmotic potential, such as Texasweed (*Caperonia palustris*) (Koger and Reddy, 2004), American sloughgrass (Rao et al., 2008), feather fingergrass (*Chloris virgata*) (Fernando et al., 2016), and *Campsis Radicans* (Chachalis and Reddy, 2000). These data indicated that germination and establishment of large crabgrass remained good in poorly drained or fairly dry soil conditions and may have a competitive advantage over other weed species under low water stress condition.

Germination was not significantly affected by salinity at five salinity levels of 85-93% (P = 0.6878) (Figure 3). Our findings are in agreement with a previous report on large crabgrass (Zhang et al., 2012). Although *Digitaria sanguinalis* was classified as glycophyte, it was reported to grow in salt-contaminated soil in China (Song, 2008) and Czech Republic (Šerá et al., 2011). These results suggested that large crabgrass may germinate under soil salinity.

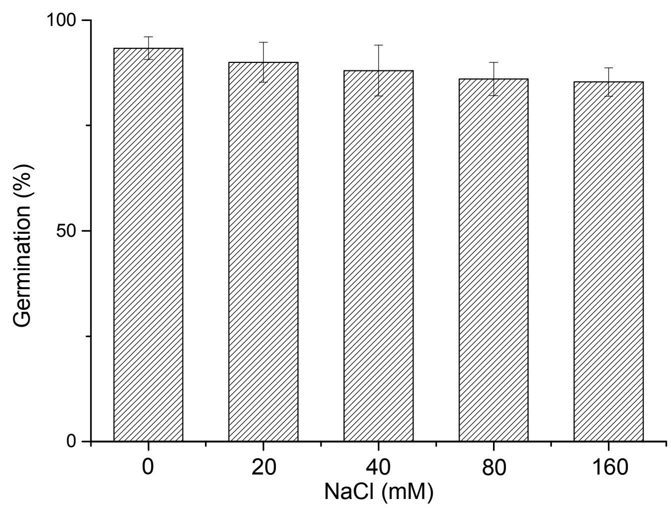


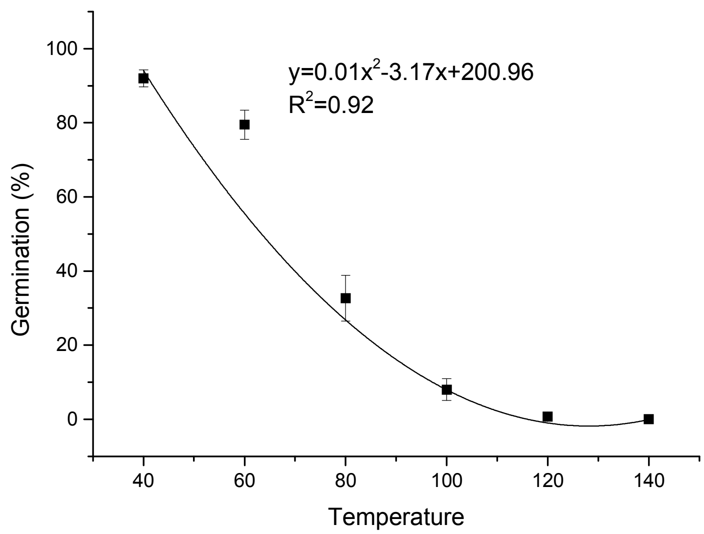
Figure 3 **-** Effect of NaCl solution on germination of large crabgrass seeds incubated at 30 °C in a 12 h photoperiod for 14 d.

