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Effect of tillage and burial depth and density of seed on viability and seedling emergence of weedy rice

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Abstract

Weedy rice (*Oryza sativa* f. *spontanea*) is one of the three worst paddy weeds in most rice growing areas. The unexpected heavy infestation is derived from a persistence of soil seed bank of weedy rice, which the shattered seeds chiefly feed back to. Information on soil seed bank dynamics is imperative to predict the infestation of weeds. In the present paper, the effect of rotary tillage on weedy rice seed bank structure was studied first, and a burial experiment of marked seeds was conducted to observe the overwintering survival, seed viability and seedling emergence of weedy rice. The results showed that the proportion of weedy rice seeds in deeper soil increased but seedling emergence decreased with increasing plowing depth. The viability of weedy rice seeds decreased as the burial duration time extended but more slowly in deeper soil layers. Additionally, there was no significant effect of burial density on seed viability. Moreover, the logistic model fitted well ($R^2 \geq 0.95$, $P \leq 0.01$) with the depressive trends of seed viability with increasing burial time under all burial depths and densities which can provide us further information about seed survival. In field experiments, number of seedling emergence significantly decreased as seed burial depth increased, conversely, proportion of seedling emergence increased as seed burial density decreased. This study has important implications for determining strategies for weedy rice management by exhausting its seed bank through the alteration of tillage practices.

Keywords: seed burial depth, seed burial density, germination, wintering survival, seed vertical distribution

1. Introduction

Weedy rice (*Oryza sativa* f. *spontanea*), sometimes known as wild rice, autogenous rice or red rice, has become a very problematic weed that infests paddy fields and is widely distributed in rice-growing areas all over the world (Ferrero *et al*. 1999; Patindol *et al*. 2006). It has been recognized as a new type of serious weed by the Food and Agriculture Organization (FAO) and is considered the third worst weed menace in cultivated rice (*Oryza sativa* L.) (Labrada 2002; Delouche *et al*. 2007). The occurrence of weedy rice is closely related to the soil seed bank, which is the important foundation for its persistence in paddy fields across seasons. It is commonly reported that the large number of seeds produced by weedy rice is the key factor that affects its population dynamics (Zhang *et al*. 2014). In terms of occurrence of the annual weed, seed persistence

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in the soil seed bank is the most important factor affecting population dynamics (Davis *et al.* 2004). Weedy rice is a detrimental weed because it produces a large quantity of seeds, matures earlier than cultivated rice and its seeds shatter easily (Vaughan *et al.* 2003; Lu and Snow 2005; Chauhan and Johnson 2010b). The shattered weedy rice seeds that fall onto the soil surface can be buried and contribute to the formation of a large seed bank, which can reach densities of thousands per m^2. Shattered seeds can be dormant and remain viable for many years in the seed bank (Cohn and Hughes 1981) allowing weedy rice to continuously occur over a long term. The study of the seed bank structure, overwintering survival and seedling emergence dynamics of weedy rice seeds in the soil seed bank and its influencing factors is useful in estimating and predicting the population dynamics of weedy rice.

Unexpected heavy infestation of weedy rice can cause a great loss of rice yield or even a harvestless paddy field (Chin 2001; Gressel and Valverde 2009). Most farmers have a misconception that the weedy rice infestation can only be sourced from contaminated rice seeds. This misconception caused a dispute with seed companies about the reason of weedy rice infestation resulting in the ignorance of the importance of the soil seed bank. Furthermore, due to the similarity in morphology and physiology of weedy rice to that of cultivated rice, weedy rice is difficult to identify at an early stage (Gressel and Valverde 2009); in addition, there is no selective herbicide available to specially target weedy rice in the field. Therefore, weedy rice control mainly relies on manual eradication, which requires more time and manpower and is usually inefficient. Understanding seed emergence patterns from the soil seed bank can be helpful to predict weedy rice occurrence, which can lead to adoption of an effective management measure in advance.

Until now, there has been little information available on the population dynamics and seed ecology of weedy rice in the soil seed bank in China. In previous reports, Gealy *et al.* (2009) and Chauhan (2012b) studied the relationship between burial depth and seedling emergence of weedy rice. Chauhan (2012b) found that the greatest emergence was observed in seeds placed on the soil surface and seedling emergence decreased with increase in burial depth. Gealy *et al.* (2009) observed that the seedling emergence was affected by both weedy rice types and the soil type. Ma *et al.* (2008) analyzed the germination dynamics of weedy rice at different sowing depths in northern China and the results showed that the elongation of mesocotyl and internode of coleoptiles played important roles in seedling emergence. However, these seed burial experiments were only conducted under laboratory conditions. Moreover, there are no reports on the influence of rotary tillage on weedy rice seed bank vertical structure and on the effect of burial depth on the change of seed survival through a winter season under field conditions.

In the present project, a field experiment was conducted to study the influence of rotary tillage on the distribution of weedy rice in the soil seed bank and field burial experiments were conducted to study the overwintering persistence and seedling emergence of weedy rice seeds at various burial depths and densities. In addition, a mathematical model was fitted to explain the change in weedy rice seed viability with burial time under various conditions. This study provides useful information for understanding the seed distribution, longevity and persistence of weedy rice in soil seed banks. This is useful not only for the prediction of weedy rice infestation and occurrence, but also to provide a scientific basis for weedy rice seed bank depletion by farming practices, which has important implications on land preparation and management.

