Weed Technology 2016 30:533–538

Influence of Environmental Factors on Seed Germination and Emergence of Asia Minor Bluegrass (*Polypogon fugax*)

Lifeng Wang,† Su Jin,† Lamei Wu, Xiaomao Zhou, Xiangying Liu, and Lianyang Bai*

Asia minor bluegrass (AmB) is a major weed impacting rapeseed production in Dongting Lake District, China. Growth chamber experiments were conducted to determine the influence of environmental factors on germination and emergence of AmB. The optimum constant temperature for germination was around 20 C. Seeds showed germination percentages above 60% under 22/15 and 24/19 C day/night temperature regimes. Seeds could germinate in the dark, but light exposure significantly enhanced the germination percentage. More than 50% of seeds germinated over a pH range between 4 and 10. Seeds were highly sensitive to osmotic stress, and germination was completely inhibited at an osmotic potential of −0.4 MPa, indicating that it was favored by a moist environment. Increasing salinity reduced germination of AmB seeds from 58% at 0 mM to 13% at 80 mM NaCl. The highest seedling emergence (62%) was observed when seeds were placed on the soil surface, and no seedlings emerged from seeds placed at a depth of 5 cm. This work shows that the climate and soil conditions in Dongting Lake District are suitable for AmB seed germination and that no-till fields, where seeds remain on the soil surface, promote the successful establishment of the weed.

Nomenclature: Asia minor bluegrass, *Polypogon fugax* Nees ex Steud.; rapeseed, *Brassica napus* L.
Key words: Burial depth, light, osmotic stress, pH, salt stress, temperature.

Polypogon fugax (AmB) es una maleza importante que impacta la producción de colza en el distrito Lago Dongting, China. Se realizaron experimentos en cámaras de crecimiento para determinar la influencia de factores ambientales en la germinación y emergencia de AmB. La temperatura constante óptima para la germinación fue alrededor de 20 C. Las semillas mostraron porcentajes de germinación superiores a 60% con regímenes de temperatura de 22/15 y 24/19 C día/noche. Las semillas pudieron germinar en la oscuridad, pero la exposición a la luz mejoró significativamente el porcentaje de germinación. Más del 50% de las semillas germinaron en un rango de pH entre 4 y 10. Las semillas fueron muy sensibles al estrés osmótico y la germinación fue completamente inhibida a un potencial osmótico de −0.4 MPa, indicando que la germinación fue favorecida en un ambiente húmedo. El aumentar la salinidad redujo la germinación de las semillas de AmB desde 58% con 0mM a 13% con 80 mM NaCl. La mayor emergencia de plántulas (62%) fue observada cuando las semillas fueron puestas sobre la superficie del suelo, y ninguna plántula emergió cuando las semillas fueron puestas a una profundidad de 5 cm. Este trabajo muestra que el clima y las condiciones de suelo en el distrito Lago Dongting son favorables para la germinación de semillas de AmB y que los campos con labranza cero, donde la semilla permanece sobre la superficie del suelo, promueven el establecimiento exitoso de esta maleza.

Asia minor bluegrass (AmB) is an annual winter grass weed widely distributed in Asia (Jung et al. 2006; Keshavarzi et al. 2007; Qureshi et al. 2009; Ranjit et al. 2011). It is a tufted grass with a height of 10 to 75 cm and reproduces by seed (Flora of China 2006). AmB seeds are small, and can be spread by wind and irrigation water (Qiang 2002, 2005). As a common weed in the farmlands of China, it occurs in most provinces and is very prominent in the lower and middle reaches of the Yangtze River (Flora of China 2006).

AmB typically germinates in autumn and matures in summer, and is an important weed of various summer-harvested crops, including wheat (*Triticum aestivum* L.) and rapeseed in the region. For example, 225 plants m^{-2} reduced wheat yield by approximately 10% (Zhang 1993). In Dongting Lake District in the central reaches of the Yangtze River, AmB has become the most problematic weed

DOI: 10.1614/WT-D-15-00043

* First, third, fourth, and sixth authors: Research Assistant, Doctor, Professor, and Professor, Biotechnology Research Center, Hunan Academy of Agricultural Sciences, Changsha, China; Collaborative Innovation Center for Field Weeds Control, Hunan Loudi, China; second author: Junior Technician, Department of Pathology, Hubei Cancer Hospital, Wuhan, China; fifth author: Associate Professor, College of Plant Protection, Hunan Agricultural University, Changsha, China. Corresponding authors' E-mails: lxy525525@163.com and bailianyang2005@aliyun.com.
† These authors contributed equally to this work.

in no-tillage rapeseed fields, and densities can reach 366 and 114 plants m^{-2} in rice (*Oryza sativa* L.)–rapeseed and cotton (*Gossypium hirsutum* L.)–rapeseed rotational fields, respectively (Zhao et al. 2001).

