Transgenic Res
DOI 10.1007/s11248-013-9687-6

ORIGINAL PAPER

Laboratory assessment of the impacts of transgenic *Bt* rice on the ecological fitness of the soil non-target arthropod, *Folsomia candida* (Collembola: Isotomidae)

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Received: 2 March 2012 / Accepted: 6 January 2013

Abstract Transgenic rice expressing *Bacillus thuringiensis* (*Bt*) endotoxins (*Bt* rice) for pest control is considered an important solution to food security in China. However, tests for potential effects on non-target soil organisms are required for environmental risk assessment. The soil collembolan *Folsomia candida* L. (Collembola: Isotomidae) is a potential non-target arthropod that is often used as a biological indicator in bio-safety assessments of transgenic crops. In the present study, the roots, stems, and leaves of *Bt* rice were exposed to *F. candida* under laboratory conditions, with survival, reproduction and growth of the collembolan as ecological fitness parameters. Significant differences in ecological fitness were found among the different treatments, including differences in the plant parts and varieties of non-*Bt* rice, presumably as the result of three factors: gene modification, plant parts and rice varieties. The fitness of *F. candida* was less affected by the different diets than by the exposure to the same materials mixed with soil. Our results clearly showed that there was no negative effect of different *Bt* rice varieties on the fitness of *F. candida* through either diet or soil exposure.

Keywords Non-target organisms · *Bt* rice · Collembola · Ecological fitness · Risk assessment

Introduction

Obtaining an in-depth understanding of the environmental consequences of the introduction of transgenic crops is critical to ensuring the success of this crop production technology, as some aspects of *Bt* toxins and transgenic crop biotechnology may adversely affect the fitness of non-target organisms (NTOs). Such fitness changes may result from differences in plant compounds between transgenic and non-transgenic varieties (Flores et al. 2005; Poerschmann et al. 2005; Saxena and Stotzky 2001b), the sustained expression of *Bt* toxins by *Bt* crops (Saxena et al.

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2004; Wu et al. 2002), long-term persistence of *Bt* toxins in soil (Tapp and Stotzky 1995; Palm et al. 1996; Sims and Ream 1997) and the fact that the *Bt* toxin is transmissible from non-target species to higher trophic levels (Obrist et al. 2006; Wandeler et al. 2002; Saxena and Stotzky 2001a).

Responses to *Bt* crops have been evaluated for a range of NTOs in several investigations, with most being unable to demonstrate significant effects of *Bt* crops on population fitness (e.g., Betz et al. 2000; Icoz and Stotzky 2008; Yuan and Ge 2010). However, studies showing different results do exist. For example, Höss et al. (2008) reported negative effects of both purified Cry1Ab protein and soil from *Bt*-maize field plots on *Caenorhabditis elegans*. Manachini and Lozzia (2002) observed that the trophic group composition of nematofauna was affected by *Bt* crops when the plants were grown in clayey soil, and Whitehouse et al. (2005) reported a decline of *Nabis* spp. in *Bt* cotton fields. Furthermore, Zwahlen and Andow (2005) found that the transgene products of *Bt* maize were taken up and persisted in all of the ground beetles examined. Thus, as the literature concerning the effects of *Bt* crops on NTOs is inconsistent, it is necessary to examine the effects of both soil and dietary exposure of *Bt* crops on NTOs.

In China, several varieties of *Bt* rice created to protect the crop from lepidopteran rice pests have been suggested as a solution to the large food demand (Chen et al. 2006; Qiu 2008; Shu et al. 2000; Tu et al. 2000; Ye et al. 2003). However, only a few research studies focusing on the effects of *Bt* rice on NTOs have been conducted to date. A field investigation and laboratory assessment undertaken by Bai et al. (2005, 2010) revealed no negative effects of *Bt* rice on collembolan species and their predators. Chen et al. (2009) recorded a significantly longer developmental time for *Pirata subpiraticus* when it preyed on *Bt* rice-fed *Cnaphalocrocis medinalis*. Other studies have reported that, although *Bt* rice had no adverse effects on microorganisms, pronounced differences in the soil fungal community composition were found between *Bt* and non-*Bt* treatments (Liu et al. 2008; Lu et al. 2010b; Wu et al. 2004; Lu et al. 2010a), implying potential effects of *Bt* rice on NTOs. However, it must be remembered that all the mentioned studies included only a limited number of *Bt* rice varieties, i.e., Kemingdao1 and/or Kemingdao2, and, as more *Bt* rice varieties will be planted in future commercial production, the inclusion of more varieties in such studies is required to ensure that *Bt* rice has no detrimental effects on NTOs.

Collembolans are key decomposers in the soil and also serve as food for polyphagous predators, rendering them important organisms in the environmental risk assessment (ERA) of transgenic crops (Guo et al. 1995; Schoenly et al. 1998; Sigsgaard 2002; Zhao et al. 2007; Zwahlen et al. 2007). *F. candida*, a common and widespread collembolan, has been used as a “standard” test organism for more than 40 years to estimate the effects of environmental pollutants and transgenic plants on non-target soil organisms (Fountain and Hopkin 2005; Heckmann et al. 2006; Ke and Krogh 2000). Whether a genetically modified (GM) plant and its use are directly and/or indirectly potentially harmful to species guilds involved in ecosystem functions is a subject to be considered in ERA (EFSA 2010). Thus far, many studies have been conducted under laboratory conditions using either roots or leaves (Clark and Coats 2006; Pont and Nentwig 2005; Heckmann et al. 2006). In addition, studies have been performed using soil to determine the direct effects of *Bt* plant material on non-phytophagous and phytophagous species, with the tests accordingly demonstrating the effects of soil and dietary exposure of *Bt* crops on NTOs (Griffiths et al. 2006; Bakonyi et al. 2006; Wandeler et al. 2002). However, plant roots, stems and leaves differ significantly in their chemical composition (Beharav et al. 2010; Uchida et al. 2007; Arango et al. 2010; Chen et al. 2011), and the evaluation of the effects of both soil and dietary exposure of *Bt* rice on *F. candida* must, therefore, include different plant parts.

In this experiment, we examined the effect of three paired lines of *Bt* rice and their near-isogenic parental control lines at the jointing stage on the fitness of *F. candida* by exposing individuals to three different plant parts (root, stem and leaf) in either artificial soil or in feeding experiments with rice material to ensure a relatively integrated evaluation. The main objective of our study was to trace the effects of unintended changes in the *Bt* rice, as previous studies have shown that *Bt* proteins (Cry1Ab and Cry1Ac) have no effects on *F. candida* (Yuan et al. 2011). In addition, artificial soil was used, as it is completely harmless to collembolans and has a more well defined and uniform composition than natural soil. Our experiments aimed to elucidate (1) whether *Bt* rice had any dietary and soil exposure effects on collembolan fitness and (2) whether different plant parts (root, stem, and leaf) of *Bt* rice had distinct effects on collembolan fitness compared to their near-isogenic counterparts.