2. Materials and methods

2.1. Experimental location and fields

Two experiments were conducted in the Jiangpu Experimental Farm of Nanjing Agricultural University (32°01–32°03´N, 118°36´–118°38´E) located in Pukou District, Nanjing, China. The Jiangpu Farm situated in subtropical climate zone, with annual average temperature of 15.4°C, annual total sunshine of 2008 h, annual precipitation of 1067 mm, and average frost-free period of 227 days. During the burial experiment (from September 2011 to May 2012), the monthly average temperature was 2.9–22.3°C, the monthly mean precipitation was 17 to 204 mm, the monthly mean sunshine was 97 to 204 h.

Two adjacent fields were chosen for the experiments. Each of the two selected experimental fields had its own history of wheat-soybean double-cropping system for more than 5 years and winter wheat was the preceding crop. Prior to the start of the experiments, no weedy rice was found in the experimental fields basing on our previous investigations. The soil type in the experimental area was clay, soil organic matter content 0.67% (determined by the $K_2Cr_2O_7$ titration method after digestion), total nitrogen 0.11% (determined by Kjeldahl method), available P 51.6 mg kg^{-1} (determined by 0.5 mol L^{-1} $NaHCO_3$ extraction colorimetric method), available K 87 mg kg^{-1} (determined by 1 mol L^{-1} NH_4OAC extraction flame photometry), pH 7.1 (determined in soil-water slurry using a combination glass electrode).

2.2. Experimental materials and experimental design

Dyeing of weedy rice seeds Matured weedy rice seeds (Japonica-like weedy rice with awnless yellow-hull) were collected in the autumn of 2010 from a previously utilized experimental rice field and were dried thereafter. Portion of these seeds were then dyed by immersion for an hour in 1% (w/v) safranine T (Sinopharm Chemical Reagent Co. Ltd., China) solution dissolved in rate 50% ethanol water solution (Qiang *et al.* 2013). The seeds were air-dried at (30±2)°C for 5 days to mark buried seeds.

The non-dyed and dyed seeds were stored at 4°C for approximately 6 months until the tillage experiment commenced on 20 June 2011. Seeds were stored at 4°C for approximately 12 months until the burial experiments were performed.

Effect of dyeing seeds with safrenine T on weight and germination rate of weedy rice The germination rate was tested to determine whether there were impacts from dying on seed viability. Five replicates of 50 seeds, either dyed or non-dyed, were placed on a substratum of two thicknesses of Whatman No. 1 filter paper in covered 9 cm diameter Petri dishes. The filter paper was moistened with 5 mL sterilized water at the beginning. The dishes were kept in an incubator at 25°C for a 16 h light/8 h dark photoperiod for 28 days. Germination was monitored daily and water was added when necessary to keep the filter paper moist. Seeds were considered germinated when the length of the radicle was longer than or equal to half the length of the seed.

Influence of tillage depth on vertical seed distribution and seedling emergence Experiment was conducted in one of the selected fields in June, 2011, to determine the effect of tillage on distribution of weedy rice seeds in the soil seed bank and on the weedy rice seedling emergence. There was no any tillage practice performed during the period between the harvest of the previous crop and the start of the experiment.

The field was first divided into nine 10 m×10 m plots (100 m^2 area per plot). Dyed weedy rice seeds were sown on the soil surface of each plot at a seeding rate of 45 kg ha^{-1}. After seed sowing, the nine plots were tilled with a rotary tillage (the width of rotary cultivator was 200 cm) at three different plowing depths: 5, 10 and 15 cm. Three replicates were presented for each plowing based on a randomized block design.

To estimate the amount of weedy rice seeds at different soil depths, 500 soil cores (2.5 cm in diameter and 15 cm deep, the coring device was a 20-cm graduated stainless steel pipe of 2.5 cm inner diameter) were randomly taken in each plot immediately after the plowing and then the earth pillar was manually divided into three depth categories: 0–5, 5–10 and 10–15 cm. The 500 subsamples of each soil depth were pooled and homogenized into a composite sample representing a total area of 0.25 m^2 (volume=12 500 cm^3). Seeds (the average length and width of the weedy rice seeds were 8.5 mm and 3.1 mm) were carefully extracted from each composite subsample by running water over a wire sieve (1-mm mesh). Once dried at ambient temperature, the remainder were placed in a Petri dish and the intact dyed seeds were counted (broken ones were discarded) under a dissecting microscope (maximum magnification of 10×4 times).

Seedling emergence in the experimental plots was recorded every week over the entire plot area from the late spring (May 2012) to early summer (July 2012), until there were no more emerging seedlings.

Influence of burial depth and burial density on seed viability and seedling emergence Seed burial experiment was conducted in another field from November 2011 to May 2012 to determine the effect of seed burial on weedy rice seedling emergence.

In the field, 45 experimental plots (2 m×2 m), each spaced 1 m from another were prepared. The dyed weedy seeds were buried in a randomized complete block design with a two-way factorial treatment design regime. Variables included seed burial depth at three levels (buried 0–5, 5–10, and 10–15 cm in the soil) and burial densities at five levels (buried with 1000, 2000, 3000, 4000 and 5000 seeds m^{-2}). The different seed densities were applied on the basis of their 1000 seed weight. Each treatment combination was replicated 3 times for a total of 45 experimental plots.