It is well known that using herbicides is an important approach in weed management programs; however, AmB has been reported to be tolerant or resistant to multiple herbicides. Tang et al. (2014) reported that the AmB populations collected from wheat and rapeseed fields under long-term herbicide use have developed resistance to clodinafop, fluazifop-butyl, haloxyfop-r-methyl, quizalofop-p-ethyl, and fenoxaprop-p.

Because AmB reproduces only by seed, germination is one of the most important phases in its life cycle (APIS 2014). Hence, a comprehensive understanding of the environmental requirements for AmB germination is essential to implement an effective management strategy. Peng et al. (2011) investigated the effect of temperature on germination of four weed species including AmB. However, other environmental factors such as light, pH, soil moisture, and seed burial depth also can affect weed seed germination (Chachalis and Reddy 2000; Koger et al. 2004; Taylorson 1987).

To date, there has been relatively little detailed research on the investigation of biology and ecology of AmB. The major objectives of this study were to determine the effect of temperature, light, pH, osmotic and salt stress, and depth of burial on germination and emergence of AmB. This information could help explain why this weed has become widespread in Dongting Lake District and provide a remedy for effective prevention and control.

Materials and Methods

Seed Description and General Experimental Procedure. AmB seeds were collected from multiple rapeseed fields from the Dongting Lake District (29.08–29.46°N; 111.59–112.18°E), Changde, Hunan province, P. R. China. Soils in this region are alluvial, with pH of 6.5 to 7.6 and organic content of 1 to 3% (Shao and Zhang 1992). Mature seeds were collected by gently shaking plants and collecting the easily dehisced seed in an envelope. Throughout our investigation, plants produced 2,702 ± 304 seeds on average, and the 100-seed weight was 8.39 ± 0.48 mg. Seeds were air dried for 7 d and then stored in sealed plastic bags at room temperature in the dark until use.

Seed germination was determined by evenly placing 30 seeds in a 9-cm-diam Petri dish containing two layers of Whatman filter paper moistened with 5 ml deionized water or treatment solution. Petri dishes were sealed with parafilm to prevent water loss, and 1 ml of deionized water was added on a regular basis (every 2 d) to ensure water saturation of the filter paper throughout the entire experiment. The seed in Petri dishes were incubated at 20 C with a 12-h light cycle, unless otherwise specified. The growth chamber was equipped with fluorescent lamps with a light intensity of 12,000 lux. Germination was monitored every 2 d for a period of 14 d and the germinated seeds removed from the dishes at each evaluation. A seed was considered to have germinated when the radicle visibly protruded through the seed coat. Germination data were analyzed as the proportion of germinated seeds from the total number of seeds in a single Petri dish.

Temperature. The effect of constant temperature on AmB germination was determined by placing the Petri dishes in growth chambers at 5, 10, 15, 20, 25, and 30 C. A separate experiment was conducted to determine the effect of alternating temperature on germination. AmB seeds typically germinate from mid-October to mid-December in Dongting Lake District. To simulate the diurnal temperature variations, AmB seed were exposed to five fluctuation temperature regimes (24/19 C, 22/15 C, 19/11 C, 15/8 C, and 13/6 C; day/night) based on the mean air temperature conditions for the previous 5 yr in Dongting Lake District during the autumn (TianQi 2013).

Light. To determine the effect of light on germination, AmB seeds were placed under 24/0, 12/12, and 0/24 h light/dark regimes per 24 h cycle, at a constant temperature of 20 C. The dark treatment was performed by covering the dishes with double layers of aluminum foil to prevent light penetration. Water additions and germination evaluations were done in a dark room with a green light.

pH. To evaluate the effects of pH on AmB germination, seeds were exposed to buffer solutions with pH 4, 6, 8, and 10. Deionized water (pH 5.8)

https://doi.org/10.1614/WT-D-15-00043 Published online by Cambridge University Press