Materials and methods

Rice varieties

All the rice varieties were grown in Dezhou, Shandong Province, China (116.81° E, 37.65° N), in 2009. Three *Bt* rice varieties and their near-isogenic parental lines were used. *Bt* rice varieties Kemingdao, Huahui1 and Btshanyou63, which were derived from their corresponding parental lines, Xiushui, Minghui63 and Shanyou63, are referred to herein as *Bt*-Xiushui, *Bt*-Minghui63 and *Bt*-Shanyou63, respectively. *Bt*-Xiushui was transformed with a Cry1Ab transgene driven by the maize ubiquitin promoter using an agrobacterium-mediated method; *Bt*-Minghui63 contains a fused *Bt* Cry1Ab/Cry1Ac transgene driven by the rice actinI promoter. *Bt*-Shanyou63 is a hybrid between Huahui1 and Zhengshan97A, an indica cytoplasmic male sterile (CMS) line (Shu et al. 1998; Xia et al. 2011).

The three varieties of transgenic *Bt* rice and their respective near-isogenic parental lines were planted in field plot trials in a randomised complete block design. The plot size was 20 m × 5 m, and there was a 1.50-m-wide unplanted walkway between the plots acting as buffer zone to minimise potential treatment effects in neighbouring plots. There were four replications for each treatment, and a 5-m-wide cotton plot acted as buffer zone among the different rice cultivars (see Table 1 for more details). The materials of six plants were randomly collected for each line in all four replications at the jointing stage of the rice crop, and all six plants were placed in a plastic bag. The jointing stage was chosen because more *Bt* protein is expressed at this stage (Zhang et al. 2004). The collected rice materials were transported to the laboratory within a few hours, and the leaves, stems, and roots were separated and frozen at −20 °C until use.

Test species

The FCDK strain of the soil collembolan *F. candida* (Simonsen and Christensen 2001; Tully et al. 2006) was obtained from the former Department of Terrestrial Ecology, Aarhus University and cultured for 10 years at the Shanghai Institute of Biological Sciences. The cultures were reared in Petri dishes (diameter of 150 mm, with plaster of Paris and activated charcoal (9:1 weight:weight) covering the bottom) and kept at 20 °C in total darkness. The relative humidity was ~100 %, which was adjusted weekly by the addition of distilled water.

Experimental design

Rice feeding experiment

In this experiment, 18 diet treatments were arranged using three types of plant parts (i.e., roots, stems and leaves) of the six rice varieties. Dry baker's yeast was selected as a control. Each treatment and control was replicated five times to assess survival, reproduction and growth. All the rice materials were lyophilised for 2 days at −60 °C under a vacuum of ~30 mTorr, ground using a plant tissue pulveriser wi50559 (Dong Xi Yi, Beijing, China) and stored at −20 °C until use. For each replicate, 10 juveniles (10–12 days old) were

Table 1 The concentrations of *Bt* protein in three *Bt* rice varieties

Bt rice variety	*Bt* protein (μg/g)*			*Bt* proteins
	Root	Stem	Leaf	
Huahui1	0.80 ± 0.07^{Bb}	0.78 ± 0.07^{Ab}	1.39 ± 0.21^{Aa}	Stacked Cry1Ab/1Ac
BtShanyou63	0.84 ± 0.04^{Ba}	1.11 ± 0.28^{Aa}	1.33 ± 0.21^{Aa}	Stacked Cry1Ab/1Ac
Kemingdao	1.75 ± 0.08^{Aa}	1.41 ± 0.23^{Aa}	2.90 ± 0.72^{Aa}	Cry1Ab

*μg/g rice leaves (fresh weight, mean ± S.E.)

The means followed by the same uppercase letter within a column are not significantly different from each other (Tukey's test, $n = 3$, $P < 0.05$)

The means followed by the same lowercase letter within a row are not significantly different from each other (Tukey's test, $n = 3$, $P < 0.05$)

placed in tightly closed, round plastic containers (diameter of 8 cm, height of 6.5 cm, with plaster of Paris and activated charcoal (8:1 weight:weight) covering the bottom). Approximately 3 mg of rice material or yeast was added to feed the *F. candida* individuals at the beginning of the experiment; the contents were inspected every second day, and new rice material/yeast was added if required. The temperature was 20 °C; the relative humidity was ~100 % and was adjusted twice weekly by the addition of distilled water and by aeration. The experiment lasted 28 days and was performed in total darkness. The number of surviving adults and juveniles in each replicate was counted under a microscope at the end of the experiment. Growth was determined as the final body length of randomly collected parent adult individuals (anaesthetised with CO_2) using a stereomicroscope (×30 magnification), and the mean body length of three individuals in each replicate was defined as the growth value.

Artificial soil experiment

Another 18 treatments were arranged consisting of artificial soil mixed with the rice materials, i.e., the three types of plant parts of the six varieties mentioned above (previously dried at 60 °C for 2 days and ground in liquid nitrogen using a mortar and pestle). Artificial soil without rice material but amended with dry baker's yeast served as the control. Each treatment and control were replicated five times. The experiment was conducted following the ISO standard 11267 (Standardization 1999), with some modifications. The artificial soil contained 10 % sphagnum peat (ground using a plant tissue pulveriser wi50559), 30 % kaolinite clay (air-dried) and 70 % industrial quartz sand, as described in ISO standard 11267. $CaCO_3$ was added to adjust the pH to 6 ± 0.5 after which the overall water content was adjusted to approximately 50 % of the total water-holding capacity. For each replicate, 25 g artificial soil was mixed with 1 g of the corresponding rice materials and placed in a close-capped container (diameter of 8 cm, height of 6.5 cm); the material was then aged for 21 days at 20 °C to initiate decomposition (Clark and Coats 2006). As a control, 25 g of the newly prepared artificial soil described above (without the rice materials) was placed in a container and approximately 1 mg yeast was added. Next, 10 juveniles (10–12 days old) were placed in each treatment and control container, and the containers were closely capped and placed in an incubator at 20 °C for 28 days in total darkness, with aeration performed once a week. The yeast in the control was replaced weekly. To determine the total number of surviving adults and juveniles at the end of the experiment, a saturated sucrose solution was used to flood each container, and a few drops of black ink were added to make the floating collembolans easier to identify. Growth was determined as described above.

Quantification of Bt *toxin and the rice material C/N ratio*

Bt toxin

The content of *Bt* protein in the rice materials was measured using enzyme-linked immunosorbent assay (ELISA) prior to the experiment. The quantification was performed using the QuanliPlate Kit for Cry1Ab/Cry1Ac (Envirologix Inc.). This "sandwich" ELISA kit included 12 strips of 8 antibody-coated wells in plate format, a Cry1Ab/Cry1Ac positive control, a Cry1Ab/Cry1Ac enzyme conjugate, the substrate and stop solution (1 N hydrochloric acid). PBS/0.05 % Tween-20 wash buffer was purchased in 1-L dry packets from Sigma Chemicals (CAT#P-3563). The extraction buffer was prepared by adding 0.5 mL Tween-20–100 mL prepared PBS/0.05 % Tween-20 wash buffer. Sample extractions were prepared by grinding approximately 2 mg rice sample in 1 mL extraction buffer. At the beginning of the experiment, 50 μL Cry1Ab/Cry1Ac enzyme conjugate was added to each well of the plate, followed by 50 μL extraction buffer blank, 50 μL Cry1Ab positive control and 50 μL of each sample extract to respective wells. After 1.5 h incubation, each well was washed with wash buffer three times and 100 μL substrate was added to each well, followed by incubation for another 4 min. Last, 100 μL stop solution was added to each well, and the absorbance was measured at 450 nm using a plate reader. A standard curve was constructed using purified Cry1Ac protein (Case Western Reserve University) to estimate the amount of *Bt* protein in the plant samples.