Plots were first dug using a spade to the designated depth (5, 10 or 15 cm), and then a fixed number of seeds were mixed with about 0.1 m^3 of the dug soil, which covered an area of 2 m^2 and reached a height of 5 cm. Then the seed mixture was sown onto each plot and covered with the leftover dug soil to the designated depth.

We sampled the soil every 15 days over a 6-month period, 12 times in total, to determine whether the dyed weedy rice seeds persisted in the soil at varying depths and densities. When sampling, 50 soil cores were sampled from the 45 plots separately. Each soil core was approximately 2.5 cm in diameter and 15 cm in depth. The 50 soil samples in each plot were then pooled and homogenized into a composite sample representing a total area of 0.025 m^2 (volume=1 250 cm^3, covered an area of 2.5 m×10^{-2} m) and then taken back to the laboratory for further analysis.

Seeds were carefully extracted using the method mentioned above. Once dried at ambient temperature, intact dyed seeds were counted (broken ones were discarded). After the weedy rice seeds retrieved from the soil samples were dried, they were stored at 4°C to prevent them from decaying between collections and the germination tests. Just before germination tests, the seeds were treated with 40°C for 5 days to break the seed dormancy.

We determined seed germination rates of buried weedy rice seeds under laboratory conditions by retrieving seeds obtained from the soil samples. The seed viability here indicated the proportion of intact seeds that survived and germinated under laboratory condition compared with the number of seeds that were buried initially. The first soil samples were taken immediately after the seeds were buried to determine the initial viability. Germination was determined by laying all the seeds retrieved from each plot in a Petri dish (9 cm diameter) lined with dense filter paper moistened with sterilized water. The seed germination test was conducted using the condition described above.

Seedling emergence in the experimental plots was monitored every week over the entire plot area from the late spring (May 2012) to early summer (July 2012), until there were no more emerging seedlings.

2.3. Data analyses

Date were statistically analyzed using SPSS 19.0 (IBM Corporation, Armonk, NY, USA), and all figures were drawn using OriginPro 9.0 (OriginLab Corporation, Northampton, MA, USA).

Model simulating Survival of weedy rice seeds in different burial depths with time was modeled using a logistic model with four parameters. Burial depth or burial density was used as the fixed effects.

$$y=a+\frac{b}{1+(\frac{x}{x_0})^c}$$

For the logistic model, a, b, c and x_0 are the curve parameters; b is the upper limit of seed viability rate changed with burial time; x_0 is the theoretical burial days when the viability rate halved compared with the initial viability rate; c is the constant; $a+b$ is the maximum seed viability rate; x is the burial duration time (in days); and y is the seed viability rate. Moreover, the confidence intervals at 5 and 95%, respectively, of the total variability were drawn for each curve.

Statistical analyses Data were examined for homogeneity (Levene's test) first and then one-way analyses of variance (ANOVA) was used to distinguish the influence on seed germination between dyed and non-dyed weedy rice seeds ($P\leq0.05$).

Correlation analysis was used to determine the correlation coefficient between weedy rice viability rate and soil seed bank density for different soil depths.

The burial data was also analyzed as a fully crossed, three-factor experiment with the seed viability as the response variable in the variance analysis.

3. Results

3.1. Effect of dyeing seeds with safranine T on weight and germination rate

The results in Table 1 indicated that there were no significant differences in weight or germination rate between safranine T-dyed and non-dyed weedy rice seeds, suggesting that the safranine T could be a candidate dyeing agent for marking weedy rice seeds. Therefore, the dyed weedy rice seeds were used in all the other experiments to ensure easy recognition of seeds in soil samples.

3.2. Influence of tillage depth on vertical seed distribution

When tilled at 5 cm plowing depth, about 185 (approximately 88% of total), 22 (10%) and 3 seeds per m^2 were retained in 0–5, 5–10 and 10–15 cm, respectively, and about 127 seedlings emerged, occupying 61% of total burial seeds. As the tillage depth increased, more of the weedy seeds were plunged into the deep soil, and density of seedling emergence was lower. When the tillage of 15-cm plowing depth applied, 88 weedy rice seeds (about 42%) were plunged into the 10–15 cm soil layer, whereas about 57 weedy rice seeds (less than 30%) were retained in the shallow 0–5 cm soil layer, and about 35 seedlings emerged, occupying 35% of total burial seeds (Fig. 1).

Table 1 Influence of dyeing weedy rice seeds with safranine T on weight and germination rate

Treatment	Thousand-kernel weight (g)	Germination rate (%)
Non-dyed	20.16±0.56	85.40±3.25
Dyed	20.34±0.68	83.20±4.13

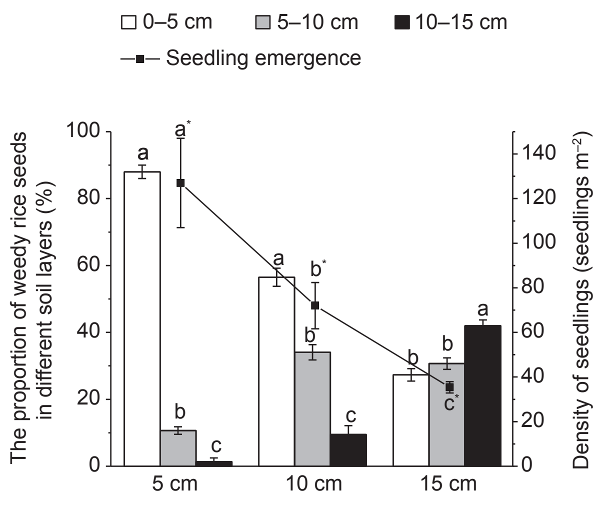

Fig. 1 Influence of tillage on vertical distribution in soil and seedling emergence of weedy rice seeds. Data indicate mean±SE. Different lowercase letters and different lowercase letters with * indicate the significant differences of seed vertical distribution at each tillage depth and the significant differences of seedling emergence among three tillage depths, respectively, at $P\leq0.05$ based on an F-LSD (Fisher's protected LSD) test.