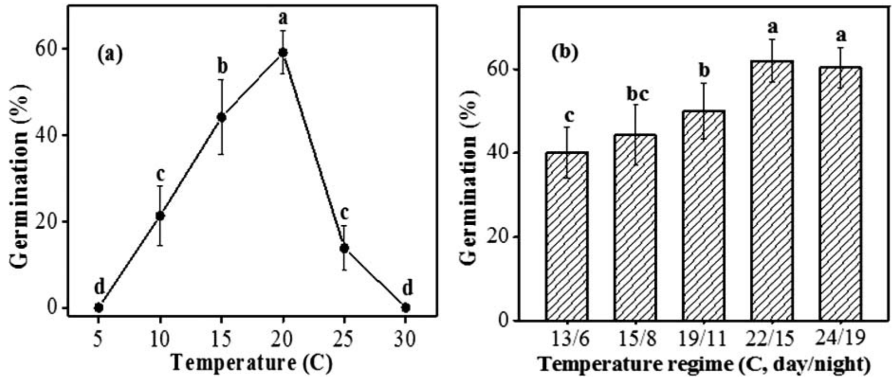

Figure 1. Effect of (a) constant and (b)alternating temperature regime on the germination of Asia minor bluegrass seeds incubated in a 12-h photoperiod for 14 d. Means with the same letter are not significantly different according to the Tukey's test ($P < 0.05$). Vertical bars represent the standard error of the mean ($n = 8$).

was used as a control. Buffer solutions were prepared as described previously (Chachalis and Reddy 2000): pH 4 buffer solution was prepared with 2 mM potassium hydrogen phthalate and 1M HCl; pH 6 buffer solution was prepared with 2 mM MES [2-(N-morpholino)ethanesulfonic acid] and 1 M NaOH; buffer solutions at pH 8 and 10 were prepared with 1 M NaOH and 2 mM HEPES [N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid)] / tricine {*N*-[tris(hydroxymethyl)methyl]glycine}.

Osmotic and Salt Stress. To test the effect of water stress on AmB germination, seeds were placed in aqueous solutions with osmotic potentials of 0, –0.05, –0.1, –0.2, –0.4, and –0.8 MPa, which were prepared by dissolving 0, 45.28, 72.48, 112.23, 169.43 and 251.03 g polyethylene glycol 6,000 in 1 L distilled water, respectively (Michel and Kaufaman 1973). The effect of salt stress on AmB germination was investigated using sodium chloride (NaCl) solutions of 0, 10, 20, 40, 80, and 160 mM.

Burial Depth. Thirty seeds of AmB were buried in soil a 2 : 1 (v/v) mixture of sterilized topsoil and sand in 13-cm-diam by 8-cm-deep plastic pots at depths of 0 (soil surface), 1, 2, 3, 4, and 5 cm, with each planting depth in a different pot. The topsoil was an alluvial soil with pH of 6.8 and organic matter content of 3.6%, which was collected from Yunyuan farm located at 122.04°E, 29.11°N in Hunan Province, China. Pots were incubated under 12 h daylength with 22 C days and 15 C nights and were watered daily. Emerged seedlings were recorded 21 d after sowing and the appearance of coleoptile was defined as seedling emergence.

Data Analysis. All experiments were conducted in a completely randomized design with four replications, and each experiment was conducted twice. The data were the mean of two runs, and there was no significant difference between two experimental runs. Data were subjected to one-way ANOVA to assess all main effects. Treatment differences were evaluated using Tukey's test ($P = 0.05$). Regression models were used to determine the effect of salinity and osmotic potential on germination and the effect of burial depth on emergence.

Results and Discussion

Temperature. When exposed to constant temperature, the cumulative germination of AmB seeds increased from 0 to 59% when the temperature increased from 5 to 20 C; thereafter, germination decreased as temperature increased and no germination was observed at 30 C (Figure 1). This result was expected because AmB is a subtropical winter annual weed that germinates at relatively cool temperatures. Tang et al. (2014) also found 20 C to be optimum for germination of a different population of AmB.

Because constant temperatures are unlikely to occur in the natural environment, we also tested the germination of AmB under alternating temperature conditions. As shown in Figure 1, germination of AmB was above 40% in all five temperature regimes. Seeds showed germination percentages above 60% under both 22/15 and 24/19 C (day/