C/N ratio

The organic carbon in the rice materials was measured following the $K_2Cr_2O_7$ method (Bao 2000). Each rice sample (0.5 g finely ground dry rice materials) was digested using 5 mL 1 mol/L $K_2Cr_2O_7$ and 10 mL concentrated H_2SO_4 ($\approx$98 %) at 150 °C for 30 min, followed by titration of the digest (with three drops of phenanthroline added as an indicator) with 0.2 mol/L $FeSO_4$. A blank (without rice sample) was processed simultaneously in a similar manner.

The nitrogen content in the rice materials was assessed using Kjeltec nitrogen analysis (Foss automated Kjeltec^{TM} instruments, Model 2100). For each sample, 0.2 g finely ground dry rice material was digested with 2 Kjeltabs Cu 3.5 and 12 mL concentrated H_2SO_4 for 60 min, the cooled digest was then diluted with 75 mL H_2O. Next, 25 mL receiver solution was added to the receiver flask, and 50 mL 40 % NaOH was added to the diluted digest. Lastly, the distillate was titrated with a standardised titrant. A blank (without rice sample) was processed simultaneously following the same method.

Statistical analyses

A three-way ANOVA, including the factors of gene modification (GM), variety and plant part (roots, stems and leaves) and their interactions, was performed to determine the survival, reproduction and growth of *F. candida* and the C/N ratio of the rice materials. The transformed gene coding for the *Bt* toxin, occurring across the varieties, was considered to be the GM factor. The normality and equality of the variances were tested using the Kolmogorov–Smirnov and Levene methods, respectively, prior to the ANOVA analysis. The binomially distributed survival data were analysed using a Generalised Linear Model (GLZ) with SAS PROC GLIMMIX for the three factors (SAS Institute Inc., 2011). Tukey's tests were then applied to compare pairs of the means. A significance level of 5 % was used for all the tests.

Results

C/N ratio and Bt *toxin*

C/N ratio

The C/N ratio of the rice was significantly affected by the factors of GM ($P < 0.001$), plant part ($P < 0.001$), all 2nd-order interactions (survival: $P_{Variety \times GM} < 0.001$, $P_{Variety \times Plant\ part} < 0.001$ and $P_{GM \times Plant\ part} < 0.001$) and the 3rd-order interaction between variety, GM and plant part ($P < 0.001$). The C/N ratio of the stems of *Bt*-Minghui63 was significantly higher than that of the near-isogenic line Minghui63 and the C/N ratios of both the roots and stems of *Bt*-Shanyou63 were significantly higher than for the near-isogenic line Shanyou63. In contrast, the C/N ratio of the roots of *Bt*-Xiushui was significantly lower than for the near-isogenic line Xiushui, though the roots and stems of Xiushui had significantly higher C/N ratios than the other parental lines, Minghui63 and Shanyou63 (Fig. 1).

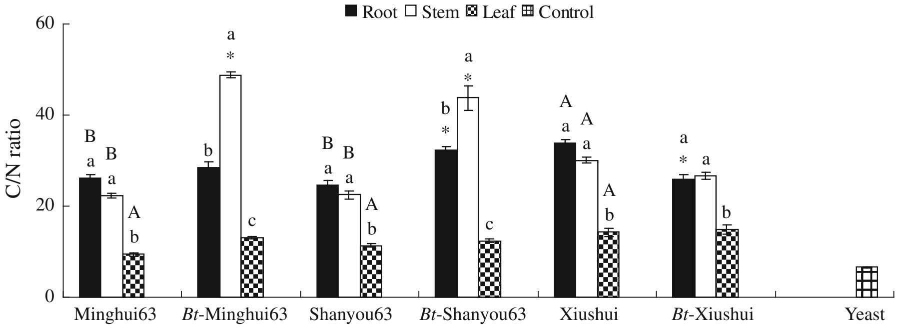

Fig. 1 C/N ratio of the roots, stems and leaves of three *Bt* rice varieties and their near-isogenic non-*Bt* rice varieties (Tukey's test, $n = 5$, $P < 0.05$). The *same uppercase letter above the same-coloured bars* indicates no significant difference from each other; the *same lowercase letter above bars within the same group* indicates no significant difference from each other. *Significantly different compared with the same plant part of the corresponding near-isogenic non-*Bt* line. The *vertical bars* indicate the standard errors. *Bt*-Minghui63: Huahui1, the transgenic *Bt* variety of Minghui63. *Bt*-Shanyou63: Btshanyou63, the transgenic *Bt* variety of Minghui63. *Bt*-Xiushui: Kemingdao, the transgenic *Bt* variety of Xiushui

Bt toxin

The content of *Bt* protein in the roots and stems of *Bt*-Minghui63 was significantly lower than in the leaves (Table 1). All three plant parts of *Bt*-Xiushui exhibited higher *Bt* toxin levels than *Bt*-Minghui63 and *Bt*-Shanyou63, but only that of the root was statistically significant.

Rice feeding experiment

Our feeding test system proved valid in that our reference food, baker's yeast, produced survival, reproductive performance and growth rates at levels usually observed for *F. candida* (e.g., Widarto et al. 2007).

No significant difference was found for *F. candida* survival among the treatments. *F. candida* reproduction was significantly affected by the GM factor ($P = 0.001$), the interaction between the variety and GM ($P = 0.007$) and the interaction between the variety, GM and plant part ($P = 0.009$). The variety ($P = 0.042$), interaction between the variety and GM ($P = 0.021$) and interaction between the three factors ($P = 0.017$) all significantly affected the growth of the collembolan.

The number of juveniles in the *Bt*-Minghui63 leaf treatment was significantly lower than in the root and stem treatments, whereas the *Bt*-Xiushui root treatment exhibited significantly lower collembolan reproduction than the root treatment of its near-isogenic non-*Bt* line. The stems of Minghui63 significantly decreased the growth of *F. candida* compared to Shanyou63 stems (Fig. 2).

Artificial soil experiment

No juveniles were found in any of the treatments with rice leaves, and adults very rarely occurred in all the leaf treatments. After the 21 days of decomposition, multiple fungi and mycelia were observed in the artificial soil for all the treatments, which may be the reason for the death of the collembolans. Consequently, all the leaf treatments were excluded from the subsequent statistical analysis.

