A THESIS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Methodology Development for *Bt* Rice (Cry1Ac) Effect Assessment on Non-target Arthropods

유전자변형벼(Cry1Ac)가 비표적절지동물에게 미치는 영향평가방법 개발

BY

SUEYEON LEE

ENTOMOLOGY PROGRAM

DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY

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UNDER THE DIRECTION OF ADVISER JOON-HO LEE
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BY
SUEYEON LEE

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APPROVED AS A QUALIFIED DISSERTATION OF SUEYEON LEE
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
BY THE COMMITTEE MEMBERS

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Methodology Development for *Bt* Rice (Cry1Ac) Effect Assessment on Non-target Arthropods

Entomology Program
Department of Agricultural Biotechnology, Seoul National University

SUEYEON LEE

ABSTRACT

To assess effects of *Bt* rice (Cry1Ac) for control *Cnaphalocrocis medinalis* on non-target arthropods, laboratory tests were conducted to evaluate the potential impacts of 1) *Bt* rice leaf expressing cry1Ac on development, survival, emergence and fitness of adult of non-target herbivores, *Nilaparvata lugens, Oxya japonica* and *Scotinophara lurida*, 2) *Bt* rice pollen on development, survival, emergence and fitness of adult of non-target pollen-feeder, *P ropylea japonica*, 3) prey fed on *Bt* rice leaf on development, survival, emergence and fitness of adult of non-target predator, *Pirata subpiraticus* and *Pachygnatha clercki*, and 4) *Bt* rice leaf on development, survival and emergence of *N aranga aenescens* close to the target pest species, *C. medinalis*, and on oviposition preference of *C. medinalis*. There were no significant differences in development, survival and emergence of *O. japonica, S. lurida* and *N. lugens* between *Bt* and non-*Bt* rice. Also, no significant differences were found in development and survival of *P. subpiraticus* and *P. clercki* feeding on *N. lugens* reared on *Bt* or non-*Bt* rice but tibia lengths of *P. subpiraticus* and *P. clercki* feeding on *N. lugens* reared on *Bt* rice were significantly longer than those feeding on *N. lugens* in non-*Bt* rice. There were no adverse effect on the development, survival and fitness of adult *P. japonica* after ingestion of *Bt* rice

pollen expressing Cry1Ac. *N. aenescens* showed significant differences in development, survival and emergence rate between *Bt* and non-*Bt* rice. *C. medinalis* did not show oviposition preference between *Bt* and non-*Bt* rice.

In a 2-year field study, a total 43 familes in 10 orders were identified from 64,099 collected insects and classified four guilds, Herbivores, Predators, Parasitoids, andDetritivores. Family richness, abundance and Shannon's index of insects were very similar between *Bt* and non-*Bt* rice. However, significantly higher abundance was observed in the non-*Bt* rice in the herbivore in 2007, and predator, and Coenagrionidae, in 2008. A total 29 species in 23 genera and 9 families were identified from 4,937 collected spiders and both *Bt* and non- *Bt* rice fields showed a typical Korean spider assemblage. Species richness, abundance and Shannon's index of spiders were very similar between *Bt* and non-*Bt* rice, although in 2008 significant difference was observed in the abundance of *P. oculiprominens, T. maxillosa and P. clercki* in the . *P. oculiprominens* and *T. maxillosa* were higher in non-*Bt* rice and *P. clercki* was higher in *Bt* rice. The results indicated that the transgenic Cry1Ac rice tested in this study had no significant adverse effects on the rice insect and spider community structure in rice fields and on the development, survival, emergence and adult fitness parameter of non-target arthropods and oviposition preference of the target species in the laboratory conditions.

To suggest appropriate sampling methods, sampling plot size, sampling timing and sampling occasion for field assessment following experiments were conducted. The efficacy of two sampling methods, sweep net and suction, were compared to survey insect and spider community, species richness and diversity of spiders in rice fields of three different plot sizes, 270 m^2, 1000 m^2 and 3300 m^2, throughout the rice growing season. Suction sampling captured more spider species and individuals than sweep net sampling, but insects were captured more by sweep net sampling. In this study, biodiversity of spiders did not increase

with increase of plot size. The similarity of the community was higher between 1000 m^2 and 3300 m^2 than others. Thus it seems that 1000 m^2 plot size test is resonable to detect *Bt* crop effects on arthropods community in rice fields. This study used cluster analysis with similarity of community to find appropriate sampling time and occasion, and sampling time were divided into five clusters with 65.6% similarity and these clusters generally corresponded to five rice growing stages in the fields.

Key words: *Bt* rice (Cry1Ac), assessment of *Bt* effects, non-target arthropods, *Cnaphalocrocis medinalis*, *Naranga aenescens*, *Nilaparvata lugens*, *Oxya japonica*, *Scotinophara lurida*, *Propylea japonica*, *Pirata subpiraticus*, *Patchygnatha clercki*, insect community, spider community,

Student number: 2006-21482

Chapter 1.

General introduction

1.1. *Bacillus thuringiensis* toxin and its mode of action

Bacillus thuringiensis (Berliner) is a facultative anaerobic and gram-positive bacterium dwelling in the soil and producing a proteinaceous parasporal crystalline inclusion during sporulation.Besides several other virulence factors, these crystalline inclusion consist of entomopathogenic proteins, called "Cry proteins" (De Maagd et al. 2001). *Bt* strains are each known as producing specific cry proteins that are toxic to different insect orders. In general, Cry1 proteins are toxic to Lepidoptera, Cry2 proteins to Lepidoptera and Diptera, Cry3 proteins to Coleoptera, and Cry4 to Diptera (Hafte and Whiteley 1989). Cry proteins of 130 kDa ingested by insects are solubilized in the alkaline environment of the insect midgut and cleaved by a gut protease to produce an active toxin of about 60kD called δ-endotoxins. δ-endotoxins consist of three main protein domains (I-III) (Grochulski et al. 1995). The domain I on the N-terminal of the protein is a bundle of 7 amino acid α-helices, some or all of which can insert into the gut cell membrane, creating a pore through which ions can pass freely. Domains II and III are located on the C-terminal of the protein and consist of amino acid β-sheets. Domain II consists of 3 amino acid β-sheets, similar to the antigen-binding regions of immunoglobulins, and is responsible for the binding on a specific midgut cell receptor in the gut. Domain III is a tightly packed beta-sandwich which is thought to protect the exposed end (C-terminus) of the active toxin, preventing further cleavage by gut proteases (Li et al. 1991, Dean et al. 1996). After the activation of the Cry toxin, δ-endotoxins bind to specific receptors on the brush border membrane of midgut epithelium cells creating pores in the cell membranes and leading to equilibration of ions (Van Rie et al. 1990). The binding on receptors leads to an insertion of α-helices of domain I into the midgut membrane which contributes to a formation of ion channels (Bravo et al. 2004, English et al. 1995). Ion