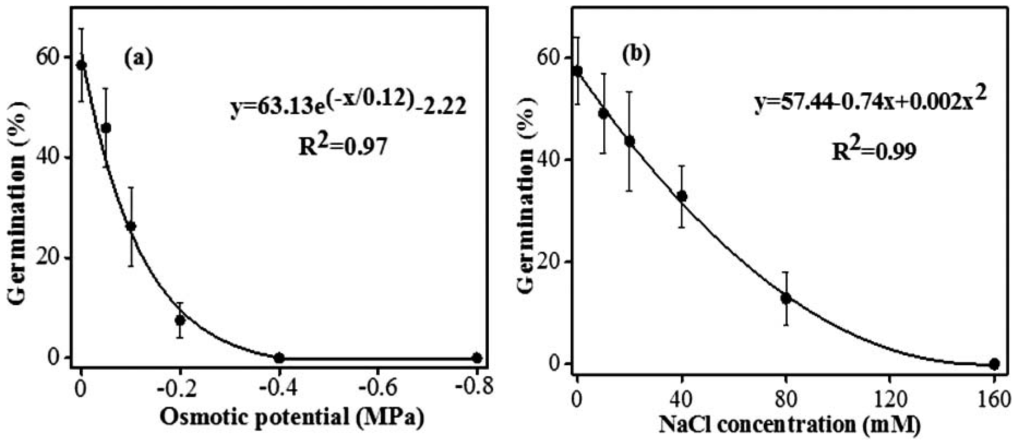

Figure 2. Effect of (a) osmotic potential and (b) NaCl concentration on the germination of Asia minor bluegrass seeds incubated at 20 C in a 12-h photoperiod for 14 d. Vertical bars represent the standard error of the mean ($n = 8$).

night) temperature regimes, which corresponds to temperatures when AmB seed germination peaks in the Dongting Lake District. Our findings support the work of Peng et al. (2011) who reported AmB germination in the Yunnan area was 58% under a temperature regime of 20/15 C (day/night), and decreased with increasing temperature.

Light. AmB germinated in both light and dark conditions, but was significantly higher when exposed to light. Germination percentage was 57 and 54% in 12-h and 24-h light-exposure treatments, respectively, whereas only 17% of seeds germinated in total darkness (data not shown). Grime et al. (1981) investigated the germination of 271 different species, and found that light is necessary for most small seeds, and the demand for light for seed germination decreases along with the increase of seed size. The seeds of AmB are small, with a mean seed weight of about 0.08 mg; thus, light exposure might significantly enhance their germination.

pH. AmB germination was between 51 and 55% over the pH range from 4 to 10 (data not shown), which implies that AmB might be adapted to a wide range of soil conditions. The soil pH of the Yangtze Delta Region and the Dongting Lake District ranges from 4 to 9 (Cheng et al. 2000; Huang et al. 2002), indicating that AmB germination is not likely to be limited by soil pH in the region.

Osmotic and Salt Stress. The relationship between AmB germination and osmotic stress can be described by an exponential curve model [G (%) $= 63.13e^{(-x/0.12)} - 2.22$, $r^2 = 0.97$] (Figure 2a). AmB germination was greatly reduced as osmotic stress increased. The highest germination (58%) was observed at 0 MPa (distilled water) and decreased by half as osmotic potential was reduced to –0.1 MPa. No germination was observed at an osmotic potential of –0.4 or –0.8 MPa. Similar results were found in texasweed [*Caperonia palustris* (L.) St. Hill] (Koger et al. 2004), redvine [*Brunnichia ovata* (Walt.) Shinners] (Shaw et al. 1991), American sloughgrass American sloughgrass [*Beckmannia syzigachne* (Steud.) Fernald] (Rao et al. 2008), and trumpetcreeper [*Campsis radicans* (L.) Seem. ex Bureau] (Chachalis and Reddy, 2000). In contrast, other weed species were very tolerant to low osmotic potential, such as buffalobur (*Solanum rostratum* Dunal) (Wei et al. 2009) and Venice mallow (*Hibiscus trionum* L.) (Chachalis et al. 2008). This indicates that AmB seeds are sensitive to osmotic stress and a moist environment is necessary for germination.

The relationship between germination and salt stress was best described by an exponential curve model [G (%) $= 57.44 - 0.74x + 0.002x^2$; $R^2 = 0.99$] (Figure 2b). The highest germination (58%) was observed in distilled water (0 mM), and germination dropped to 13% as salinity increased to 80 mM NaCl. No germination was recorded at 120 mM NaCl.