The survival and reproduction of *F. candida* were significantly affected by all 2nd-order interactions (survival, $P_{\text{Varieties} \times \text{GM}} = 0.002$, $P_{\text{Varieties} \times \text{Plant parts}} < 0.001$, $P_{\text{GM} \times \text{Plant parts}} = 0.039$; reproduction, $P_{\text{Varieties} \times \text{GM}} = 0.006$, $P_{\text{Varieties} \times \text{Plant parts}} < 0.001$, $P_{\text{GM} \times \text{Plant parts}} < 0.001$) and the 3rd-order interaction between variety, GM and plant parts (survival, $P = 0.002$; reproduction, $P < 0.001$). The growth of *F. candida* was markedly impacted by the variety ($P < 0.001$), GM ($P < 0.001$) and plant parts ($P < 0.001$) and also by their two-and three-way interactions ($P_{\text{Varieties} \times \text{GM}} < 0.001$, $P_{\text{Varieties} \times \text{Plant parts}} = 0.012$, $P_{\text{GM} \times \text{Plant parts}} < 0.001$, $P_{\text{Varieties} \times \text{GM} \times \text{Plant parts}} < 0.001$).

The stems of Minghui63 significantly decreased the survival and reproduction of *F. candida* compared to its roots, but the body length of *F. candida* in the Minghui63 stem treatment was significantly higher than in the Minghui63 root treatment. The stem treatments of *Bt*-Minghui63, Xiushui and *Bt*-Xiushui resulted in a significantly higher body length than that recorded in the root treatments. In the *Bt*-Shanyou63 stem treatment, *F. candida* exhibited significantly higher survival, reproduction, and growth than in the *Bt*-Shanyou63 root treatment. In a comparison between the three non-*Bt* isogenic line treatments, the Minghui63 root treatment demonstrated a significantly higher number of surviving adults than the Shanyou63 root treatment. The Minghui63 root treatment exhibited significantly more juveniles than the root treatment of both Shanyou63 and Xiushui, whereas *F. candida* in the Minghui63 stem treatment exhibited significantly lower reproduction than in the Shanyou63 stem treatment. The Shanyou63 root treatment showed significantly higher growth than the Xiushui root treatment. The *Bt*-Minghui63 root treatment of significantly decreased the survival, reproduction and growth of *F. candida* compared to the root treatment of its near-isogenic non-*Bt* line, Minghui63. The *F. candida* individuals in the *Bt*-Shanyou63 treatment exhibited a significantly lower body length than in the Shanyou63 treatment (Fig. 3).

Discussion

Bt rice has some significant advantages that may potentially decrease insecticide use and increase the yield and profitability of crops (High et al. 2004). However, the long-term and widespread use of *Bt* rice may have an adverse impact on agro-ecosystems, thus it is very important to evaluate the risk associated with

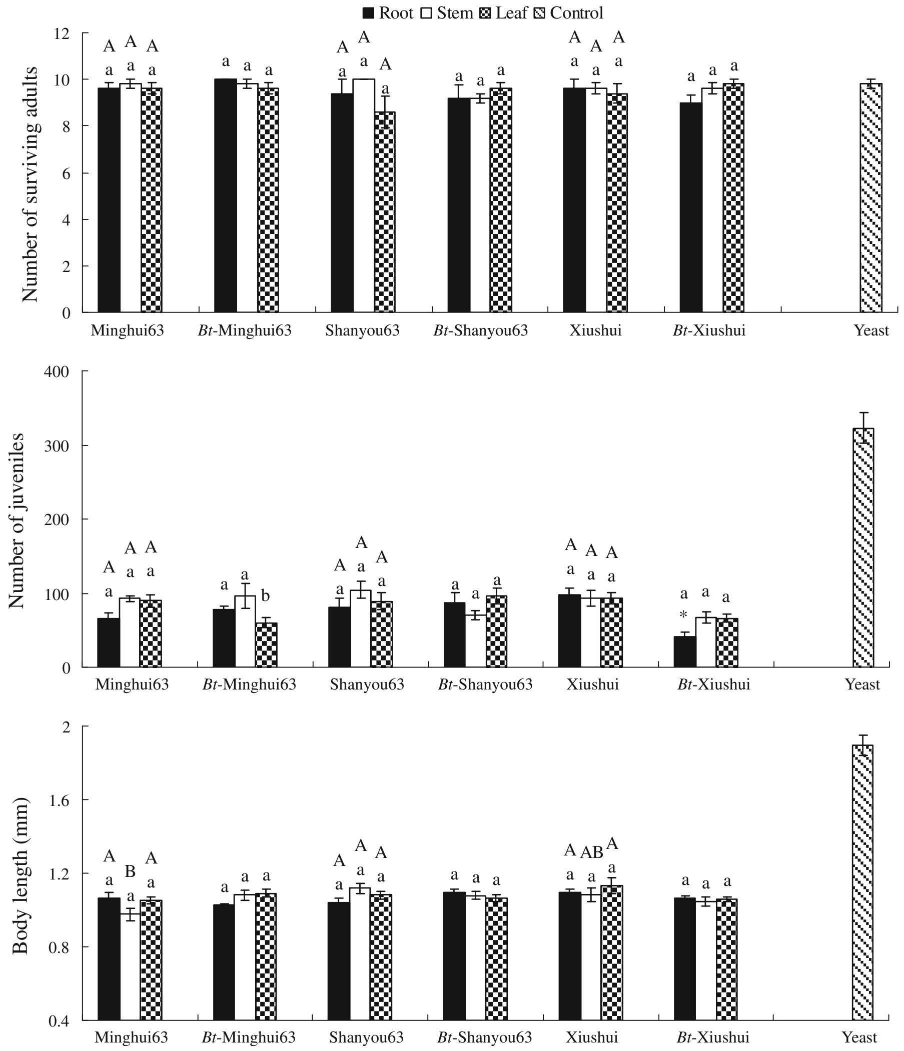

Fig. 2 The mean number of surviving adults, mean number of juveniles and mean body length (mm) of *F. candida* exposed to the roots, stems and leaves of three *Bt* rice varieties and their near-isogenic non-*Bt* rice lines in a rice feeding experiment (Tukey's test, $n = 5$, $P < 0.05$). The *same uppercase letter above the same-coloured bars* indicates no significant difference; the *same lowercase letter above bars within the same group* indicates no significant difference. *Significantly different compared with the same plant part treatment of the corresponding near-isogenic non-*Bt* line. The *vertical bars* indicate the standard errors. *Bt*-Minghui63: Huahui1, the transgenic *Bt* variety of Minghui63. *Bt*-Shanyou63: Btshanyou63, the transgenic *Bt* variety of Minghui63. *Bt*-Xiushui: Kemingdao, the transgenic *Bt* variety of Xiushui

Bt plants for NTOs, particularly with regard to the soil ecosystem. Collembolans constitute an abundant and diverse group of decomposers in the soil ecosystem and perform many functional processes, together with other soil-dwelling invertebrates, for instance, in the nutrient cycling and decomposition of organic matter (Moore et al. 1988). Therefore, testing the effects of *Bt* rice on collembolans using *F. candida* as a "standard" soil arthropod is commonly applied to detect possible effects on soil arthropods. Our study is the first to evaluate the impacts of *Bt* rice on the ecological fitness of *F. candida* under laboratory conditions using relatively many *Bt* rice varieties and discriminating between their root, stem and leaf organs.