channels mediate an influx of ions into the midgut membrane cells which is followed by an osmotic water influx and results in the burst of midgut epithelium cells (Knowles and Ellar 1987). As a result, the gut is rapidly immobilized, the epithelial cells lyse, the larva stops feeding, and the gut pH is lowered by equilibration with the blood pH. This lower pH enables the bacterial spores to germinate, and the bacterium can then invade the host, causing a lethal septicaemia (Heimpel and Angus 1959, Gringorten 2001, Glare and O'Callaghan 2000). Due to the insecticidal effectiveness and the specificity to pest targets of Cry proteins, commercially available pesticides based on *B. thuringiensis* were used in agriculture since the 1930s (Van Frankenhuyzen 1993).

1.2. *Bt* crops and cry1Ac rice in Korea

Since genetically modified (GM) crops were first commercialized in 1996, farmers have consistently increased their plantings of GM crops by 10% or more each year worldwide (reference!). It is generally expected that commercial cultivation of GM crops will further increase over the coming years. Between 1996 and 2011, a total of 29 countries – 10 industrial and 19 developing – contributed to more than a 94 fold increase in the global area of transgenic crops from 1.7 million hectares in 1996 to 160 million hectares in 2011 (James 2011). In general, gene transfer to plants occurs in two main methods. One technique uses a natural soil bacterium, *agrobacteria tumefaciens*, which naturally infects plants, causing plant tumor. The *Agrobacterium* method exploits the tumor-indicing (Ti) plasmid which naturally is occurring and enabling DNA transfer called T-DNA into the gene of wounded plant cells. For the plant transformation, these genes are replaced with those carrying the useful GM trait, and used (Cristou et al. 2006). Another method is based on the bombardment of plant tissue with microprojectiles called a gene gun which fires tiny gold particles coated with genes directly into a plant cells (Twyman and Christou 2004).

Nowdays GM crops are altered for agronomic traits, such as herbicide-tolerance, insect-or virus-resistance. One of the first and still the most widespread is *Bt* crops resistant to common insect pests. Until now, *Bt* was deployed in cotton, maize, soybean, potato and tomato for their transformation.

The potential of *Bt* crops to make important contributions to food security and agricultural sustainability worldwide is indisputable. Through increasing productivity and economic benefits sustainably, *Bt* crops can contribute to food, feed and fiber security and self sufficiency, including more affordable food at the farmer level.

Rice, *Oryza sativa* L., is the most important staple food for a large part of the world's human population with the third-highest worldwide production, after sugar cane and maize. More than 90% of the world's rice is produced and consumed in Asia (FAO 2010). Significant yield losses in rice have been documented by insect pests, especially stem borers, leaffolder and planthoppers (Sheng et al. 2002, Chen et al. 2003, Wang et al. 2010). Rice stem borers are chronic pests in rice. Of them, the yellow stem borer, *Scirpophaga incertulas* (Pyralidae), is the most critical pest, while the striped stem borer, *Chilo suppressalis* (Crambidae), is the most abundant in Asia. They damage tillers, resulting in deadhearts and whiteheads which are the production of panicles of unified grains. The most serious foliage feeding Lepidoptera is the rice leffolder, *Cnaphalocrocis medinalis* (Pyralidae) (Wada et al. 1980, Bautista et al. 1984, Dale 1994). Because of the important pest status of stem borers and leaffolders and the limited sources of resistance to these pests in rice germplasm, numerous transgenic rice lines carrying single genes derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) have been developed since 1993. Fujimoto et al. (1993) reported the first transformation of rice with a *Bt* gene and many papers reported the development and evaluation of *Bt* rice lines and reviewed by High et al. (2004) and Chen et al. (2006). Rice lines expressing cry1Aa, cry1Ab, cry1Ac, cry1Ab/cry1Ac fusion, cry1B, cry1C, cry1Ca1, cry2A and cry9C have been shown to confer resistance to stem borers and to leaffolders and other foliage-feeding Lepidoptera (Table 1).

In Korea, *Bt*-rice line expressing cry1Ac1 for control of *C. medinalis* (Lepidoptera: Pyralidae) was developed. To increase the more expression of *Bt* genes in rice, the coding sequences of cry1Ac was modified to the codons preferred by the plants. The promoter of the small subunit gene of ribulose biphosphate carboxylase/oxygenase (rbcS) and its transit peptide (TP) were used as a transformation vector for effective expression of genes in green

tissues. Several fertile transgenic lines were generated through the Agrobacterium transformation procedure and examined on the expression levels of transgenic lines during the period of T2 to T5 generations. Cry1Ac1 7-1-9-1 expressing high and stable resistance against natural and artificial infestations by the rice leaf folder (RLF) was selected. Shin et al. (2009) carried out to provide the molecular data for the risk assessment of GM rice containing insect-resistant gene, modified cry1Ac (cry1Ac1). The molecular analysis with cry1Ac1 induced GM rice confirmed the steady integration and expression of transgene, the transgene copy number, adjacent region sequences of inserted gene into rice genome, and the transgene stability in progenies.