The weather in Dongting Lake District is humid, with the average annual rainfall of 1,250 to 1,450 mm, and most of the soils in this area are mildly saline (Peng et al. 2006). Moreover, the major crops

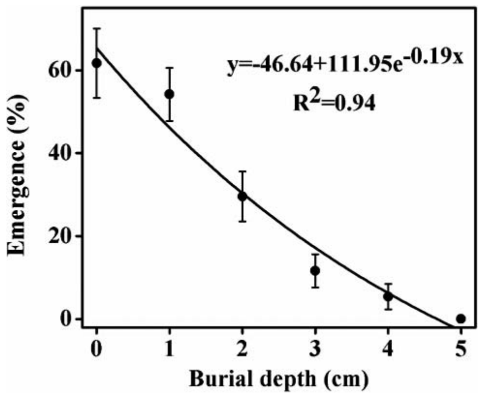

Figure 3. Effect of burial depth on emergence of Asia minor bluegrass seeds incubated at 20 C in a 12-h photoperiod for 14 d. Vertical bars represent the standard error of the mean ($n = 8$).

of wheat and rapeseed are often rotated with rice. These fields often have relatively high soil moisture due to the irrigation conducted in periods of paddy planting, which provides a suitable environment for AmB seed germination.

Burial Depth. No seedling emergence was observed in the blank control pots (nonseeded) after 21 d, which demonstrates no background AmB seed bank in the soil used. Burial depth significantly affected AmB emergence (Figure 3). An exponential model [G (%) = $-46.64 + 111.95e^{-0.19x}$; $R^2 = 0.94$] described the relationship between AmB emergence and burial depth. The greatest emergence (62%) was observed when seeds were placed on the soil surface, and emergence decreased sharply with an increase in burial depth. No emergence was observed from the seeds placed at a depth of 5 cm. Germination at shallow soil depths has been reported in several weed species such as giant sensitiveplant *(Mimosa diplotricha* C. Wright ex Sauvalle) (Chauhan and Johnson 2008) and smutgrass [*Sporobolus indicus* (L.) R. Br.] (Rana et al. 2012) that were notably all small-seeded species. Small-seeded weeds often need light exposure to enhance their germination; however, increasing burial depth greatly decreases the light penetration (Benvenuti 1995). This might be one major reason why burial depth caused a reduction in AmB seedling emergence. In Dongting Lake District, a high proportion of the crop fields are under a no-tillage or minimal-tillage cropping system (Huang 2007), conditions that permit AmB seeds to remain near the soil surface and favor successful germination and establishment.

In summary, this study showed that AmB seeds from the Dongting Lake District of China could germinate under a wide range of pH conditions and is tolerant to a moderate level of salinity stress but did not germinate well under osmotic stress. These results suggest that soil factors are unlikely to limit the potential range of AmB in this region. Temperature influenced AmB germination and the optimum condition was a constant temperature of 20 C or an alternating temperature regime of 22/15 C and 24/19 C, which indicates that AmB seeds could germinate from October to early December and with peak emergence likely during mid- to late-October in the region. Although some germination occurred under complete darkness, AmB germination was higher when imbibed seed were exposed to light. Additionally, emergence was greatest when seeds were at the soil surface, and dropped dramatically with burial depth. The temperature, soils, and cropping systems of the Dongting Lake District are suitable for the germination of AmB and have contributed to the spread across the region. Furthermore, no-tillage or minimal-tillage cropping systems that have been adopted in this district further promoted the germination and establishment of this weed. Therefore, some sort of treatment (tillage or other) to the soil surface might be a feasible option for limiting germination in AmB and reducing successful establishment in the Dongting Lake District in China.

Acknowledgments

This work was supported by a grant from the Special Fund for Agro-Scientific Research in the Public Interest, P.R. China (No. 201303031), Natural Science Funds of Hunan Province (No. 14JJ3096), Hunan Provincial Key Laboratory for Biology and Control of Weeds (2015TP1016), and Scientific-Innovative team of Hunan Academy of Agricultural Sciences (2014TD01).

Literature Cited

[APIS] Agriculture Pest Information System (2014) Database of Dry Land Weeds in China, dw00196. http://www.agripests.cn/show3_2.asp?id=128. Accessed May 4, 2014

Benvenuti S (1995) Soil light penetration and dormancy of jimsonweed (*Datura stramonium*) seeds. Weed Sci 43:389–393

https://doi.org/10.1614/WT-D-15-00043 Published online by Cambridge University Press

Chachalis D, Korres N, Khah EM (2008) Factors affecting seed germination and emergence of Venice mallow (*Hibiscus trionum*). Weed Sci 56:509–515

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

Chauhan BS, Johnson DE (2008) Seed germination and seedling emergence of giant sensitiveplant (*Mimosa invisa*). Weed Sci 56:244–248