Bt *proteins in rice materials*

Because *Bt*-Shanyou63 is a hybrid between *Bt*-Xiushui and Zhengshan97A, it is not surprising that

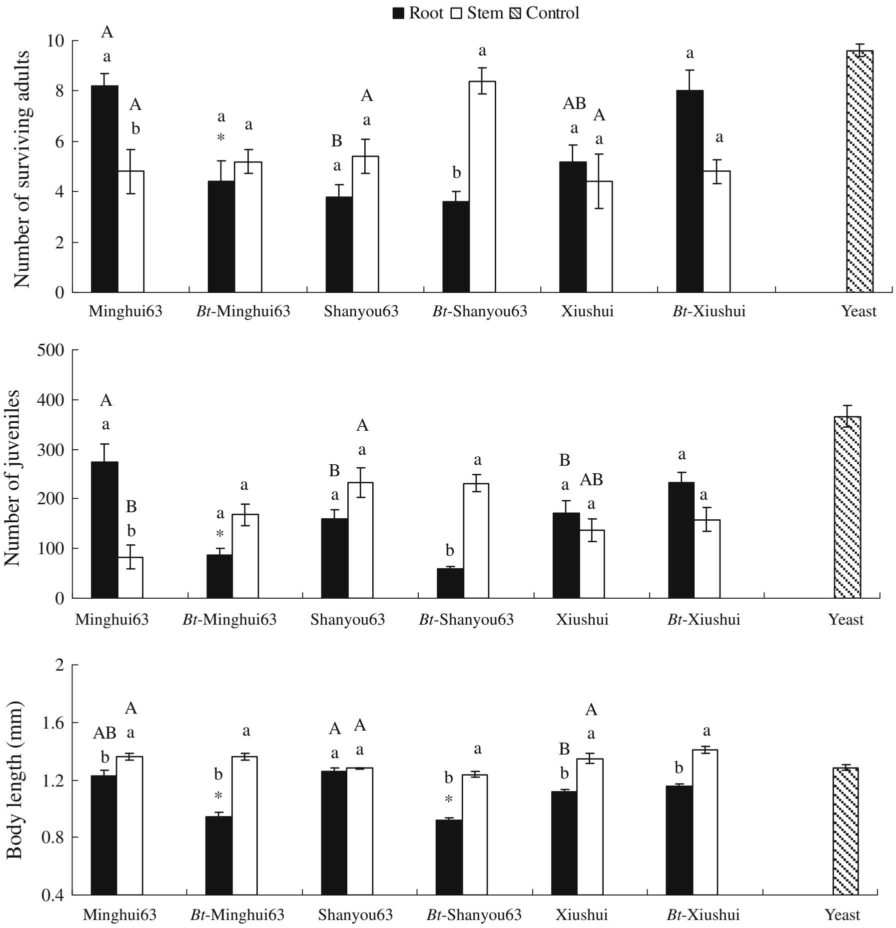

Fig. 3 The mean number of surviving adults, mean number of juveniles and mean body length (mm) of *F. candida* exposed to the roots and stems of three *Bt* rice varieties and their near-isogenic non-*Bt* rice lines in an artificial soil experiment (Tukey's test, $n = 5$, $P < 0.05$). The *same uppercase letter above the same-coloured bars* indicates no significant difference; the *same lowercase letter above bars within the same group* indicates no significant difference. *Significantly different compared with the same plant part treatment of the corresponding near-isogenic non-*Bt* line. The *vertical bars* indicate the standard errors. *Bt*-Minghui63: Huahui1, the transgenic *Bt* variety of Minghui63. *Bt*-Shanyou63: Btshanyou63, the transgenic *Bt* variety of Minghui63. *Bt*-Xiushui: Kemingdao, the transgenic *Bt* variety of Xiushui

Bt-Shanyou63 and *Bt*-Xiushui had similar levels of *Bt* proteins. Furthermore, the higher *Bt* protein level in *Bt*-Xiushui indicates that different transgene methods may result in different *Bt* protein expression levels. However, the results for the ecological fitness of *F. candida* did not correlate with the *Bt* protein level in the rice materials. Previous studies on the effects of the Cry1Ab and Cry1Ac proteins on the population fitness of several Collembola species, such as *F. candida*, *Xenylla grisea* and *Protaphorura armata*, have not revealed any negative effects (Sims and Martin 1997; Heckmann et al. 2006). Our previous study also confirmed the assumption that the effects of *Bt* rice on *F. candida* were created by the differences in plant composition, depending on the variety of rice and not on the *Bt* protein level (Yuan et al. 2011). In contrast, the levels of *Bt* proteins (Cry1Ab and Cry1Ac) in several *Bt* rice varieties, such as MSB and KMD, decreased rapidly (≈50 %) in the soil after approx. 30 days (Bai et al. 2004; Li et al. 2007; Wang et al. 2007). Hence, we recommend that studies focusing on the effect of *Bt* rice on *F. candida* address the impact of unintended changes in plant components rather than on the intended insertion of *Bt* proteins.

Rice feeding experiment

Several experiments on the effect of *Bt* crops on collembolans have been performed under laboratory conditions to test the dietary effects on the collembolan community, with the collembolans usually being fed *Bt* and non-*Bt* plant material or purified *Bt* proteins. For example, Bakonyi et al. (2006) found no dietary effect of *Bt* maize on the survival of three collembolan species. No significant effect on reproduction and growth was found when *F. candida* and *Oppia nitens* (an orbatid mite) were fed the leaves of *Bt* cotton or *Bt* potato and their non-*Bt* near-isogenic lines (Yu et al. 1997). Heckmann et al. (2006) fed the collembolan *Protaphorura armata* with root tissues of *Bt* and non-*Bt* maize and purified Cry1Ab, and no significant impact was observed on growth and reproduction. Thus, all the mentioned studies show that feeding collembolans with biomass of *Bt* cotton, *Bt* potato and *Bt* maize has no marked effect, though the impact of a *Bt* rice diet has not been evaluated until now.