Table 1. Representative rice lines transformed with genes for resistance to Lepidoptera

Gene	Promoter	Cultivar	References
cry1Aa	*Ubiquitin*	Ariete, Senia	Breitler et al., 2004
cry1Ab	*Ubiquitin* *PEPC*	Xiushui 11, Tarom Molaii	Shu et al., 2000, Ye et al. 2001, Wu et al., 2002 Ghareyazie et al., 1998, James, 2005
cry1Ac	*Ubiquitin*	Basmati	Bashir et al. 2004
cry1Ac1	*RbcS*	Nakdong	Shin et al., 2009
cry1Ab/cry1Ac fusion	*Actin1*	Minghui 63, IR72	Tu et al., 2000, Huang et al., 2005
cry1B	*Ubiquitin*	Ariete, Senia	Breitler et al., 2004
cry1C	*Ubiquitin* *RbcS*	Minghui 63 Zhonghua 11	Wei et al., 2006 Ye et al. 2009
cry1Ca1	*D35S*	Xiushui 11	Zaidi et al. 2009
cry2A	*CaMV*	Minghui 63	Chen et al., 2005, Riaz et al. 2006
cry9C	*Ubiquitin*	Minghui 63	Chen et al. 2008

1.3. Risk assessment of insect-resistant GM crops

Since GM crops may have negative effects on the environment, and the area of GM crops has been increasing worldwide (James 2011), public and scientific concerns have been expressed against the approval of GM crop varieties. Several countries developed regulatory systems for investigation of the potential toxicity of GM crops as well as for post-release monitoring of GM crops in order to assess their potential long-term or unexpected effects (Conner 2003). Environmental risk assessment (ERA) was defined in the European Union (EU) legislation, EU Directive 2001/18/EC (European Parliament and Council 2001) as evaluation of 'risks to human health and the environment, whether direct or indirect, immediate or delayed, which the deliberate release or the placing on the market of GMOs may pose' (Annex II). The EU Directive 2001/18 explains that 'Direct effects' are primary effects on human health and the environment which are the result of the GMO itself and which do not occur through a causal chain of events. 'Indirect effects' are effects 'occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management of the crop' (EC 2001, Annex II). 'Immediate effects' refer to effects 'which are observed during the period of the release of the GMO'. Immediate effects may be direct or indirect. 'Delayed effects' are effects 'which may not be observed during the period of the release of the GMO but become apparent as a direct or indirect effect either at a later stage or after termination of the release'. However, standardized protocols for assessing potential risks of GM crops do not exist and several approaches are proposed (Jepson et al. 1994, Dutton et al. 2003, Wolt et al. 2003, Howard and Donnelly 2004, Romeis et al. 2008, Hillbeck et al. 2011). Broadly, risk assessment for GM crop is largely classified into "ecological approach" and "ecotoxicological approach", and the respective risk research and assessments are largely and

still based on ecotoxicological laboratory approaches due to the good reproducibility of experiments, easy breeding of those organisms and low costs of the work (Meyer 2011). ERA of GM crops is designed to explain the potential risks of introducing such plants into the environment, and includes three main phases: problem formulation, analysis which consists estimates of the likelihood of the hazards being realized (exposure) and analysis of the potential severity of the consequences of the hazards being realized, and risk characterization (USEPA 1998, Raybould 2006, Garcia-Alonso et al. 2006, Carstens et al. 2010, Wolt et al. 2010, Romeis et al. 2011). Problem formulation, the initial step in the risk assessment, defines the scope of risk assessment and the environmental assessment endpoints that are to be protected against a potential stressor for relevant decision-making (USEPA 1998, Raybould 2006 Carstens et al. 2010, Wolt et al. 2010, Romeis et al. 2011). Problem formulation leads to an analysis plan that is consistent with the risk hypotheses and establish the relationship between the stressor and the ecological impacts of concern. According to the risk hypotheses, risk assessment should be carried out in a tiered system or step-wise system (Dutton et al. 2003, Andow and Zwahlen 2006, Garcia-Alonso et al. 2006, Rose 2007, Romeis et al. 2008, 2011, Hilbeck et al. 2011). Lower-tier laboratory studies are conducted under worst-case exposure conditions where species representative of non target arthropods (NTAs) present in the receiving environment that are likely to be exposed to the arthropod-active protein are exposed to concentrations of the protein in excess of exposure in the field. Lower tiers allow tighter control over experimental variables and exposure conditions, resulting in a greater ability to produce statistically reliable results at relatively low cost though realism in terms of exposure pathway or level is usually relatively low. Higher-tier studies are conducted under a larger temporal and/or spatial scale than in the lower tier studies like greenhouses, semi-field or field studies, and can more realistically assess

potential exposure to the insecticidal protein in the field than lower-tier studies. However, it is difficult to achieve adequate replication, to control due to extraneous variables, and to find appropriate control treatments. Also higher-tier studies can lead to the possibility that no differences were detected because of lack of statistical power, not by the insecticidal protein. In addition, sampling and analysis can be labor intensive and expensive (Rose 2007, Romeis et al. 2009). Moving from lower to higher tiers is proceeded by the need and the ability to test hypotheses resulting from the problem formulation and by the need for additional data to satisfy regulatory requirements. The decision to move to a higher tier of testing is first and foremost determined by the results of lower tier tests. Generally ERA on NTAs for GM crops is conducted as following:

Tier I is laboratory tests of selected non-target species using exposure levels representing at least 10x the highest Expected Environmental Concentration (EEC). Insecticidal protein mixed with artificial diet is the preferred test compound. The most appropriate protein is similar to that expressed in GM crops. The protein is often produced microbially and may be in an activated form where GM crops data indicate of this to be appropriate.

Tier II is laboratory tests using plant material alone or mixed with artificial diet like in pollen or leaf discs from the GM crops. Because plant materials are used, exposure levels generally reflect 1x the EEC.

Tier III is long-term laboratory and/or semi-field tests. Examples of long-term laboratory tests include full life-cycle tests and “tri-trophic tests.” Extended laboratory or semi-field tests may be conducted under greenhouse conditions. Controlled semi-field tests employ cages or other techniques to provide some measure of experimental control under simulated or restricted field conditions.

Tier IV is field tests. These tests may use plots of a hectare or more, which can be distributed across an area or region. Studies may involve looking at specific groups of sentinel organisms or census studies (Rose 2007, Romeis et al. 2008, Hilbeck et al. 2011).