Effect of pH value on seed germination

Large crabgrass seed germination was not affected by pH value (data not shown). Germination was greater than 90% over a pH range of 4.0-10.0. There was maximum germination (98%) at pH 6.0. However, Pierce (1999) reported that germination of large crabgrass was increased with decreasing pH in soil. The different conclusion might be due to the different experimental condition. Many studies have indicated that the effect of pH on seed germination is dependent on weed species. Other weed species have been reported to germinate over a wide range of pH values, including redvine (*Brunnichia ovata*) (Shaw et al., 1991), Venice mallow (*Hibiscus trionum*) (Chachalis et al., 2008), buffalobur (*Solanum rostratum*) (Wei et al., 2009), minor bluegrass (*Polypogon fugax*) (Wang et al., 2015). By contrast, poor germination was reported at extreme pH values in several weed species (Chachalis and Reddy, 2000; Zhou et al., 2005). High seed germination potential over a broad range of pH indicated that pH should not be a limiting factor for seed germination in most soil conditions.

Effect of heat-shock on germination

Germination was significantly influenced after exposure to the heat-shock treatment ($p<0.001$) (Figure 4). A quadratic polynomial model [$G(\%)=0.01x^2$ $3.17x+200.96$, $R^2=0.92$] was used to describe the relationship between large crabgrass emergence and heat-shock temperature. Germination was greater than 90% at 40 °C, and sharply decreased to 32% at 80 °C. No germination was found at more than 140 °C. The temperature for 50% inhibition of the maximum germination was 58.4 °C according to the fitted model mentioned above.



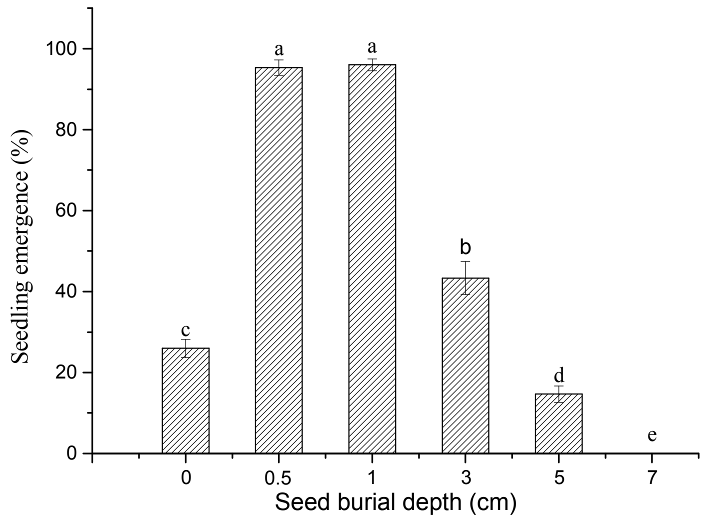
The data represent the mean ± standard errors of the mean (n=6).

Figure 4 - Effect of heat-shock on germination of large crabgrass seeds incubated at 30 °C in a 12 h photoperiod for14 d.

In China, burning is an important step to clear debris from the land after crop harvest by many farmers. According to a previous report, soil surface temperature raised to 550 °C for 6 min, when crop was burned (Cook, 1939). However, temperature can decrease from 550 °C to 50 °C at a rate of 100 °C cm^{-1} in surface soil (0-5 cm depth) (Sanchez, 1976). Assuming these values, seeds of the crabgrass species may remain ungerminated on surface soils above 4 cm and they were killed by high temperatures (140 °C).

Seedling emergence response to burial depth

Seedling emergence of large crabgrass was significantly influenced by burial depth (Figure 5). Maximum emergence (96%) was measured for seeds placed on the burial depth of 1 cm, and



The vertical bars represent the standard errors of the mean (n=6).