3.3. Influence of burial depth and burial density on seed viability

The logistic model of the survival rate of buried weedy rice seeds with different depths and densities described the response variable significantly well (adjusted $R^2 \geq 0.95$, $P \leq 0.01$) (Fig. 2). The average initial germination rate of weedy rice seeds (buried for 0 days) was approximately 80%, and the seed survival rate decreased to only 5–20% after a 5-month burial period. After 5 months, weedy rice seed survival rate decreased to 5% in the shallow soil layer (0–5 cm), whereas in the deeper soil layer (10–15 cm),

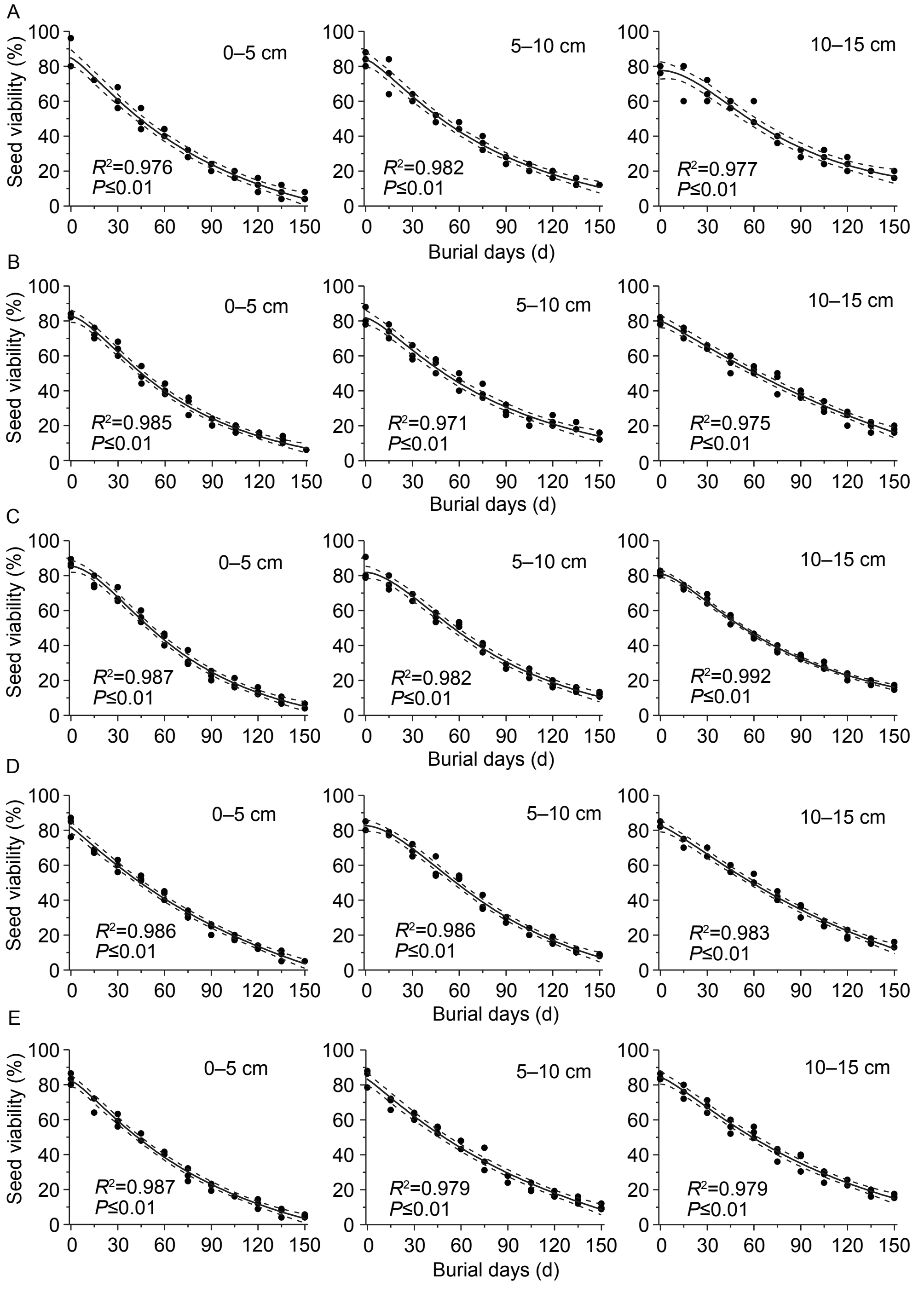

Fig. 2 Seed viability of weedy rice with time under different burial depths at different seed burial densities. A, B, C, D and E, the burial density was constant with 1 000, 2 000, 3 000, 4 000 and 5 000 seeds m^{-2}, respectively. – was the logistic regression line and the space between the "----" was at a 95% confidence interval.

approximately 20% remained viable.