For a risk assessment of GM crops, it is impossible to test all species that are potentially present in the receiving environment and exposed to the arthropod-active protein, e.g. *B. thuringiensis*, therefore, testing organisms should be selected that represent different habitats (e.g., soil- or plant-dwelling arthropods) or different ecosystem services such as ecological functions (e.g., predator, parasitoid or decomposer), taxonomic groups, the availability of the test organism or the likelihood of exposure to GM crops as well as a possible sensitivity to products of transgenic plants (Jepson et al. 1994, Dutton et al. 2003, Hilbeck and Andow 2002, Rose 2007, Romeis et al. 2008, 2011, Hilbeck et al. 2011). Also ranking of species according to its geographic distribution, habitat specialization, abundance, phenology, linkage and association with the crop can reduce the number of potential testing species existing in a given cropping system and surrounding habitats while acknowledging the limitations of the available knowledge about species and their function and identifying important gaps of information (Hilbeck et al. 2011). The test substance should be characterized and formulated in a way that allows precise calculation of the amount that is delivered to the test organism. Therefore, the following should be considered about the test substance, which is typically either in a purified form or GE plant material expressing the protein of interest: biological activity of the test substance, purity of the test substance, test substance equivalence (e.g., for purified protein), test substance stability and homogeneity, method of delivery (e.g., artificial diets, treatment of non-GM food items, GM plant material or GE plant-fed herbivores) or test concentration selection (e.g., maximum hazard dose

(MHD), EEC, LOAEC or NOEC, LC_{50}, LD_{50}, EC_{50}, or $ED5_0$) (Rose 2007, Romeis et al. 2011).

Before testing, the objectives of the risk assessment and specific measurement endpoints (measures of response) need to be defined. Appropriate measurement endpoints should be easy to evaluate on risk assessment and likely to indicate the possibility of adverse effects on the assessment endpoints. Typical measurement endpoints are divided into ecotoxicological endpoints and biologiacal endpoints. Ecotoxicological endpoints are mortality (e.g., estimated as LD50) or developmental rate and biological endpoints include binary response which is recorded as response/no response (e.g., mortality or survival) and continuous response which is recorded as a measured response (e.g., development duration, body mass or behavior change) or as a count (e.g., fecundity or adult emergency) (Candolfi et al. 2000, Rose 2007, Stacey et al. 2006, Duan et al. 2002, 2006, 2008, Romeis et al. 2011). Determination of the measurement endpoint should consider the impact of the trait (e.g., *Bt* protein) on the target organisms and its mode of action (MOA), the biology of the selected NTAs and their life-stages, test duration and the availability of reliable test protocols (Romeis et al. 2011). Generally, laboratory tests (lower-tier) have a shorter duration than semi-field or field tests (higher tier) with higher protein doses/concentrations. The duration of a specific laboratory test depends on the measurement endpoints and the test duration must be sufficiently long enough to detect the response to adverse effects of test substance. Also, the test duration should allow for the test substance (e.g., MOA, test concentration or expression characteristic in plant), the selected test species and its lifestages, their development rate under the specific experimental conditions (incl. experimental set-up, environmental conditions) and the suitability of the test system (Rose 2007, Romeis et al. 2011).

Controls typically play a role as indicators of the suitability of the test system and for comparison to the data from the treatment of interest in an experiment. Using negative controls is able to assess the suitability (health) of test organism, the test conditions (e.g., temperature and diet) and the natural background effects on the measurement endpoints within the test system. Thus negative controls allow an assessment to be made of how the test system and test conditions are influencing on the measurement endpoints (e.g., mortality, development, and/or behavior of the test species) (EPA 1996, Rose 2007, Romeis et al. 2011). Using positive controls is able to confirm that the test organism is exposed to the test protein and test substance is actually ingested, assess the test system is able to detect treatment effects and compare to other results conducted previously, so it is useful for test protocol development and standardization (Duan et al. 2007, Rose 2007, Romeis et al. 2011). The test for risk assessment must be sensitive enough to detect treatment-related and eliminate effects of most common design problems. Suitable statistical methods and statistical powers should be a function of the test system and its inherent variability, experimental design, and the level of replication. Statistical approaches which are commonly used for non-target effects test as following,

Analysis of variance (ANOVA) is applied to determine whether or not differences in mean response among treatments are greater than expected by chance. A proportions test (e.g., z test) is applied to binary responses, such as mortality or number of affected individuals to determine if the proportion of individuals exhibiting a response is significantly different from some pre-determined. The last possible approach is the analysis of survivorship curves, though this is less often used because it requires substantially more effort to track the response of every individual (Candolfi et al. 2000, Rose 2007, Romeis et al. 2011).

1. 4. Objectives of this study

In chapter II, the potential impacts of *Bt* rice (Cry1Ac) for control of *C. medinalis* on development, survival, reproduction and Biological characteristics or growth parameter were evaluated against three different non-target herbivores, *N. lugens, O. japonica* and *S. lurida,* two predators *P. subpiraticus* and *P. clercki*, and one pollen feeder, *P. japonica,* and fecundity preference and survival of two different target species, *C. medinalis* and *N. aenescens* under laboratory conditions.

In chapter III, 2-year study was conducted to determine potential impacts of *Bt* rice on the spider and insect community in the rice field. We assessed the effects of *Bt* rice on biodiverty, guild structure and dominant families and species in insect and spider community.

In chapter IV, a tiered assessment system of *Bt* rice on non-target arthropods was suggested for Korean rice fields based on my laboratory and field study. For selection of non-target test species for the lower tier test, it should be considered expression sites of insecticidal protein in plant tissue, exposure possibility to insecticidal expression sites, inhabiting sites on rice plant, ecologically functional role, relatively importance on rice, easiness and control for tests and feeding mode of test species. For the field trial, the result of comparing the efficiency of two sampling methods, the influence of plot size in three different plot sizes and various sampling time and sampling occasions with arthropods diversity.

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Chapter 2.