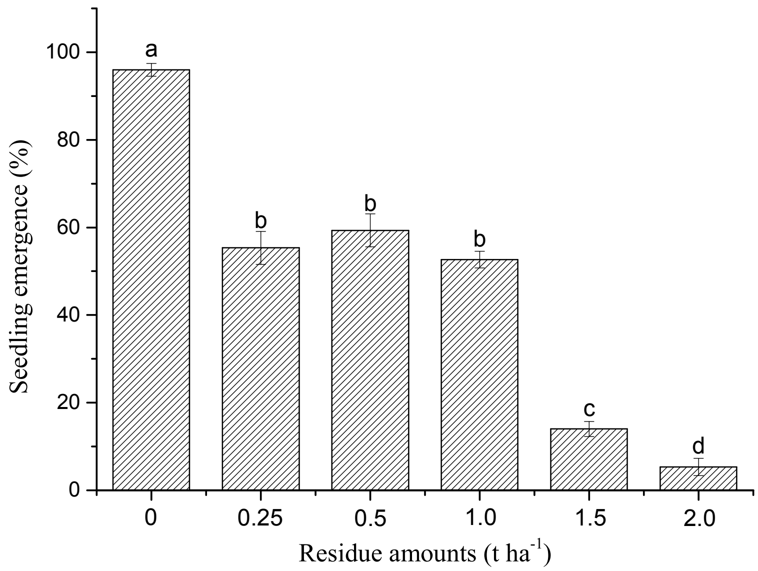
Figure 5 **-** Effect of burial depth on emergence of large crabgrass seeds incubated at 30 °C in a 12 h photoperiod for 14 d.

emergence gradually decreased with the increase of burial depth when the depths were more than 1 cm. Large crabgrass did not emerge when seeds were found to be deeper than 7 cm to the soil surface. Our findings were identical to those reported in Hoyle's study (2013). Similarly to large crabgrass, for several weed species there was maximum emergence above the depth of 2 cm, whereas emergence was less than 10% at a depth of 7 cm (Benvenuti et al., 2001; Zhou et al., 2005; Chauhan and Johnson, 2008; Rana et al., 2012; Wang et al., 2015). Notably, the weed species possessed the same characteristic with small seeds, whose emergence was limited by light (Baskin and Baskin, 1998; Mennan and Ngouajio, 2006). Thus, light penetration and resources in seeds are the probable reason for reduced emergence at deeper depths.

Seedling emergence response to sugarcane residue

In China, sugarcane leaves were peeled off and left in field at harvest time. Thus, leaves were added to evaluate their effect on seedling emergence. Our results showed that emergence was reduced by the addition of the chopped sugarcane leaves (Figure 6). Maximum emergence (96%) was measured without sugarcane leaf residue, and emergence gradually decreased with the increase of residue amounts. Meanwhile, we found that seedling emergence became lower and slower with the addition of residues. This might be due to the decreasing soil thermal amplitude and light penetration (Dyer, 1995). In addition, residues may be a physical barrier to seedling emergence. Similar results have also been reported for many weed species (Mohler and Calloway, 1992; Chauhan and Johnson, 2008a,b). Further research will be performed in field conditions to effectively suppress weeds by controlling the amount of residue without reducing crop yield.

In conclusion, this research demonstrated that temperature and light are the critical factors affecting seed germination of large crabgrass. The temperature tested on the successful seed germination of large crabgrass ranged from 15 to 45 °C, which indicated that large crabgrass seeds germinated from March to November in the Province of Guangxi, in China. Light exposure significantly enhanced seed germination compared with the dark condition at all temperatures. Large crabgrass seeds were rather tolerant to low osmotic potential and 160 mM NaCl, and adapted to a broad range of pH levels, which indicated that large crabgrass could adapt to diverse habitats and soil conditions. The fact that this species had widespread distribution worldwide was likely due to its broad requirement for seed germination. Seedling emergence was optimal for seeds placed at 1 cm soil depth rather than those placed on the soil surface, and no seedlings emerged when burial depth reached 7 cm. Deep tillage would bury the seeds to deeper soil depth (7 cm) to reduce seedling emergence. Additionally, emergence was dropped dramatically with



Means with the same letter are not significantly different on the basis of LSD at P=0.05.

Figure 6 **-** Effect of residue amount on large crabgrass seedlings.

the addition of crop residues. This information could help explain why large crabgrass was abundant in fields, and provided a successful management and control such as seed burial by deep tillage, heated treatment, and crop residues.

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