Cheng J, Pan G, Cang L, Yang J, Wang J (2000) Effect of simulation acid rain on plant and pH of paddy soils in Taihu area. J Nanjing Agric Univ 23:116–118 [In Chinese]

Flora of China (2006) Flora of China Branch; Poaceae, *Polypogon*. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200026068. Accessed December 27, 2013

Grime JP, Mason G, Curtis AV, Rodman J, Band SR, Mowforth MAG, Neal AM, Shaw S (1981) A comparative study of germination characteristics in a local flora. J Ecol 69:1017–1059

Huang H (2007) Current situation on direct seeding and no-tillage cultivation of rape field in China. Hunan Agric Sci 4:158–160 [In Chinese]

Huang Y, Jiao Y, Zong L, Zhou Q, Zheng X, Sass R (2002) N_2O emission from wheat cultivated soils as influenced by soil physicochemical properties. Acta Sci Circ 22:598–602 [In Chinese]

Jung M, Soreng RJ, Kuoh C (2006) Notes on grasses (Poaceae) for the Flora of Taiwan (II). Taiwania 51:240–248

Keshavarzi M, Kharazian FA, Seifali M (2007) Morphological variations of genus *Polypogon* in Iran. Iran J Weed Sci 3:118–128

Koger CH, Reddy KN, Poston DH (2004) Factors affecting seed germination, seedling emergence, and survival of texasweed (*Caperonia palustris*). Weed Sci 52:989–995

Michel BE, Kaufaman MR (1973) The osmotic potential of polyethylene glycol 6000. Plant Physiol 51:914–916

Peng PQ, Wu JS, Huang DY (2006) Microbial biomass C, N, P of farmland soils in different land uses and cropping systems in Dongting Lake region. Acta Ecol Sin 26:2261–2266 [In Chinese]

Peng W, Zhu Q, Li Y, Tang D (2011) Temperature effect on seed germination of four weeds. Weed Sci (China) 29:26–29 [In Chinese]

Qiang S (2002) Weed diversity of arable land in China. J Korean Weed Sci 22:187–198

Qiang S (2005) Multivariate analysis, description, and ecological interpretation of weed vegetation in the summer crop fields of Anhui Province, China. J Integr Plant Biol 47:1193–1210

Qureshi R, Waheed A, Arshad M (2009) Weed communities of wheat crop in district Toba Tek Singh, Pakistan. Pak J Bot 41:239–245

Rana N, Wilder BJ, Sellers BA, Ferrell JA, MacDonald GE (2012) Effects of environmental factors on seed germination and emergence of smutgrass (*Sporobolus indicus*) varieties. Weed Sci 60:558–563

Ranjit JD, Bellinder R, Benidict C, Kumar V (2011) Green house studies on the biology of winter annual grasses and the use of cover crops and herbicides for their control. Agron J Nepal 2:139–148

Rao N, Dong L, Li J, Zhang H (2008) Influence of environmental factors on seed germination and seedling emergence of American sloughgrass (*Beckmannia syzigachne*). Weed Sci 56:529–533

Shao G, Zhang J (1992) Characteristic and management of agricultural ecosystem in Dongting Lake wetland. Resour Sci 14:48–52 [In Chinese]

Shaw DR, Mack RE, Smith CA (1991) Redvine (*Brunnichia ovata*) germination and emergence. Weed Sci 39:33–36

Tang W, Zhou F, Chen J, Zhou X (2014) Resistance to ACCase-inhibiting herbicides in an Asia minor bluegrass (*Polypogon fugax*) population in China. Pestic Biochem Physiol 108:16–20

Taylorson RB (1987) Environmental and chemical manipulation of weed seed dormancy. Rev Weed Sci 3:135–154

TianQi (2013) Historical Synoptic Chart, Changde. http://lishi.tianqi.com/changde/index.html. Accessed December 19, 2013

Wei S, Zhang C, Li X, Cui H, Huang H, Sui B, Meng Q, Zhang H (2009) Factors affecting buffalobur (*Solanum rostratum*) seed germination and seedling emergence. Weed Sci 57:521–525

Zhang H (1993) Studies on the production loss of wheat affected by *Polypogon fugax* and the economic thresholds. Weed Sci (China) 4:15–16 [In Chinese]

Zhao Y, Yi S, Yang X, Pi C (2001) Investigation of weeds occurring in rapeseed fields of Dongting Lake region. Crop Res 2:35–36 [In Chinese]

Received April 7, 2015, and approved November 3, 2015.

Associate Editor for this paper: Bradley Hanson, University of California, Davis.