Effects of *Bt* rice on arthropods in laboratory conditions

2.1. Abstract

The commercial release of rice genetically engineered to express a Cry1Ab/Ac protein from Bacillus thuringiensis (*Bt*) for control of Lepidoptera is a subject of debate. One major point of the debate has focused on the ecological safety of *Bt* rice on non-target organisms and selecting appropriate test organisms for assessment of *Bt* effects. Test non-target arthropods were selected by considering 1) ecological function such as herbivore, pollen-feeder and predator, 2) various taxonomic group such as Araneae, Coleoptera, Heteroptera, Lepidoptera and Othoptera, 3) their feeding or foraging behavior such as chewing, pierce sucking, hunting and web-building, and 4) the site and the time of protein expression in the *Bt* rice such as pollen and leaf of 20 day and 60 day old rice seedlings. Laboratory tests were conducted to evaluate the potential impacts of 1) *Bt* rice leaf expressing cry1Ac on development, survival, emergence and fitness of adult of non-target herbivore, *N. lugens, O.japonica* and *S. lurida*, 2) *Bt* rice pollen on development, survival, emergence and fitness of adult of non-target pollen-feeder, *P. japonica*, 3) prey fed on *Bt* rice leaf on development, survival, emergence and fitness of adult of non-target predator, *P. subpiraticus* and *P. clercki*, and 4) *Bt* rice leaf on development, survival and emergence of *N. aenescens* close to the target pest species, *C. medinalis*, and on oviposition preference of *C. medinalis*. There were no significant differences in development, survival and emergence of *O. japonica*, *S. lurida* and *N. lugens* between *Bt* and non- *Bt* rice. There no significant differences in development and survival of *P. subpiraticus* and *P. clercki* feeding on *N. lugens* reared on *Bt* or non-*Bt* rice although tibia length of *P. subpiraticus* and *P. clercki* with *N. lugens* feeding on *Bt* rice showed significantly longer than in non-*Bt* rice. There were no adverse effect on the development, survival and fitness of adult *P. japonica* after ingestion of *Bt* rice pollen expressing Cry1Ac

when compared with pollen from the corresponding non-transformed rice plant. *N. aenescens* showed significant differences in development, survival and emergence rate between *Bt* and non- *Bt* rice. *C. medinalis* did not show oviposition preference between *Bt* and non- *Bt* rice. Overall, the results indicated that the transgenic Cry1Ac rice lines tested in this study had no adverse effects on the development, survival, emergence and adult fitness parameter of non-target arthropods and oviposition preference of the target species in the laboratory conditions.

2.2. Introduction

Rice, *Oryza sativa* L., is the most important staple food for a large part of the world's human population with the third-highest worldwide production, after sugar cane and maize (FAO 2010). But critical yield losses have been documented by insect pests, especially stem borers, leaffolder and planthoppers (Sheng et al. 2002, Chen et al. 2003, Wang et al. 2010).

Since 1993, numerous transgenic rice lines carrying single genes derived from the soil bacterium *Bacillus thuringiensis* Berliner (*Bt*), namely cry1Aa (Breitler et al. 2004), cry1Ab (Ye et al. 2001), cry1Ac (Bashir et al. 2004), cry1B (Breitler et al. 2004), cry1C (Ye et al. 2009), cry1Ca1 (Zaidi et al. 2009), cry2A (Riaz et al. 2006) and cry9C (Chen et al. 2008), have been developed against lepidopteran rice pests. In Korea, *Bt*-rice line expressing cry1Ac1 for control of *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae), an important rice insect pest in Asia (Wada et al. 1980, Bautista et al. 1984, Dale 1994) was developed (Shin et al. 2009). However, *Bt* rice has not been approved for commercial release yet in the world and the potential adverse effects of *Bt* crops on non-target arthropods, as part of the environmental risk assessment process, should be carefully assessed before the decision is made to release novel *Bt* crops commercially (Romeis et al. 2008).

One major concern has been raised regarding the direct or indirect impacts that *Bt* crops may have on various groups of non-target organisms of ecological and economic value through crop-based food chains (Snow and Palma 1997, Schuler et al.1999, Poppy 2000, Wolfenbarger and Phifer 2000, Obrycki et al. 2001). Such concerns, however, have centered mainly on natural enemies, including predators and parasitoids, of target or non-target pests of *Bt* crops, and little on non-target herbivore such as planthoppers, leafhoppers, thrips, and aphids, many of which are important crop pests (Shieh et al. 1994, Lozzia et al. 1998, Riddick et al. 1998, Cui and Xia 2000, Ashouri et al. 2001, Reed et al. 2001). Prey-mediated effects of

Bt crops on higher trophic levels are well documented in the laboratory study (Hilbeck et al. 1998, Bernal et al. 2002, Dutton et al. 2002, Romeis et al. 2004, 2006, Lövei and Arpaia 2005, Hilbeck and Schmidt 2006, Torres and Ruberson 2006, 2008, Chen et al. 2009, Naranjo 2009) and in the field study (Orr and Landis 1997, Pilcher et al. 1997, Wold et al. 2001, Bourguet et al. 2002, Hassell and Shepard 2002, Musser and Shelton 2003, Jasinski et al. 2003, Pons and Stary 2003, Candolfi et al. 2004, Toth et al. 2004, Meissle and Lang 2005, Pons et al. 2005, Li et al. 2008, Farinós et al. 2008, Han et al. 2011). Reduction of insecticide application due to *Bt* crop and high host quality with absence of target species may increase other non-target herbivores in the fields. Thus more chances may occur that natural enemies are exposed to prey taken up *Bt* toxin. Besides, some studies conducted with *Bt* rice reported that *Bt* protein could be taken up by herbivore that fed on transgenic *Bt* rice and transfer to its natural enemy (Bernal et al. 2002, Chen et al. 2005, 2007, Bai et al. 2006).

Previous studies related to effects of *Bt* rice on nontarget herbivores have been focused on several piercing-sucking species including the planthoppers, *N. lugens* (Stål), *Sogatella furcifera* (Horvath), and *Laodelphax striatella* (Falle´n) (Homoptera: Delphacidae), and the leafhoppers *Nephotettix cincticeps* (Uhler) and *N. virescens* (Distant) (Hemiptera: Cicadellidae) because Bt proteins may be ingested by these nontarget insects and transported to their natural enemies through tritrophic interactions (Chen et al. 2005, 2006, 2007, 2009, Bai et al. 2006). No adverse effects on the fitness and population densities of the planthoppers and leafhoppers were observed under laboratory and field conditions in previous studies (Bernal et al. 2002, Liu et al. 2002, 2007, Chen et al. 2003, 2004, 2006, Fu et al. 2003, Bai et al. 2006, Zhou et al. 2006, Tan et al. 2006), except one case in which *N. cincticeps* actually performed better on *Bt* rice KMD1 and KMD2 under laboratory and field conditions (Zhou et al. 2005). None of the previous studies have paid attention to the effects

of *Bt* rice on various non-target herbivore considering the mode of feeding and the part of protein expression in the plant.

