J. Ecol. Field Biol. 30 (4): 319~324, 2007

Effects of Transgenic Rice on Life History Traits of *Daphnia magna* in Life Table Experiments

Nam, Sungjin[1], Dongwoo Yang[1], Chang-Gi Kim[2] and Sangkyu Park[1]*

[1]*Department of Biological Science, Ajou University, Suwon 443-749, Korea*

[2]*Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology, Chungbuk 363-883, Korea*

ABSTRACT: To investigate the impacts of transgenic rice on freshwater organisms, we conducted two life table experiments using *Daphnia magna* for fifteen and twenty days, respectively. We examined life history traits such as population growth rates (r), reproductive rates (R_0), generation times, and survivorship. In the first experiment, we used non-drought-stressed transgenic and non-transgenic rice harvested in 2005. In the second study, we used non-transgenic and transgenic rice harvested in 2006 following drought stress. Each experiment involved three treatments in which *D. magna* neonates were fed with *Selenastrum capricornutum* (control treatment) and *S. capricornutum* with 5% aqueous extracts of non-transgenic rice (N-T) and transgenic rice (T). In the first experiment, *D. magna* showed reduced population growth rates and lowered fecundity in the N-T and T treatments. In the second experiment, *D. magna* receiving both transgenic and non-transgenic rice extracts showed very high mortality, low population growth rates and reproduction rates. We could not detect any significant negative effects of extracts from transgenic rice on *D. magna* life history traits at 95%.

Key words: *Daphnia magna*, Life table experiment, Life history traits, Transgenic rice

INTRODUCTION

Large areas of natural wetlands have been converted for other land uses such as urban development in South Korea. Therefore, rice paddy fields have become important temporary wetlands, occupying approximately 10% of the total area of South Korea. Rice fields also play important roles in global biogeochemistry exchanging significant amounts of carbon dioxide, methane and nitrous oxide (Kanno et al. 1997, Tsuruta et al. 1997, Kimura et al. 2004). Recently, many transgenic crops have been introduced to agro-ecosystems all over the world (Nap et al. 2003). Transgenic crops can affect agro-ecosystems directly or indirectly through gene flow, invasions and food web changes (Dale et al. 2002).

Recently, we have been involved in risk assessment for a transgenic rice line that overexpresses a trehalose-6-phosphate synthase/phosphates (TPSP) fusion gene, potentially resulting in superior tolerance for a variety of abiotic stresses (Seo et al. 2000). As unknown secondary metabolites of transgenic rice may affect aquatic food webs in rice paddy fields in addition to human health, it is necessary to develop protocols to detect impacts of transgenic rice on aquatic organisms (Park 2007). We conducted standard life table experiments using *Daphnia magna* to detect any negative impacts of TPSP rice on the life history traits, such as population growth rate, generation time and survivorship, of freshwater organisms. We selected *D. magna* as a model organism to assess the impacts of transgenic rice on aquatic food webs, since *Daphnia* plays a central role in aquatic food webs (Weisse and Stockner 1993, Gaedke and Straile 1998) and has been used as a "standard" aquatic test species for toxicological research (Feldmannová 2006, Martins et al. 2007).

Since toxicity from transgenic rice may not be easily detected by acute toxicity tests with short exposures, we chose to conduct chronic toxicity tests using *D. magna*. Life table experiments are among the most comprehensive chronic toxicity tests (Tanaka and Nakanishi 2001), and can examine the effects of toxic substances on life history traits such as survivorship, reproduction rates, and population growth rates over several generations. In this study, we conducted life table experiments to examine the effects of chronic exposure to transgenic rice extracts on life history traits of *D. magna*.

MATERIALS AND METHODS

We conducted two consecutive life table experiments to investigate the effects of exposure to transgenic rice on *D. magna*. The first experiment was conducted for 15 days (except for the control treatment, which was extended to 75 days) while the second experiment was conducted for 20 days. We obtained a strain of *D. magna* from the Han-River Environment Research Laboratory and maintained the organisms in a growth chamber at 26℃ with a 16:8 h light: dark cycle using L16 medium (Lindström 1982). We collec-