Some significant differences were found in our rice feeding experiment between the *Bt* rice variety and non-*Bt* rice variety treatments and also between the different plant part treatments and between the different non-*Bt* rice variety treatments. No effect of *Bt* rice was detected on the survival of *F. candida*, whereas reproduction and growth were significantly affected by the GM and variety factors and the three-factor interaction. These results reveal that the reproduction and growth of *F. candida* are more sensitive parameters and, thus, more suitable than survival for studying the dietary effects of *Bt* rice. Furthermore, reproduction and growth may indicate different aspects of effects, i.e., of the GM insert and the variety, respectively. Specifically, the roots of *Bt*-Xiushui had a negative effect on the reproduction of *F. candida* compared to its near-isogenic line (GM factor), whereas the stems of Minghui63 decreased the growth of *F. candida* compared to the stems of Shanyou63 (variety factor). Although a negative effect on *F. candida* was observed for the *Bt*-Xiushui roots, a similar effect could not be detected for its stems or leaves or for the roots of the other two *Bt* rice varieties. In addition, our results showed that the differences between plant parts led to reduced reproduction in *F. candida*. Accordingly, we conclude that *Bt* rice did not have a negative effect on *F. candida*. However, the effects of three-factor interactions on *F. candida* showed that the variety, plant part and GM may interact with each other and that different varieties and plant parts can induce different effects on *F. candida* for GM. This finding suggests that all new *Bt* rice varieties ought to be assessed for environmental safety, even if all other *Bt* rice varieties have passed an environmental risk assessment. Finally, it is necessary to discuss the reasons behind the observed differences. In our experiment, the collembolans fed yeast, having a significantly lower C/N ratio than rice tissue, exhibited significantly higher reproduction and growth than those fed the rice materials. This observation corresponds well with previous studies showing that food quality had a notable impact on collembolans (Zimmer and Topp 2000; Heckmann et al. 2006). However, the collembolans fed the leaves, with a significantly lower C/N ratio than the stems and roots, of all the rice varieties did not exhibit higher reproduction and growth. Saxena and Stotzky (2001b) stated that the altered expression of secondary plant metabolites following transformation events may influence the overall impact of a GM crop. In a previous study, we suggested that unintentional changes in plant metabolism and/or the composition of GM rice due to genetic modification may affect the *F. candida* (NTO)–plant relationship (Yuan et al. 2011). The C/N ratio results of the present study have verified that the composition of other chemicals in plants may change after *Bt* gene modification. On the one hand, differences in the components or secondary plant metabolites between *Bt* rice and their near-isogenic non-*Bt* rice lines, between non-*Bt* rice varieties and between different plant parts are very important for the ecological fitness of collembolans. On the other hand, the results of the rice feeding experiment appear to confirm the general perception that the performance of NTOs may be influenced by differences both within and among GM and non-GM varieties (Griffiths et al. 2007; Pont and Nentwig 2005; Wandeler et al. 2002). Finally, although significant differences appeared between the different plant parts or rice varieties, the results of the rice feeding experiment revealed only very few significant differences between the exposure treatments. In addition, the *F. candida* body length was relatively similar in all the treatments included in the rice feeding experiment yet was significantly lower than in the control. The *F. candida* individuals were observed to consume only a very small quantity of rice residues during the exposure period, which is consistent with its diet preference for microorganisms (Fountain and Hopkin 2005). Consequently, the lack of significant differences in the rice feeding experiment presumably resulted from the limited feeding on the rice materials.

Artificial soil experiment

Most previous laboratory experiments with soil have been designed mainly to test effects during the exposure to soil used as a GMO growth substrate or GMO plant material amended to the soil. Griffiths et al. (2006) studied the responses of natural soil microbial and faunal communities to *Bt* maize in two soils and found that artificially grown *Bt* maize decreased collembolan abundance. Clark and Coats (2006) conducted a laboratory study to test the soil-mediated effects of *Bt* corn litter exposed to *F. candida*, and a reduced number of *F. candida* offspring was found in the *Bt* corn treatment (Bt11-90) compared to the non-*Bt* isoline treatment. All studies to date indicate that the soil exposure effects of *Bt* plants on collembolans may be greater than those of dietary exposure, but laboratory assessments of soil exposure effects of *Bt* rice on collembolans are rare. For most collembolans, including *F. candida*, plant residues are not the preferred food item (Fountain and Hopkin 2005). Therefore, soil experiments may more closely resemble natural conditions than rice feeding experiments by reflecting the soil exposure effects of *Bt* rice on *F. candida*. This assertion is supported by our data showing that the highest number of juveniles and highest body length of *F. candida* occurred in our artificial soil experiment (274.4 juvenile offspring; 1.41 mm) compared to the rice feeding experiment (104.4 juvenile offspring; 1.13 mm) and our observation of microorganisms growing on the soil and plant materials after 21 days of decomposition. These results suggest that the collembolans in the artificial soil experiment grazed on these microorganisms during the decomposition of the rice residues and possibly also ate the sphagnum constituting the organic matter component of the artificial soil.

All the main factors and interactions between the survival, reproduction and growth of *F. candida* were affected by the exposure to the rice material in soil. No effects of diet were observed on collembolan survival; however, dramatic impacts on survival were recorded when *F. candida* was exposed to rice material in soil (Figs. 2, 3). Although the two different drying methods, i.e., lyophilisation and heating at 60 °C, used in this study may weaken the comparability between rice feeding experiments and artificial soil experiments, the distinct way in which the rice materials affected the collembolans suggests a greater impact of soil-mediated effects of rice than effects of dietary exposure. As the 60 °C drying method may produce a certain plant composition due to the specific denaturation and decomposition of the plant material, we suggest that the lyophilised or fresh rice materials may have had stronger effects on the collembolans. In addition, the significant interaction indicates that the effect of the GM trait depended strongly on the rice variety and plant part; indeed, it is known that plant species and varieties may alter the composition and diversity of soil microbial communities (Icoz and Stotzky 2008). Through our research results, we demonstrate that such effects can occur at the plant part level because significant differences appeared between the root and stem treatments for each variety. Hence, we suggest that *Bt* rice had no detrimental soil exposure effect on *F. candida*. The reason for the lack of *F. candida* reproduction in the leaf treatments of the artificial soil experiment could be explained by rice leaf-induced changes in the composition and diversity of the soil microbial communities, rendering the conditions unfavourable to *F. candida*.

In addition, the soil type may affect the soil microbial community. For example, Blackwood and Buyer (2004) found that the impact of the soil type on the composition of the microbial community was greater than that of the *Bt* gene insertion. Fang et al. (2005) reported that the differences in bacterial diversity in the rhizosphere between transgenic and non-transgenic maize were mainly caused by soil texture. Considering that the artificial soil used in our study differs substantially from natural soil, the change in soil type may likely have altered the relationship among the *Bt* plant material, microorganisms and collembolans.

Conclusion

Overall, our study provides evidence for a lack of detrimental effects of *Bt* rice on *F. candida*. However, some components of *Bt* rice were unintentionally changed upon gene insertion and may have triggered both diet and soil exposure effects on the collembolans. Different plant parts may affect NTOs differently, and we, therefore, suggest using separate plant parts depending on the feeding ecology of the focal species in future safety evaluations of transgenic crops.

Acknowledgments This project was supported by the Special Program for New Transgenic Variety Breeding of the Ministry of Science and Technology, China (No. 2012ZX08011002) and the Innovation Program of Chinese Academy of Science(KSCX2-EW-Z-6). Paul Henning Krogh was supported by grant no 3304-FVFP-09-B-004 from The Danish Food Industry Agency programme "Future crops—application of biotechnology with special focus on genetically engineering for the benefit of the whole society".