In this study, the potential impacts of *Bt* rice on development, survival, reproduction and adult characters were evaluated for three different non-target herbivores, two predators and one pollen feeder and fecundity preference and survival of two different target species under laboratory conditions.

2. 3. Material and Methods

Plant materials

Transgenic *Bt*-rice line with a synthetic cry1Ac gene, C7-1-9-1 was used with its non-*Bt* isoline japonica rice cultivar Nakdong. C7-1-9-1 line was developed to express insecticidal action derived from *B. thuringiensis* to control *C. medinalis*.

Selection of arthropod

Arthropod species for laboratory tests were selected considering expression sites of insecticidal protein in plant tissue, exposure possibility to insecticidal expression sites, inhabiting sites on rice plant, ecologically functional role, relative importance on rice, and easiness and control for tests. From the considered factors, 8 test species were selected; 1) *C. medinalis* (Lepidoptera: Crambidae) and *Naranga aenescens* (Lepidoptera: Noctuidae) for target pests, 2) *Scotinophara lurida* (Hemiptera: Pentatomidae), *Oxya japonica* (Orthoptera: Acrididae), and *Nilaparvata lugens* (Hemiptera: Delphacidae) for non-target pests, 3) *Propylea japonica* (Coleoptera: Coccinellidae) for non-target pollen feeder, 4) *Pirata subpiraticus* (Araneae: Lycosidae), *Pachygnatha clercki* (Araneae: Tetragnathidae) for natural enemies, predator. *P. japonica* identified as insect pests and predators generally, since they feed rice pollen and nymphs of leafhoppers and planthoppers (Figure 1).

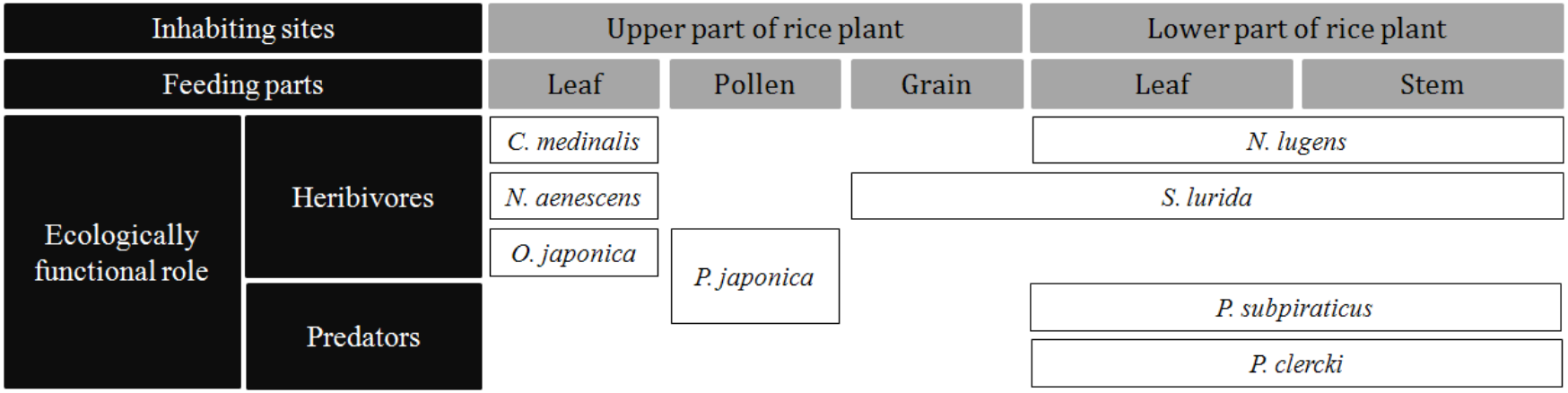

Figure 1. Selection standard of test species

Laboratory experiment conditions

All the laboratory experiments were conducted in the incubator at 25°C±1°C, 60%±10% RH, and a photoperiod of 16:8 (L:D) h.

Fecudity and oviposition preferrence of C. medinalis

Pupae of *C. medinalis* were obtained from the National Academy of Agricultural Science. Fifty newly emerged female *C. medinalis* per a replication were allowed to mate with seventy newly emerged male *C. medinalis* for 24 hours, and then placed in a transparent acrylic cage (50 cm long×50 cm wide×50 cm high) without light. Six sugar-water cotton balls (sugar:water = 1:1) were hanged in the cage for diet. *Bt* rice leaves, non-*Bt* rice leaves and floral foams were tested for oviposition preferance of *C. medinalis*. They were randomly placed in the cage at regular intervals. Sixty day old rice seedlings were supplied for oviposition place and its size was 1.2-1.5 cm wide and 30 cm long. Floral foams were supplied with the same dimension of rice plants. Rice plants and floral foams were replaced every 12 hours. Then, eggs laid on these test materials were counted. The oviposition test was conducted for 72 hours, and was replicated 3 times.

Larval mortality and emergence rate of *N. aenescens*

Adult *N. aenescens* were collected from rice fields in Gimpo, Gyeonggi-do. They were brought to the laboratory and allowed to lay eggs. Newly hatched larvae were transferred to acryl cages (5 cm diameter, 1.5 cm high). On the bottom of cages, filter paper (size??) and water-saturated cotton ball were placed. Before the test, larvae of different ages were reared individually with 30 day old rice seedlings (Chucheong) and were checked every 12 hours due to 100% mortality of test species fed on *Bt* rice. Cutted leaves of 30 day rice

seedling (3 cm long, 1 cm wide) and 60 day rice seedling (5 cm long, 1 cm wide) of *Bt* and non-*Bt* rice were supplied for 1st to 3rd instars and for 4th to 5th instars, respectively. Supplied leaves were wrapped with moistened cottons on either side to prevent withering of the rice plant. Mortality and feeding were checked for the first 48 hours after inoculation. Survival and development were checked every 24 hours with replacing rice plants. Molting was determined by exuviae of head capsule. Tests were conducted with 20 individuals with 5 replications for 1st and 2nd instars, 20 individuals with 3 replications for 3rd instar, 20 individuals with 4 replications for 4th and 5th instars, respectively.