The change in seed viability over time depended mainly on both seed burial depth and burial density. Our analyses presented that there was a highly significant positive relationship between burial depth and weedy rice seed viability, but a highly significant negative relationship between burial time (days) and weedy rice seed viability; however, there was no correlation between the burial density and seed viability (Tables 2 and 3).

Approximately 6 months after the initial burial, the weedy rice seed emergence began and lasted for about 1 month. In field conditions, under the same burial density, the seedlings emerged most from the shallow soil (Fig. 3-A). When the seeds of three different burial depths but with the same burial density were treated as a whole, more than 75% of the seedlings emerged from the 0–5 cm soil layer, approximately 20% emerged from the 5–10 cm layer and less than 5% emerged from the 10–15 cm layer (Fig. 4-A). However, under laboratory conditions, the seed viability (germinability) increased when the burial depth increased (Fig. 3-B). When the viable (germinable) seeds from the three different burial depths but with the same burial density were treated as a whole, proximately 50% of the germinable seeds came from the 10–15 cm soil layer and only approximately 15% of the germinable seeds came from the 0–5 cm soil layer (Fig. 4-B). The number of emerged seedlings (Fig. 3-A) in the field condition was much lower than the amount of viable (germinable) seeds discovered under laboratory conditions (Fig. 3-B).

Table 2 Variance analysis for the influences of seed burial depth, burial density and burial duration time on seed viability of weedy rice[1)]

Effect	SS	*df*	MS	*F*-statistic	*P*-value
Density	0.014	4	0.004	2.968	0.020
Burial duration time (days)	28.073	10	2.807	2 368.669	<0.001
Depth	0.468	2	0.234	197.630	<0.001
Density×Days	0.063	40	0.002	1.330	0.095
Density×Depth	0.034	8	0.004	3.555	<0.001
Days×Depth	0.181	20	0.009	7.642	<0.001
Density×Days×Depth	0.076	80	0.001	0.805	0.879
Error	0.391	330	0.001		
Total	113.493	494			

[1)] SS, sum of squares; *df*, degrees of freedom; MS, mean square.

4. Discussion

With the development and prevalence of the rice direct-seeding, the labor-saving rice cultivation method, weedy rice infestation became a major problem for rice production all over the world, particularly in Asia, South and North America, and southern Europe (Ferrero *et al.* 1999; Bres-Patry *et al.*

Table 3 Correlation coefficients among the seed burial depth, burial density and burial duration time for both seed viability under laboratory conditions and for seedling emergence in the field

Item	Burial density	Burial depth	Burial time
Viability	0.006	0.126**	–0.967**
Density of seedling emergence	0.258*	–0.914**	

* and ** indicate significant differences at $P \le 0.05$ and $P \le 0.001$, respectively.

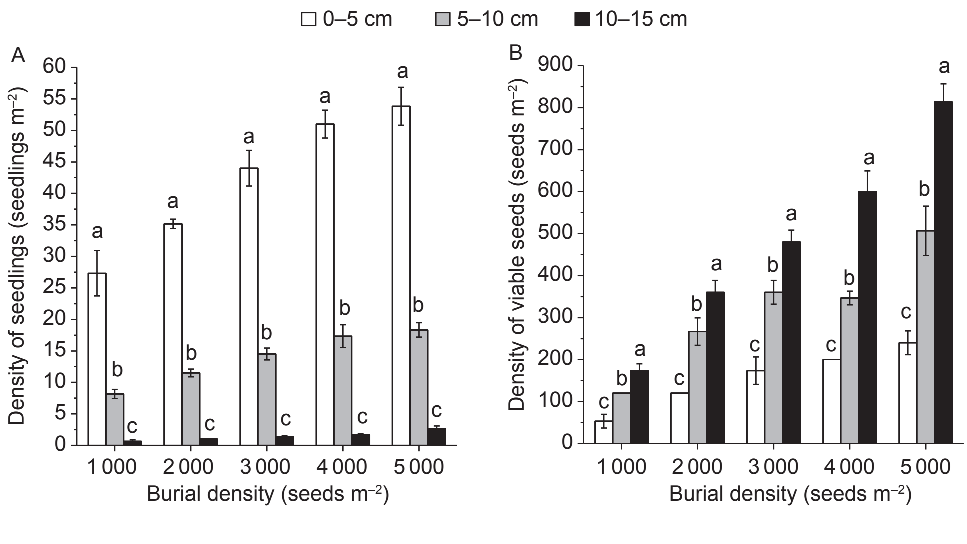

Fig. 3 Comparison of weedy rice seedling emergence in the field and seed viability under laboratory conditions. A, the density of seedling emergence found in the field. B, the density of viable (germinable) seeds after a 150-days' burial experiment tested under laboratory conditions. Data indicate mean±SE. Different lowercase letters indicate significant differences at $P \le 0.05$ among the constant seed burial densities based on an F-LSD (Fisher's protected LSD) test.