References

Arango J, Wust F, Beyer P, Welsch R (2010) Characterization of phytoene synthases from cassava and their involvement in abiotic stress-mediated responses. Planta 232(5):1251–1262

Bai YY, Jiang MX, Cheng JA, Shen HM, Yang P, Chen ZX, Jiang YH, Shu QY (2004) Degradation of Cry1Ab toxin protein expressed by *Bt* transgenic rice in paddy soils. Chinese J Rice Sci 18(3):255–261

Bai YY, Jiang MX, Cheng JA (2005) Impacts of transgenic cry1Ab rice on two collembolan species and predation of *Microvelia horvathi* (Hemiptera: Veliidae). Act Entomol Sin 48(01):42–47

Bai YY, Yan RH, Ye GY, Huang FN, Cheng JA (2010) Effects of transgenic rice expressing *Bacillus thuringiensis* Cry1Ab protein on ground-dwelling collembolan community in postharvest seasons. Environ Entomol 39(1):243–251

Bakonyi G, Szira F, Kiss I, Villanyi I, Seres A, Szekacs A (2006) Preference tests with collembolas on isogenic and Bt-maize. Eur J Soil Biol 42:S132–S135

Bao S (2000) Agricultural and chemical analysis of soil. China Agricultural, Beijing (in Chinese)

Beharav A, Ben-David R, Malarz J, Stojakowska A, Michalska K, Dolezalova I, Lebeda A, Kisiel W (2010) Variation of sesquiterpene lactones in *Lactuca aculeata* natural populations from Israel, Jordan and Turkey. Biochem Syst Ecol 38(4):602–611

Betz FS, Hammond BG, Fuchs RL (2000) Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Reg Tox Pharm 32(2):156–173

Blackwood CB, Buyer JS (2004) Soil microbial communities associated with *Bt* and non-*Bt* corn in three soils. J Environ Qual 33(3):832–836

Chen M, Zhao JZ, Ye GY, Fu Q, Shelton AM (2006) Impact of insect-resistant transgenic rice on target insect pests and non-target arthropods in China. Insect Sci 13(6):409–420. doi:10.1111/j.1744-7917.2006.00071.x-i1

Chen M, Ye GY, Liu ZC, Fang Q, Hu C, Peng YF, Shelton AM (2009) Analysis of Cry1Ab toxin bioaccumulation in a food chain of Bt rice, an herbivore and a predator. Ecotoxicology 18(2):230–238

Chen F, Wu G, Ge F, Parajulee MN (2011) Relationships between exogenous-toxin quantity and increased biomass of transgenic *Bt* crops under elevated carbon dioxide. Ecotoxicol Environ Safe 74(4):1074–1080

Clark BW, Coats JR (2006) Subacute effects of Cry1Ab *Bt* corn litter on the earthworm *Eisenia fetida* and the springtail *Folsomia candida*. Environ Entomol 35:1121–1129

EFSA (2010) EFSA Panel on Genetically Modified Organisms (GMO); guidance on the environmental risk assessment of genetically modified plants. EFSA J. 8(11), 1879. [111 pp.]. Available online: www.efsa.europa.eu/efsajournal

Fang M, Kremer RJ, Motavalli PP, Davis G (2005) Bacterial diversity in rhizospheres of nontransgenic and transgenic corn. Appl Environ Microbiol 71(7):4132–4136

Flores S, Saxena D, Stotzky G (2005) Transgenic *Bt* plants decompose less in soil than non-*Bt* plants. Soil Biol Biochem 37(6):1073–1082

Fountain MT, Hopkin SP (2005) *Folsomia candida* (Collembola): a "standard" soil arthropod. Annu Rev Entomol 50:201–222

Griffiths BS, Caul S, Thompson J, Birch ANE, Scrimgeour C, Cortet J, Foggo A, Hackett CA, Krogh PH (2006) Soil microbial and faunal community responses to *Bt* maize and insecticide in two soils. J Environ Qual 35(3):734–741

Griffiths BS, Heckmann LH, Caul S, Thompson J, Scrimgeour C, Krogh PH (2007) Varietal effects of eight paired lines of transgenic *Bt* maize and near-isogenic non-*Bt* maize on soil microbial and nematode community structure. Plant Biotechnol J 5(1):60–68

Guo YJ, Wang NY, Jiang JW, Chen JW, Tang J (1995) Ecological significance of neutral insects as nutrient bridge for predators in irrigated rice arthropod community. Chin J Biol Cont 11(1):5–9

Heckmann LH, Griffiths BS, Caul S, Thompson J, Pusztai-Carey M, Moar WJ, Andersen MN, Krogh PH (2006) Consequences for *Protaphorura armata* (Collembola: Onychiuridae) following exposure to genetically modified *Bacillus thuringiensis* (*Bt*) maize and non-*Bt* maize. Environ Pollut 142(2):212–216

High SM, Cohen MB, Shu QY, Altosaar I (2004) Achieving successful deployment of *Bt* rice. Trends Plant Sci 9(6):286–292

Höss S, Arndt M, Baumgarte S, Tebbe CC, Nguyen HT, Jehle JA (2008) Effects of transgenic corn and Cry1Ab protein on the nematode Caenorhabditis elegans. Ecotoxicol Environ Safe 70(2):334–340

Icoz I, Stotzky G (2008) Fate and effects of insect-resistant *Bt* crops in soil ecosystems. Soil Biol Biochem 40(3):559–586

ISO (1999) Soil quality—inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. Rep. No. ISO 11267: 1999(E) Geneva: Int. stand. Organ. 16 pp

Ke X, Krogh PH (2000) Effects of *Bacillus thuringiensis* (*Bt*) corn on soil *Folsomia fimetaria*, *Folsomia candida* (Collembola), *Hypoaspis aculeifer* (Acarina) and *Enchytraeus crypticus* (Oligochaeta). 7 Int Symp, Biosafety GMO-s, Beijing, China

Li YH, Wu KM, Zhang YJ, Yuan GH (2007) Degradation of Cry1Ac protein within Transgenic *Bacillus thuringiensis* rice tissues under field and laboratory conditions. Environ Entomol 36(5):1275–1282

Liu W, Hao LuH, Wu W, Kun Wei Q, Xu Chen Y, Thies JE (2008) Transgenic Bt rice does not affect enzyme activities and microbial composition in the rhizosphere during crop development. Soil Biol Biochem 40(2):475–486

Lu HH, Wu WX, Chen YX, Wang HL, Devare M, Thies JE (2010a) Soil microbial community responses to *Bt* transgenic rice residue decomposition in a paddy field. J Soil Sediment 10(8):1598–1605

Lu HH, Wu WX, Chen YX, Zhang XJ, Devare M, Thies JE (2010b) Decomposition of *Bt* transgenic rice residues and response of soil microbial community in rapeseed-rice cropping system. Plant Soil 336(1–2):279–290

Manachini B, Lozzia GC (2002) First investigations into the effects of *Bt* corn crop on Nematofauna. Boll ZoolAgrar Bachic 34(1):85–96

Moore JC, Walter DE, Hunt HW (1988) Arthropod regulation of microbiota and mesobiota in belowground detrital food webs. Annu Rev Entomol 33:419–439

Obrist LB, Dutton A, Albajes R, Bigler F (2006) Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. Ecol Entomol 31(2):143–154

Palm CJ, Schaller DL, Donegan KK, Seidler RJ (1996) Persistence in soil of transgenic plant produced *Bacillus thuringiensis* var. *kurstaki* delta-endotoxin. Can J Microbiol 42(12):1258–1262