* Corresponding author; Phone: +82-31-219-2967, e-mail: daphnia@ajou.ac.kr

ted neonates born within a 12-hour period for the experiment. We prepared aqueous extracts of dried and ground powder of Nakdong (non-transgenic) rice and ABC promoter TPSP (transgenic) rice using the extraction methods of Rice et al. (2005). For the first experiment, we used rice harvested in October, 2005 that had not been exposed to drought stress. For the second experiment, we used drought-exposed rice harvested in October, 2006. We added 6 g of rice powder to 90 mL of distilled water in a 250 mL flask and shook the mixture at 4℃ for 24 hours. The extracted solution was then centrifuged at 100×g (3,000 rpm) for 10 minutes and filtered through 47 mm GF/C (Whatman) and 0.45 μm nitrocellulose (Millipore) filters. We conducted three experimental treatments in which 15 neonates of *D. magna* were treated with *Selenastrum capricornutum* (Control), *S. capricornutum* with a 5% aqueous extract from non-transgenic rice (N-T treatment), or *S. capricornutum* with a 5% aqueous extract from transgenic rice (T treatment). We chose 5% for the extract concentration because 5% was the highest concentration that did not significantly affect algal growth as a result of shading in preliminary experiments. Each day of the experiment, we fed *S. capricornutum* produced from a chemostat (final algal concentration: 0.5 mg C L^{-1}) in a beaker with L16 (volume: 200 mL) to individual *D. magna*, checked their length, molting, and survivorship, and recorded the appearance of eggs and the number of neonates produced. *D. magna* growth experiments usually use 0.5 mg C L^{-1} above incipient limiting level (ILL) as a food concentration (Lampert 1987, Park et al. 2002). Survivorship was defined as common logarithm of the ratio of animals alive to the initial animal number on a given day. *D. magna* in the first experiment were transferred to fresh medium every three days (due to a limited supply of extracts) while in the second experiment they were transferred to fresh medium every day. We then estimated the population growth rates (r), reproductive rates (R_0), generation time (T_c), molting number, and age at first reproduction (*AFR*).

The intrinsic rate of population increase (r) for each treatment was estimated from reproductive rates (R_0) and cohort generation time (T_c):

$$r = R_0 / T_c \quad (1)$$

$$T_c = \Sigma x l_x m_x / \Sigma l_x m_x \quad (2)$$

$$R_0 = \Sigma l_x m_x \quad (3)$$

Where l_x indicates the proportion of original cohort surviving to day x and m_x indicates the mean number of offspring produced per survivor on day x. We used pseudo r values generated by jackknifing for standard errors and statistical comparisons of population growth rates (r) (Meyer et al. 1986, Park et al. 2003). All statistical analyses were performed with S-Plus 6 for Windows (Insightful Corp., USA).

RESULTS

In the first experiment, *D. magna* in the control treatment outlived those in the nontransgenic (N-T) treatment and transgenic (T) treatments by 60～64 days (Fig. 1). We continued our observations of the *D. magna* in the control treatment to provide reference data for other life table experiments. The mean length of *D. magna* in the control treatment was 0.8 mm at 1 day old and length increased almost linearly until the 10th day. The rate of length increase approached the maximum level of 4.4 mm by the 40th day. *Daphnia*