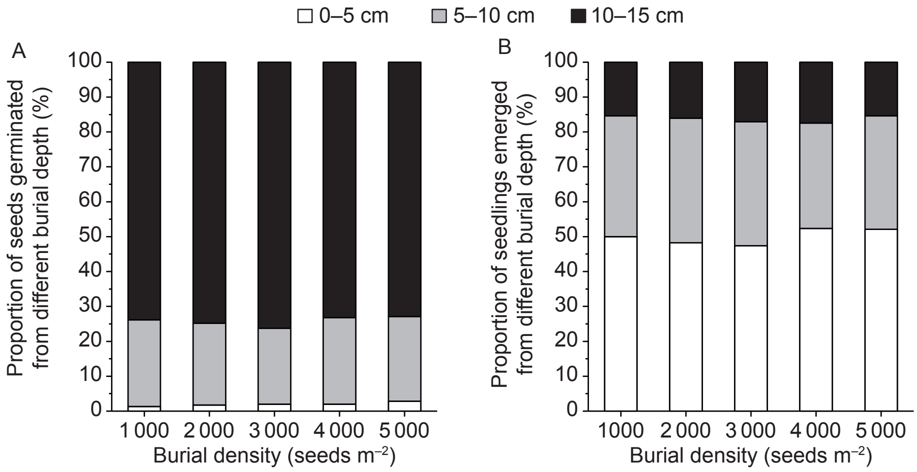

Fig. 4 Proportion of emerged seedlings from different soil depths or viable (germinable) seeds from three different burial depths. A, the seedlings emergence in the field. B, the viable (germinable) seeds in the laboratory.

2001). In Jiangsu Province, China, under continuous wheat-rice double cropping systems, the direct-seeded rice has to be widely adopted because of labor shortages, tight season schedules and water saving restrictions (Zheng 2000). As a result, weedy rice has become one of the most troublesome weeds in paddy fields and recently has caused great economic losses to the local farmers in this province. However, farmers usually attribute the heavy weedy rice infestation to contaminated rice seeds. The misconception of the real source of weedy rice, usually leads to ignorance regarding the weedy rice seed bank, which eventually leads to a serious weedy rice infestation.

4.1. The dyeing technique used for easy retrieving of weedy rice

Tracking the weedy rice seeds in soil seed bank is helpful to reveal the source of the serious weedy rice infestation. Dyeing weedy rice seed with safranine T was firstly used to track weedy rice seeds. This seed dyeing method is easy to operate and the color of dyed weedy rice seeds can last for more than one year when buried in the soil as we observed. In addition, seed weight and germination rate were not affected by dyeing treatment. Furthermore, because the safranine T dyeing method is simple, environmentally safe, inexpensive, and effective, it can be widely used to dye light-colored weed seeds. The seed dying technique can be also used in the quantitative analysis of seed bank and seed dispersal. In this project, the weedy rice seeds were dyed for easy retrieving. The spatial distribution of weedy rice seeds in the soil seed bank affected by tillage and the dynamics of viability of seeds buried at different soil depths under both laboratory and field conditions were revealed by tracking the dyed weedy rice seeds.

4.2. Weedy rice seed distribution was influenced by agronomic practices

Spatial distribution of weed seeds in soil seed banks was closely related to agronomic practices, including the tillage practice (Chauhan *et al.* 2006). In wheat-rice cropping system, rotary tillage would occur after rice harvest but before wheat sowing. The results in this study indicated that rotary tillage can plunge the newly shattered weedy rice seeds into the soil at different positions in the soil profile. Shallow tillage plowed most of the weedy rice seeds into shallow soil, whereas deep tillage plowed some of the weedy rice seeds into deeper soil. Seed persistence in the soil seed bank can compensate for effects of unfavorable environmental conditions on seedling emergence (Holmgren *et al.* 2006) and increase the odds of seed survival for recruitment when conditions are suitable (Wijayratne and Pyke 2012).

4.3. Seed viability and seed emergence of buried weedy rice was influenced by burial depth

In this study, it was observed that the weedy rice seed survival rate decreased significantly as the burial time increased, and the viability (survival rate) of weedy rice seeds was higher when buried in deeper soil compared to seeds buried in shallow soil. Though a greater depletion of viable seeds was found in shallow soil than in deep soil, there was still a large number of weedy rice seeds survived overwintering, especially in deep soil. The present study also find that the weedy rice seedlings emerged most from shallow soil while the seed viability was higher when buried in deeper soil. These findings indicated that the deep soil could conserve the seed better and most of the viable seeds were dormant in the field when buried in deep soil. This information gives us the reason to believe that the great outbreak and persistence of weedy rice infestation in rice fields could be caused by the presence of the large-scale seed bank.