Poerschmann J, Gathmann A, Augustin J, Langer U, Gorecki T (2005) Molecular composition of leaves and stems of genetically modified *Bt* and near-isogenic non-*Bt* maize-characterization of lignin patterns. J Environ Qual 34(5):1508–1518

Pont B, Nentwig W (2005) Quantification of Bt-protein digestion and excretion by the primary decomposer *Porcellio scaber*, fed with two Bt-corn varieties. Biocontrol Sci Tech 15(4):341–352

SAS Institute Inc. (2011). SAS/STAT® 9.3 User's Guide. SAS Institute Inc., Cary, NC

Saxena D, Stotzky G (2001a) *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biol Biochem 33(9):1225–1230

Saxena D, Stotzky G (2001b) *Bt* corn has a higher lignin content than non-*Bt* corn. Am J Bot 88(9):1704–1706

Saxena D, Stewart CN, Altosaar I, Shu QY, Stotzky G (2004) Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B. thuringiensis* corn, potato, and rice but not of *B. thuringiensis* canola, cotton, and tobacco. Plant Physiol Biochem 42(5):383–387

Schoenly KG, Justo HD, Barrion AT, Harris MK, Bottrell DG (1998) Analysis of invertebrate biodiversity in a Philippine farmer's irrigated rice field. Environ Entomol 27(5):1125–1136

Shu QY, Ye GY, Cui HR, Xiang YB, Gao MW (1998) Development of transgenic *Bacillus thuringiensis* rice resistant to rice stem borers and leaf folders. J Zhejiang Agri Univ 24: 579–580

Shu QY, Ye GY, Cui HR, Cheng XY, Xiang YB, Wu DX, Gao MW, Xia YW, Hu C, Sardana R, Altosaar I (2000) Transgenic rice plants with a synthetic cry1Ab gene from *Bacillus thuringiensis* were highly resistant to eight lepidopteran rice pest species. Mol Breed 6(4):433–439

Sigsgaard L (2002) Early season natural biological control of insect pests in rice by spiders-and some factors in the management of the cropping system that may affect this control. Eur Arachn 2000:57–64

Simonsen V, Christensen PG (2001) Clonal and genetic variation in three collembolan species revealed by isozymes and randomly amplified polymorphic DNA. Pedobiologia 45(2): 161–173

Sims SR, Martin JW (1997) Effect of the *Bacillus thuringiensis* insecticidal proteins CryIA(b), CryIA(c), CryIIA, and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola). Pedobiologia 41(5):412–416

Sims SR, Ream JE (1997) Soil inactivation of the *Bacillus thuringiensis* subsp. *kurstaki* CryIIA insecticidal protein within transgenic cotton tissue: laboratory microcosm and field studies. J Agr Food Chem 45(4):1502–1505

Tapp H, Stotzky G (1995) Insecticidal activity of the toxins from *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* adsorbed and bound on pure and soil clays. Appl Environ Microbiol 61(5):1786–1790

Tu JM, Zhang GA, Datta K, Xu CG, He YQ, Zhang QF, Khush GS, Datta SK (2000) Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* delta-endotoxin. Nat Biotechnol 18(10):1101–1104

Tully T, D'Haese CA, Richard M, Ferriere R (2006) Two major evolutionary lineages revealed by molecular phylogeny in the parthenogenetic collembola species *Folsomia candida*. Pedobiologia 50(2):95–104

Uchida H, Sugiyama R, Nakayachi O, Takemura M, Ohyama K (2007) Expression of the gene for sterol-biosynthesis enzyme squalene epoxidase in parenchyma cells of the oil plant, Euphorbia tirucalli. Planta 226(5):1109–1115

Wandeler H, Bahylova J, Nentwig W (2002) Consumption of two *Bt* and six non-*Bt* corn varieties by the woodlouse *Porcellio scaber*. Basic Appl Ecol 3(4):357–365

Wang HY, Ye QF, Gan J, Wu LC (2007) Biodegradation of Cry1Ab protein from *Bt* transgenic rice in aerobic and flooded paddy soils. J Agric Food Chem 55(5):1900–1904

Widarto TH, Krogh PH, Forbes VE (2007) Nonylphenol stimulates fecundity but not population growth rate ([lambda]) of *Folsomia candida*. Ecotoxicol Environ Safe 67(3): 369–377

Wu G, Cui H, Ye G, Xia Y, Sardana R, Cheng X, Li Y, Altosaar I, Shu Q (2002) Inheritance and expression of the *cry1Ab* gene in Bt (*Bacillus thuringiensis*) transgenic rice. Theor Appl Genet 104(4):727–734

Wu WX, Ye QF, Min H, Duan XJ, Jin WM (2004) Bt-transgenic rice straw affects the culturable microbiota and dehydrogenase and phosphatase activities in a flooded paddy soil. Soil Biol Biochem 36(2):289–295

Xia H, Lu BR, Xu K, Wang W, Yang X, Yang C, Luo J, Lai F, Ye W, Fu Q (2011) Enhanced yield performance of *Bt* rice under target-insect attacks: implications for field insect management. Trans Res 20(3):655–664

Ye GY, Yao HW, Shu QY, Cheng X, Hu C, Xia YW, Gao MW, Altosaar I (2003) High levels of stable resistance in transgenic rice with a *cry*1Ab gene from *Bacillus thuringiensis* Berliner to rice leaffolder, *Cnaphalocrocis* *medinalis* (Guenee) under field conditions. Crop Prot 22(1):171–178

Yu L, Berry RE, Croft BA (1997) Effects of *Bacillus thuringiensis* toxins in transgenic cotton and potato on *Folsomia candida* (Collembola: Isotomidae) and *Oppia nitens* (Acari: Orbatidae). J Econ Entomol 90(1):113–118

Yuan YY, Ge F (2010) Effects of transgenic *Bt* crops on non-target soil animals. Chin JAppl Ecol 21(05):1339–1345

Yuan Y, Ke X, Chen F, Krogh PH, Ge F (2011) Decrease in catalase activity of *Folsomia candida* fed a Bt rice diet. Environ Pollut 159(12):3714–3720

Zhang YJ, Zhao HY, Wu KM, Zhao KJ, Peng YF, Guo YY (2004) Expression of Cry1Ac protein in cry1Ac/CpTI transgenic rice and its resistance in different developmental stages to *Chilo suppressalis*. Chin J Agric Biotechnol 1(3):149–153

Zhao WC, Wang GH, Cheng JA, Chen ZX (2007) Development of a monoclonal antibody to Collembola and its application on predation evaluation. Acta Ecol Sin 09:3694–3700

Zimmer M, Topp W (2000) Species-specific utilization of food sources by sympatric woodlice (Isopoda: Oniscidea). J Anim Ecol 69(6):1071–1082

Zwahlen C, Andow DA (2005) Field evidence for the exposure of ground beetles to Cry1Ab from transgenic corn. Environ Biosafety Res 4(02):113–117

Zwahlen C, Hilbeck A, Nentwig W (2007) Field decomposition of transgenic *Bt* maize residue and the impact on non-target soil invertebrates. Plant Soil 300(1–2):245–257