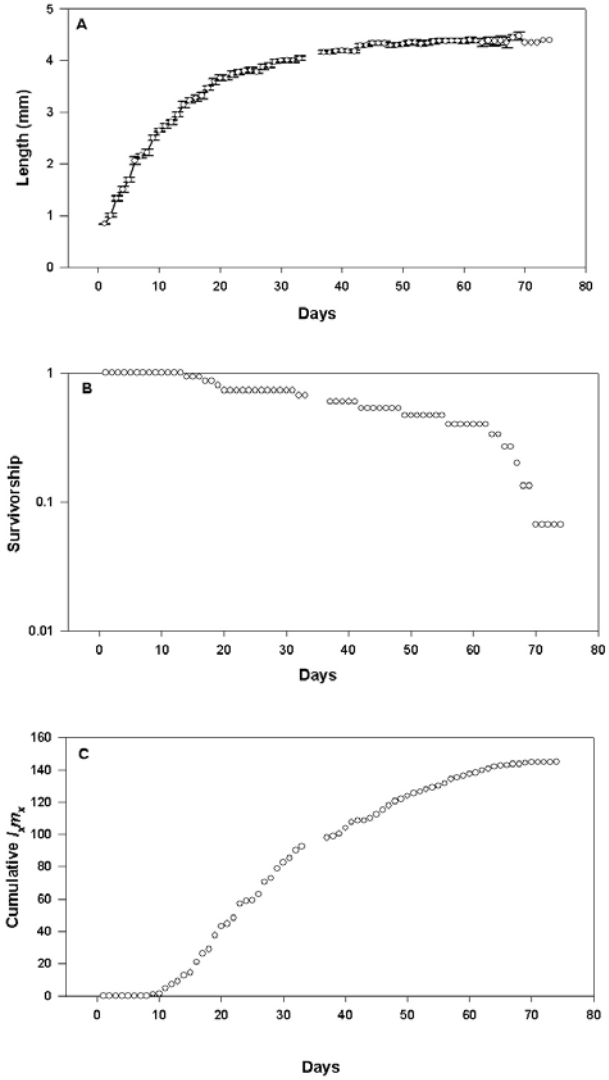

Fig. 1. Length growth (A), survivorship (B) and cumulative l_xm_x (C) of *Daphnia magna* in the control treatment in the first experiment. Average lengths are shown at all ages. Final values of cumulative l_xm_x are equal to reproductive rate (R_0).

magna displayed type 1 survivorship, with a dramatic decrease in survivorship beyond the 60th day. Cumulative l_xm_x reached 145, meaning that one healthy female *D. magna* could produce 145 offspring during her lifetime if supplied with enough green algae as food.

In the first experiment, the length of *D. magna* individuals grew significantly more slowly in the N-T and T treatments than in the control treatment (Fig. 2A). There was little difference in survivorship of *D. magna* in the control, N-T and T treatments (Fig. 2B). However, the cumulative l_xm_x of daphniids was much lower in the N-T and T treatments than in the control treatment. Cumulative l_xm_x was slightly higher in the T treatment than the N-T treatment (Fig. 2C).

In the second experiment, *D. magna* exhibited similar length growth patterns, with *D. magna* in the N-T and T treatments growing significantly more slowly than *D. magna* in the control treatment, and no difference in growth between the N-T and T treatments (Fig. 3A). Survivorship in both the N-T and T treatment declined rapidly after the 9th day while survivorship in the control survivorship changed little throughout the experiment (Fig. 3B). *D. magna* in both the N-T and T treatments did not produce any offspring (Fig. 3C).

We compared life history traits of *D. magna* in the control, N-T and T treatment in the first and second experiments using the Jack-knife method (Table 1 and Table 2). In the first experiment,

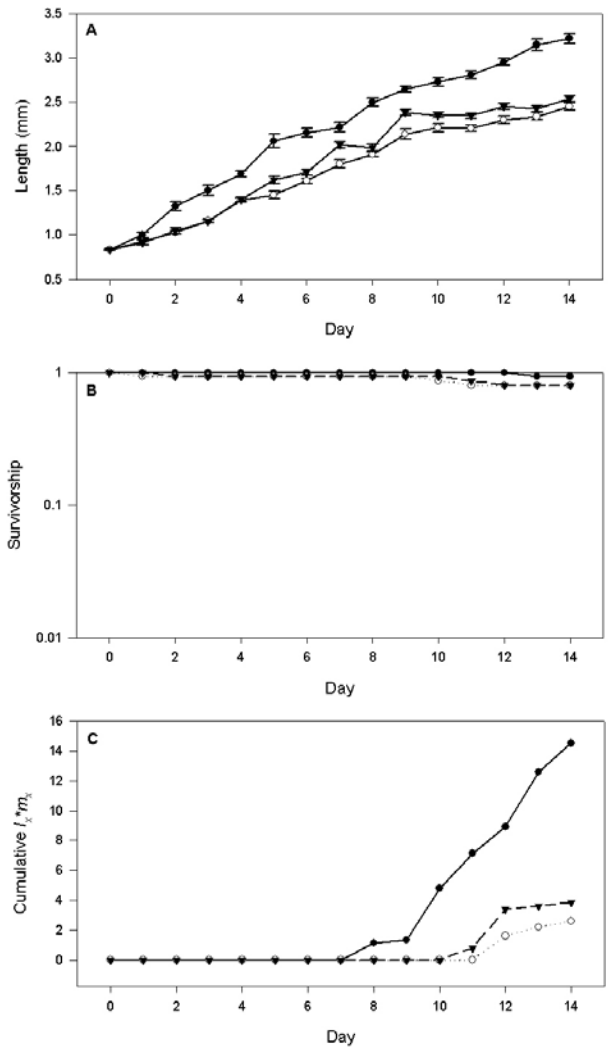

Fig. 2. Length growth (A), survivorship (B) and cumulative l_xm_x (C) of *Daphnia magna* in the control treatment (●), non-transgenic treatment (○) and transgenic treatment (▼) in the first experiment. For length of *D. magna*, average lengths were shown with bars for S.E. Final values of cumulative l_xm_x are equal to reproductive rate (R_0).