Weedy rice seeds in the soil seed bank would be depleted or would lose their vitality due to various factors during the autumn and winter season (Delouche *et al.* 2007), e.g., predation by insects and vertebrates, bacterial and fungal decay (Wagner and Mitschunas 2008) and the constant consumption of nutritive substances. However, there would still be a large number of seeds that survived up to the next rice growing season. The seed survival rate differed in different seed burial depths, but all followed a similar downtrend that can be described with a logistic model. In general, on the soil surface or in shallow soil, seed depletion was greater and seed retention was lower. As expected, seed longevity was usually longer and the viability was higher for deeply buried seeds (Zorner *et al.* 1984; Skoglund 1992; Bekker *et al.* 1998). On the soil surface or in shallow soil, seeds have greater opportunities for granivore fauna predation (Chauhan *et al.* 2012) and seed decay is easily caused by ever-changing environmental factors such as temperature, moisture and gas exchange on/near soil surface (Taylorson 1970). In addition, when seed dormancy is assumed to be one explanation for seed persistence in soil seed bank, the adequate light and the frequent temperature fluctuations in the shallow soil layer can easily break the seed dormancy, which would lead to earlier seed germination in adverse seasons. In deeper soils, there are several factors that keep seeds dormant for a longer time: less gas exchange, lower oxygen, higher carbon dioxide and lack of light (Botha *et al.* 1992; Benvenuti and Macchia 1995; Keeley and Fotheringham 1997; Chauhan and Johnson 2010a). The deeper the seed buried in soil, the greater the inducement of seed dormancy (Benvenuti *et al.* 2001). Seed germination would be hindered as well, which may be the mechanism of seed enforced dormancy that evolved to avoid germination under adverse conditions and to ensure survival in the soil seed bank (Mapes *et al.* 1989; Baskin and Baskin 2001). These deeply buried weedy rice seeds formed the seed bank which was not active. Relatively constant temperature and humidity in deep soils are more conducive for the conservation of dormant weedy rice seeds. These deeply buried seeds showed a great germination potential under laboratory condition when compared to the seeds buried in shallow soil. The present study showed that the weedy rice seedlings emerged most under field condition from the shallowest soil layer. The seed germination and seedling emergence would increase greatly as the burial depth decreased (Fogliatto *et al.* 2010; Chauhan 2012a) for the change of light, temperature, moisture and other environment factors (Holmes and Smith 1975) as it is found in this study. Therefore, when the deeply buried seeds were turned over to shallow soil, the seed dormancy would be broken, and the seeds would germinate under favorable conditions. As a result, the deeply buried seeds can become the potential source of weedy rice infestation.

4.4. Model simulation of weedy rice survival rate with time

The logistic model for the survival rate of weedy rice seeds buried in different soil layers fitted very well. If environmental factors, such as soil moisture, temperature, illumination and other factors are measured further, the number of surviving seeds over a winter season could be more accurately estimated through a logistic model. Moreover, the weedy rice occurrence can be predicted with high accuracy. Because the longevity of weedy rice seeds was significantly shorter on the soil surface than in deep soil, in practice, some agronomical measures could be performed to exhaust the seed bank to control weedy rice.

The results obtained from the seed burial experiments that the deeper burial would conserve weedy rice seeds well and the tillage practice could influence the seed vertical distribution could provide valuable information for weedy rice control. After rice harvest, no-tillage or shallow tillage should be applied instead of deep tillage, so that the chance of weedy rice seeds being plunged into the deeper soil seed bank could be reduced. This precaution could lead to more of the shattered weedy rice seeds being concentrated in the shallow soil, which could increase the overwinter consumption of weedy rice seeds and accelerate the depletion of the seed bank. Furthermore, the seed bank investigation could be conducted before rice planting to determine the weedy rice seed distribution in the soil seed bank. In the case of seeds being mainly concentrated in the topsoil layer, control methods could include weedy rice germination induction directly by pre-sowing irrigation (Fogliatto *et al.* 2010); thereafter, weedy rice can be killed by chemical herbicide or removed through rotary tilling before rice planting. In the case of seeds being mainly concentrated in the deep soil layer, deep tillage could be performed first to plow the seeds back into the shallow soil. Germination-inducing and eradication methods could be applied as well. Conditions permitting, winter irrigation could also be adopted to increase soil moisture and facilitate depletion of the seed bank (Chauhan *et al.* 2012). If some of these management measures could be adopted, the density of weedy rice may dramatically decrease in the next rice growing season.

5. Conclusion

Early and easy shattering is usually the important trait of weedy rice. The shattered weedy rice seeds can be incorporated in the soil to vary depths with the help of disturbances of the soil, e.g., the cultural operations (tillage) preparatory to sowing the next crop. The shallow buried weedy rice seeds could be the cause of current infestation, but the deep buried seeds would be potential source of weedy rice infestation in the future.

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References

Baskin C C, Baskin J M. 2001. *Seeds*: *Ecology*, *Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego.

Bekker R, Bakker J, Grandin U, Kalamees R, Milberg P, Poschlod P, Thompson K, Willems J. 1998. Seed size, shape and vertical distribution in the soil: Indicators of seed longevity. *Functional Ecology*, **12**, 834–842.

Benvenuti S, Macchia M. 1995. Effect of hypoxia on buried weed seed germination. *Weed Research*, **35**, 343–351.

Benvenuti S, Macchia M, Miele S. 2001. Light, temperature and burial depth effects on *Rumex obtusifolius* seed germination and emergence. *Weed Research*, **41**, 177–186.

Botha F, Potgieter G, Botha A M. 1992. Respiratory metabolism and gene expression during seed germination. *Plant Growth Regulation*, **11**, 211–224.

Bres-Patry C, Lorieux M, Clement G, Bangratz M, Ghesquière A. 2001. Heredity and genetic mapping of domestication-related traits in a temperate japonica weedy rice. *Theoretical and Applied Genetics*, **102**, 118–126.

Chauhan B S. 2012a. Weed ecology and weed management strategies for dry-seeded rice in Asia. *Weed Technology*, **26**, 1–13.

Chauhan B S. 2012b. Weedy rice (*Oryza sativa*) II. Response of weedy rice to seed burial and flooding depth. *Weed Science*, **60**, 385–388.

Chauhan B S, Gill G, Preston C. 2006. Influence of tillage systems on vertical distribution, seedling recruitment and persistence of rigid ryegrass (*Lolium rigidum*) seed bank. *Weed Science*, **54**, 669–676.