Growth of *O. japonica*

Adult *O. japonica* were collected from rice fields in Icheon, Gyeonggi-do. They were brought to the laboratory and eggs were obtained from them. Eggs were stored in the incubator at 4°C for 50 days for diapause termination. Stored eggs were placed under incubator condition and increased 1 °C every two days to reach 25 °C for hatching. Then newly hatched nymphs (1st instars) were placed into acrylic cages (3 cm diameter, 20 cm high) with water-saturated sponge at the bottom for water supply. The top of the cage was blocked with the sponge cap. Thirty day old rice seedlings for 1st instars and 60 day old rice seedlings for 2nd to 6th instars of *Bt* and non-*Bt* rice were supplied, respectively. Plant materials were replaced every 48 hours for 1st to 3rd instars and 24 hours for 4th to 6th instars, respectively. Survival and development were checked every 24 hours and tests were conducted with 20 individuals with 3 replications. Body length, dry weight (dried 72 hours in the drier) and head width index were measured after adult emergence. Head width index was calculated as head height (length)/head width.

Growth of *S. lurida*

Adult *S. lurida* were collected from rice fields in Hongseong, Chungcheongnam-do. They were brought to the laboratory and eggs were obtained from them. Newly hatched larvae were transferred to acrylic cages (5 cm diameter, 1.5 cm high). Tests were initiated with the 2nd instar nymphs because the mortality of 1st instar nymphs was too high. The 2nd instar nymphs were placed into acrylic cages (3 cm diameter, 20 cm high) with moistened sponge at bottom for water supply. The top of cages were blocked with sponge cap. Sixty day old rice seedlings of *Bt* and non-*Bt* rice were supplied and plant materials were replaced every 48 hours. Survival and development were checked every 24 hours and tests were conducted with 20 individuals with 5 replications. Body length, dry weight (dried 72 hours in the drier) and head width index were measured after adult emergence.

Growth of *N. lugens*

Adult *N. lugens* were obtained from the National Academy of Agricultural Science. *N. lugens* were reared on 15 day old rice seedlings (Chucheong) in acrylic cages (3 cm diameter, 9 cm high) and were checked every 12 hours. Then newly hatched nymphs (1st instars) were collected and used in the experiments. Experiments were conducted on the 20 day old and 60 day old rice seedlings of *Bt* rice and non-*Bt* rice. Plant materials were replaced every 48 hours. Two types of transparent insect breeding acrylic cages with moistened sponge at bottom for water supply and sponge cap to prevent escaping were used; 9 cm in diameter with 9 cm high cages in 20 day rice seedlings test and 3 cm in diameter with 20 cm high ones in 60 day rice seedlings test, respectively. Survival and development were checked every 24 hours and tests were conducted with 20 individuals with 3 replications.

Growth of *P. japonica*

Eggs of *P. japonica* were obtained from the National Institute of Horticultural and Herbal Science. Eggs were transferred to acrylic cages (5 cm in diameter with 1.5 cm high) and newly hatched nymphs (1st instars) with every 12 hours check were used in the experiments. The bottom of the insect breeding acrylic cages was laid with filter paper and moistened cotton ball for water supply. Three types of preys were supplied; *Bt* rice pollen with *Aphis gossypii*, non-*Bt* rice pollen with *A. gossypii*, and *A. gossypii* only. *Bt* and non-*Bt* rice pollen were respectively collected in the field by small sweep net (18 cm in diameter). The pollens were collected and sieved through a screen of 60-mesh (250 ㎛) size, and frozen at -20^{o}C until use. Adults of *A. gossypii* obtained from National Institute of Horticultural and Herbal Science and mass reared in transparent insect breeding acrylic cage (40 × 40 cm, 40 cm in height) supplied seedlings of *Glycine max*. The pollens were thawed for 1 hour before use and grinded finely with a pestle. The pollens were supplied solely in the first 16 hours just after molting and 5 aphids were added with leaf of *G. max* (3 cm long and 3 cm wide) to next molting. Prey materials were renewed every 8 hours. Survival rate, mortality and development were checked every 8 hours and tests were conducted with 20 individuals per 3 replications. Dry weight (dried 72 hours in the drier) was measured after adult emergence.

Growth of *P. subpiraticus*

Adults of *P. subpiraticus* were collected from local fields in Suwon, Gyeonggi-do. Collected spiders were reared in Chucheong rice planted and *N. lugens* inoculated rectangular rice pot (50 × 40 cm, 30 cm in height). Females with eggsac were transferred

to petri dishes (10 cm in diameter with 4 cm high) individually. Tests were initiated with dispersed 2nd spiderlings emerging from eggsac because emerged spiderlings from the eggsac have molted once in the eggsac. Transparent insect breeding acrylic cages (3 cm in diameter with 9 cm high) with sponge plate at an angle of 45^{o} at bottom and water was supplied in 1 cm deep below the sponge plate, and sponge cap to prevent escaping were used. Ten individuals of *N. lugens* fed *Bt* and non-*Bt* rice seedlings between 15 and 20 day old were supplied every 48 hours. Survival rate, mortality and development were checked every 24 hours and tests were conducted with 20 individuals per 3 replications. Dry weight (dried 72 hours in the drier), tibia length and carapace index were measured after adult emergence. Carapace index was calculated as carapace width / carapace length.

Growth of *P. clercki*

Experimental mothods were same as those for *P. subpiraticus*.

Data analysis

One-way ANOVA (Proc GLM) in SAS 9.2 (SAS Institute 2004) was used to analyze the effect of "*Bt* status" (i.e. *Bt*-rice and non-*Bt* rice) on the fecundity, developmental period, survival rate, emergence and fitness parameters (body length, dry weight, head width index, tibia length and carapace index. Mean separation was conducted with the Tukey HSD test. Survival and adult emergence rate were arcsine transformed before analysis.