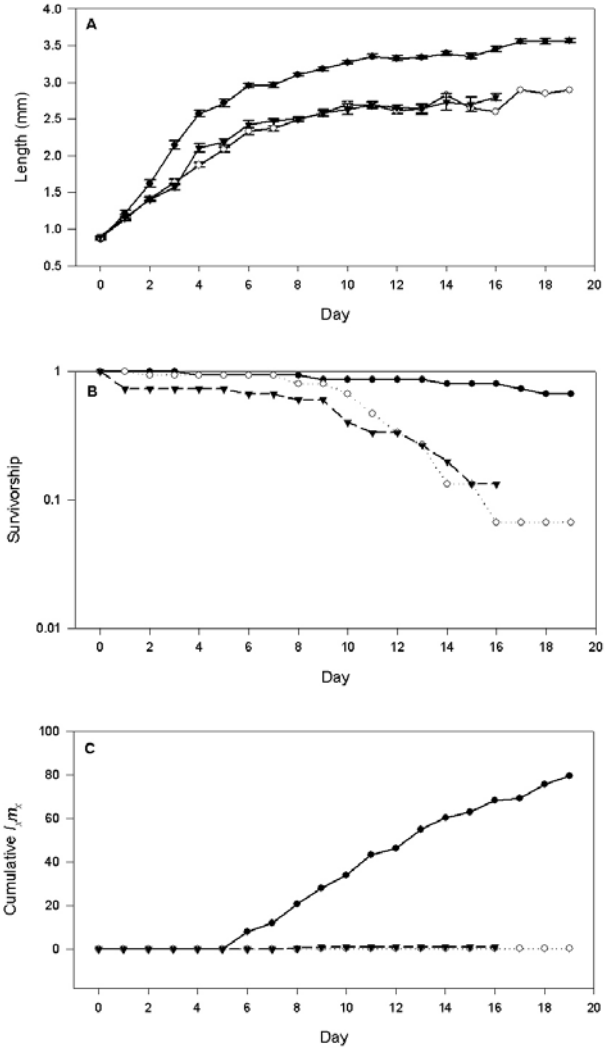

Fig. 3. Length growth (A), survivorship (B) and cumulative l_xm_x (C) of *Daphnia magna* in the control treatment (●), non-transgenic treatment (○) and transgenic treatment (▼) in the second experiment. For length of *D. magna*, average lengths were shown with bars for S.E. Final values of cumulative l_xm_x are equal to reproductive rate (R_0).

D. magna in the N-T and T treatments showed much lower population growth rates, delayed reproduction, somewhat longer generation times and much lower reproductive rates than *D. magna* in the control condition (Table 1). However, the number of molting events did not significantly differ among animals in the control, N-T and T treatments. *D. magna* in the T treatment showed similar or slightly better performance than animals in the N-T treatment. In the second experiment, animals in the N-T and T treatments again showed much lower population growth rates, delayed reproduction, and much lower reproductive rates than animals in the control treatment (Table 2). However, we could not detect any significant difference in life history traits between the N-T and T treatments.

DISCUSSION

This report describes one of the first studies to conduct life table experiments using *D. magna* to assess the effects of chronic exposure to transgenic crops on aquatic non-target organisms. We are aware of a few additional experiments using the life table approach for ecological assessment of transgenic crops such as transgenic cottons using aphids (Liu et al. 2005) and transgenic maize using thrips (Obrist et al. 2005), and an assessment of ecological risk from acid mine drainage using life table experiments using *D. pulex* (Jooste and Thirion 1999).

Our results indicate that plant extracts from rice affected several life history traits of *D. magna*, including growth rate, survivorship and reproductive rates, but we could not detect any significant differences between *D. magna* life history traits in organisms treated with non-transgenic and transgenic rice. In fact, *D. magna* treated with transgenic rice extracts appeared to show slightly better performance than *D. magna* treated with non-transgenic rice extracts in almost every life history trait examined. Therefore, we could not detect any significant negative effects of exposure to transgenic TPSP rice, as opposed to non-transgenic rice, on *D. magna* life history traits in the present study.