Chauhan B S, Johnson D E. 2010a. The role of seed ecology in improving weed management strategies in the tropics. *Advances in Agronomy*, **105**, 221–262.

Chauhan B S, Johnson D E. 2010b. Weedy rice (*Oryza sativa*) I. Grain characteristics and growth response to competition of weedy rice variants from five Asian countries. *Weed Science*, **58**, 374–380.

Chauhan B S, Singh R G, Mahajan G. 2012. Ecology and management of weeds under conservation agriculture: A review. *Crop Protection*, **38**, 57–65.

Chin D V. 2001. Biology and management of barnyardgrass, red sprangletop and weedy rice. *Weed Biology and Management*, **1**, 37–41.

Cohn M A, Hughes J. 1981. Seed dormancy in red rice (*Oryza sativa*) I. Effect of temperature on dry-afterripening. *Weed Science*, **29**, 402–404.

Davis A S, Dixon P M, Liebman M. 2004. Using matrix models to determine cropping system effects on annual weed demography. *Ecological Applications*, **14**, 655–668.

Delouche J C, Labrada R, Rosell C. 2007. *Weedy Rices*: *Origin*, *Biology*, *Ecology and Control*. FAO of the United Nations, Rome, Italy.

Ferrero A, Vidotto F, Balsari P, Airoldi G. 1999. Mechanical and chemical control of red rice (*Oryza sativa* L. var. *sylvatica*) in rice (*Oryza sativa* L.) pre-planting. *Crop Protection*, **18**, 245–251.

Fogliatto S, Vidotto F, Ferrero A. 2010. Effects of winter flooding on weedy rice (*Oryza sativa* L.). *Crop Protection*, **29**, 1232–1240.

Gealy D R, Saldain N E, Talbert R E. 2009. Emergence of red rice (*Oryza sativa*) ecotypes under dry-seeded rice (*Oryza sativa*) culture. *Weed Technology*, **14**, 406–412.

Gressel J, Valverde B E. 2009. A strategy to provide long-term control of weedy rice while mitigating herbicide resistance transgene flow, and its potential use for other crops with related weeds. *Pest Management Science*, **65**, 723–731.

Holmes M, Smith H. 1975. The function of phytochrome in plants growing in the natural environment. *Nature*, **254**, 512–514.

Holmgren M, Stapp P, Dickman C R, Gracia C, Graham S, Gutiérrez J R, Hice C, Jaksic F, Kelt D A, Letnic M. 2006. Extreme climatic events shape arid and semiarid ecosystems. *Frontiers in Ecology and the Environment*, **4**, 87–95.

Keeley J E, Fotheringham C. 1997. Trace gas emissions and smoke-induced seed germination. *Science*, **276**, 1248–1250.

Labrada R. 2002. Major weed problems in rice-red/weedy rice and the *Echinochloa* complex. *FAO Rice Information*, **3**, 11.

Lu B R, Snow A A. 2005. Gene flow from genetically modified rice and its environmental consequences. *BioScience*, **55**, 669–678.

Ma D R, Wang N, Wang Y, Jia D T, Chen W F. 2008. Germination dynamics of weedy rice in northern China at different sowing depths. *Chinese Journal of Rice Science* **22**, 215–218. (in Chinese)

Mapes G, Rothwell G W, Haworth M. 1989. Evolution of seed dormancy. *Nature*, **337**, 645–646.

Patindol J, Flowers A, Kuo M I, Wang Y J, Gealy D. 2006. Comparison of physicochemical properties and starch structure of red rice and cultivated rice. *Journal of Agricultural and Food Chemistry*, **54**, 2712–2718.

Qiang S, Zhang Z, Li R H. 2013. *The Application of Safranine T Dyeing Technique for Labeling and Tracing*. Chinese Patent, CN 102523785 B. (in Chinese)

Skoglund J. 1992. The role of seed banks in vegetation dynamics and restoration of dry tropical ecosystems. *Journal of Vegetation Science*, **3**, 357–360.

Taylorson R. 1970. Changes in dormancy and viability of weed seeds in soils. *Weed Science*, **18**, 265–269.

Vaughan D A, Morishima H, Kadowaki K. 2003. Diversity in the *Oryza* genus. *Current Opinion in Plant Biology*, **6**, 139–146.

Wagner M, Mitschunas N. 2008. Fungal effects on seed bank persistence and potential applications in weed biocontrol: A review. *Basic and Applied Ecology*, **9**, 191–203.

Wijayratne U C, Pyke D A. 2012. Burial increases seed longevity of two *Artemisia tridentata* (Asteraceae) subspecies. *American Journal of Botany*, **99**, 438–447.

Zhang Z, Dai W M, Song X L, Qiang S. 2014. A model of the relationship between weedy rice seed-bank dynamics and rice-crop infestation and damage in Jiangsu Province, China. *Pest Management Science*, **70**, 716–724.

Zheng J G. 2000. Rice-wheat cropping system in China. In: Hobbs P R, Gupta R K, eds. *Soil and Crop Management Practices for Enhanced Productivity of the Rice-Wheat Cropping System in the Sichuan Province of China*. *Rice-Wheat Consortium Paper Series No. 9*. Rice-Wheat Consortium for the Indo-Gangetic Plains, New Delhi. pp. 1–10.

Zorner P S, Zimdahl R L, Schweizer E E. 1984. Effect of depth and duration of seed burial on kochia (*Kochia scoparia*). *Weed Science*, **32**, 602–607.

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