In the second experiment, the TPSP rice was grown under water stress (drought), which activates the ABC promoter to over-express the TPSP fusion gene and possibly to promote superior tolerance to several abiotic stressors. The ABC promoter over-expresses the TPSP fusion gene in transgenic plants only when the plant experiences stressors such as drought (Su et al. 1998). However, while the results from the first and second experiments differed slightly, the transgenic and non-transgenic rice treatments produced similar results in each experiment. Therefore, we conclude that the over-expression of the TPSP gene induced by an artificial drought did not have any significant effect on the life history traits of *D. magna* in our study. It is also important to note that the first and second experiments employed different feeding schedules which might have

Table 1. Comparison of life history traits of *Daphnia magna* such as molting rates (Molting, ± S.E.), rates of population increase (r_j, ± S.E., using the Jack-knife method), age at first reproduction (*AFR*, ± S.E.), cohort generation time (T_{cj}, ± S.E., using the Jack-knife method) and reproduction rates (R_0, ± S.E., using the Jack-knife method) for the control treatment (Control), non-transgenic treatment (N-T), and transgenic treatment (T) in the first life table experiment. Superscripts indicate results from Tukey multiple comparison tests among Control, N-T and T treatment

	Molting (d^{-1})	r_j (d^{-1})	*AFR* (d)	T_{cj}	R_{0j}
Control	0.389 ± 0.0185^a	0.214 ± 0.0057^a	9.80 ± 0.262^a	12.527 ± 0.0224^a	14.53 ± 0.180^a
N-T	0.408 ± 0.0340^a	0.055 ± 0.0012^c	12.30 ± 0.213^b	13.723 ± 0.0093^c	2.20 ± 0.083^c
T	0.367 ± 0.0286^a	0.104 ± 0.0013^b	11.91 ± 0.163^b	12.983 ± 0.0056^b	3.87 ± 0.066^b

Table 2. Comparison of life history traits of *Daphnia magna* such as molting rates (Molting, ± S.E.), rates of population increase (r_j, ± S.E., using the Jack-knife method), age at first reproduction (*AFR*, ± S.E.), cohort generation time (T_{cj}, ± S.E., using the Jack-knife method) and reproduction rates (R_0, ± S.E., using the Jack-knife method) for the control treatment (Control), non-transgenic treatment (N-T), and transgenic treatment (T) in the second life table experiment. Superscripts indicate results from Tukey multiple comparison tests among Control, N-T and T treatment

	Molting (d^{-1})	r_j (d^{-1})	*AFR* (d)	T_{cj}	R_{0j}
Control	0.363 ± 0.0185^a	0.345 ± 0.0010^a	7.286 ± 0.125^a	12.718 ± 0.0845^a	80.11 ± 1.511^a
N-T	0.217 ± 0.0261^b	−0.125	10	10	0.27 ± 0.019^b
T	0.161 ± 0.0337^b	0.012 ± 0.0047^b	9.667 ± 0.333^b	9.712 ± 0.0396^b	1.13 ± 0.045^b

affected our results. The second experiment, with everyday replacement of the culture medium, showed higher mortality of *D. magna* than the first experiment, which might be due to increased exposure of *D. magna* to fresh rice extract, rather than to the effects of drought treatment.

Our experiments involved some limitations which may have affected the results. First, it is possible that we used an excessively high concentration of rice extract in our life table experiments, which might have masked the effects of transgenic rice. For example, the high concentration of rice extracts might have caused *D. magna* to die within 15 to 20 days due to toxicity from the rice extracts, regardless of their transgenic status. In addition, the *D. magna* in our experiments did not have enough time to produce many offspring. Therefore, we suggest that further experiments should be conducted to determine appropriate plant extract concentrations to permit much longer survivorship of *D. magna* before conducting additional life table experiments with transgenic rice extracts.

Developers of transgenic crops are sensitive to the publication of studies demonstrating negative impacts of transgenic products. Therefore, we think there may be some publication bias in favor of studies that show no significant impacts of transgenic crops. We believe that our results should not be interpreted as indicating that the use of transgenic crops is safe. Rather, many safety issues regarding transgenic crops have not yet been resolved, and we should continue to improve our tools for assessment of the risks of transgenic crops.

In conclusion, we showed that it was possible to use *Daphnia* life table experiments to assess the chronic impacts of TPSP rice on aquatic organisms. We also provide key baseline information on the life history traits of *D. magna* fed with green algae *Selenastrum capricornutum*, which may be used to assess the impacts of exposure to transgenic crops.

ACKNOWLEDGEMENTS

This research was supported by the grants from the Crop Functional Genomics Center and the Korea Science and Engineering Foundation (KOSEF) funded by the Ministry of Science and Technology.

LITERATURE CITED

Dale PJ, Clarke B, Fontes EMG. 2002. Potential for the environmental impacts of transgenic crops. Nat Biotechnol 20: 567-574.

Feldmannová M, Hilscherová K, Maršálek B, Bláha L. 2006. Effects of N-Heterocyclic polyaromatic hydrocarbons on survival, reproduction, and biochemical parameters in *Daphnia magna*. Environ Toxicol 21: 425-431.

Gaedke U, Straile D. 1998. Daphnids: Keystone species for the pelagic food web structure and energy flow - a body sized-related analysis linking seasonal changes at the population and ecosystem levels. Arch Hydrobio Spec Issues Advanc Limnol 53: 587-610.

Jooste S, Thirion C. 1999. An ecological risk assessment for a South African acid mine drainage. Water Sci Tech 39: 297-303.

Kanno T, Miura Y, Tsuruta H, Minami K. 1997. Methane emission from rice paddy fields in all of Japanese prefecture. Nutr Cycl Agroecosys 49: 147-151.

Kimura M, Murase J, Lu Y. 2004. Carbon cycling in rice field ecosystems in the context of input, decomposition and translocation of organic materials and the fates of their end products (CO_2 and CH_4). Soil Biol Biochem 36: 1399-1416.

Martins J, Teles O, Vasconcelos V. 2007. Assays with *Daphnia magna* and *Danio rerio* as alert systems in aquatic toxicology. Environ Internat 33: 414-425.

Lampert W. 1987. Feeding and nutrition in *Daphnia*. In Daphnia, (Peters RH, De Bernardi R, eds). Mem Ist Ita Idrobiol 45, pp 143-192.

Lindström K. 1983. Selenium as a growth factor for plankton algae in laboratory experiments and in some Swedish lakes. Hydrobiologia 101: 35-48.

Liu XD, Zhai BP, Zhang XX, Min J. 2005. Impact of transgenic cotton plants on a non-target pest, *Aphis gossypii* Glover. Ecol Entomol 30: 307-315.

Meyer JS, Ingersoll CG, McDonald LL, Boyce MS. 1986. Estimating uncertainty in population growth rates: Jackknife vs. Bootstrap techniques. Ecology 67: 1156-1166.

Nap JP, Metz PLJ, Escaler M, Conner AJ. 2003. The release of genetically modified crops into the environment. Part I. Overview of current status and regulations. Plant J 33: 1-18.

Obrist LB, Klein H, Dutton A, Bigler F. 2005. Effects of Bt maize on *Frankliniella tenuicornis* and exposure of thrips predators to prey-mediated Bt toxin. Entomol Exp Appl 115: 409-416.

Park S, Brett MT, Müller-Navarra DC, Goldman CR. 2002. Essential fatty acid content and the phosphorus to carbon ratio in cultured algae as indicators of food quality for *Daphnia*. Freshw Biol 47: 1377-1390.

Park S, Brett MT, Oshel ET, Goldman CR. 2003. Seston food quality and *Daphnia* production efficiencies in an oligo-mesotrophic subalpine lake. Aquat Ecol 37: 123-136.

Park S. 2007. Methods for environment risk assessment of transgenic crops. In: Plant Transformation (Korean Society for Plant Genetic Transformation ed). Jungmunkag, Seoul. pp. 463-475.

Rice CP, Park YB, Adams F, Abdul-Baki AA, Teasdale JR. 2005. Hydroxamic acid content and toxicity of rye at selected growth stages. J Chem Ecol 31: 1887-1905.

Seo HS, Koo YJ, Lim JY, Song JT, Kim CH, Kim JK. 2000. Characterization of a bifunctional enzyme fusion of trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase of *Escherichia coli*. Appl Environ Microb 66: 2484-2490.

Su J, Shen Q, Ho THD, Wu R. 1998. Dehydration-stress-regulated transgene expression in stably transformed rice plants. Plant Physiol 117: 913-922.

Tanaka Y, Nakanishi J. 2001. Life history elasticity and the popula-

tion-level effect of *p*-nonylphenol on *Daphnia glaeata*. Ecol Res 16: 41-48.

Tsuruta H, Kanda K, Hirose T. 1997. Nitrous oxide emission from a rice paddy field in Japan. Nutr Cycl Agroecosys 49: 51-58.

Weisse T, Stockner JG. 1993. Eutrophication: the role of microbial food webs. Mem Ist Ital Idrobiol 52: 133-150.

(Received October 29, 2007; Accepted November 9, 2007)