2.4. Results

2.4.1 Effects of *Bt* rice on target herbivore

2.4.1.1 Effects of *Bt* rice on oviposition preference of *Cnaphalocrocis medinalis*

The number of eggs of *C. medinalis* in the back-side of rice leaves were a little higher than the front-side, but there was no significant difference in oviposition preference *C. medinalis* between *Bt* rice and non-*Bt* rice. ($F_{1,4}$=0.00, P=0.9735) (Fig. 2A). The number of eggs of *C. medinalis* was higher in *Bt* and non-*Bt* rice than in floral foam, no difference was found between *Bt* and non-*Bt* rice ($F_{2,6}$= 3.44, P=0.1013). Oviposition of *C. medinalis* fluctuated over the time, was highest in both *Bt* rice and non-*Bt* rice 12 hours after exposure, and there was no significant between *Bt* rice and non-*Bt* rice at each time (Fig. 2B).

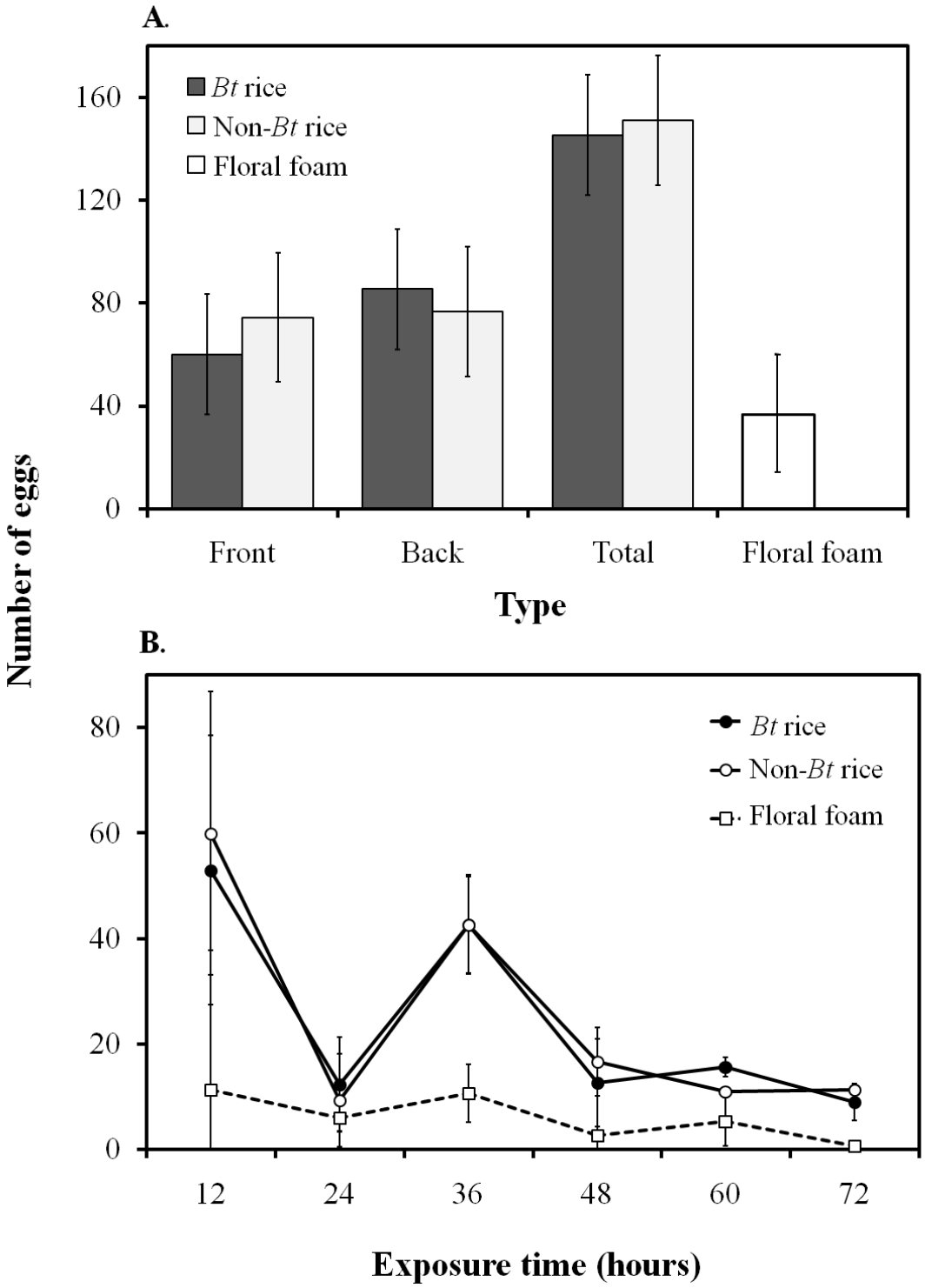

Figure 2. Fecundity of *C. medinalis* between *Bt* rice, non-*Bt* rice and floral foam (Total number of eggs for 72 hrs, B. Egg numbers according to time, replication = 3)

2.4.1.2 Effects of *Bt* rice leaf on growth of *Naranga aenescens*

All of the 1st and 2nd instar larvae of *N. aenescens* were dead within 48 hours and the 3rd instar larvae were dead within 60 hours in *Bt* rice (Table 1). Mortality of the 4th instar larvae was sharply increased between 48 hours and 120 hours in *Bt* rice and reached to 100% at 240 hours. On the other hand, mortality of the 4th instar larvae fed non-*Bt* rice was 58.5% at 240 hours (Fig. 3). Mortality of the 5th instar larvae was sharply increased after exposure of *Bt* rice and reached to 100% at 192 hours. On the other hand, survival rate of the 5th instar larvae fed on non-*Bt* rice was 89.8% at 192 hours which was the time 100% mortality of larvae fed on *Bt* rice. Pupation of the 5th instar larvae fed on non-*Bt* rice started at 120 hours and was 62.5% at 192 hours (Fig. 3)

Table 1. Survival rate (mean±SE) of *N. aenescens* feeding off *Bt* rice and non-*Bt* rice (n=20, replication=5)

Larval stage	*Bt* rice		Non-*Bt* rice	
	at 48 hours	at 60 hours	at 48 hours	at 60 hours
1st instar	0.00±0.00*	-	0.82±0.06	-
2nd instar	0.00±0.00*	-	0.79±0.05	-
3rd instar	0.18±0.07*	0.00±0.00*	0.97±0.02	0.92±0.02

* Significantly different (one-way Anova, $P<0.05$)

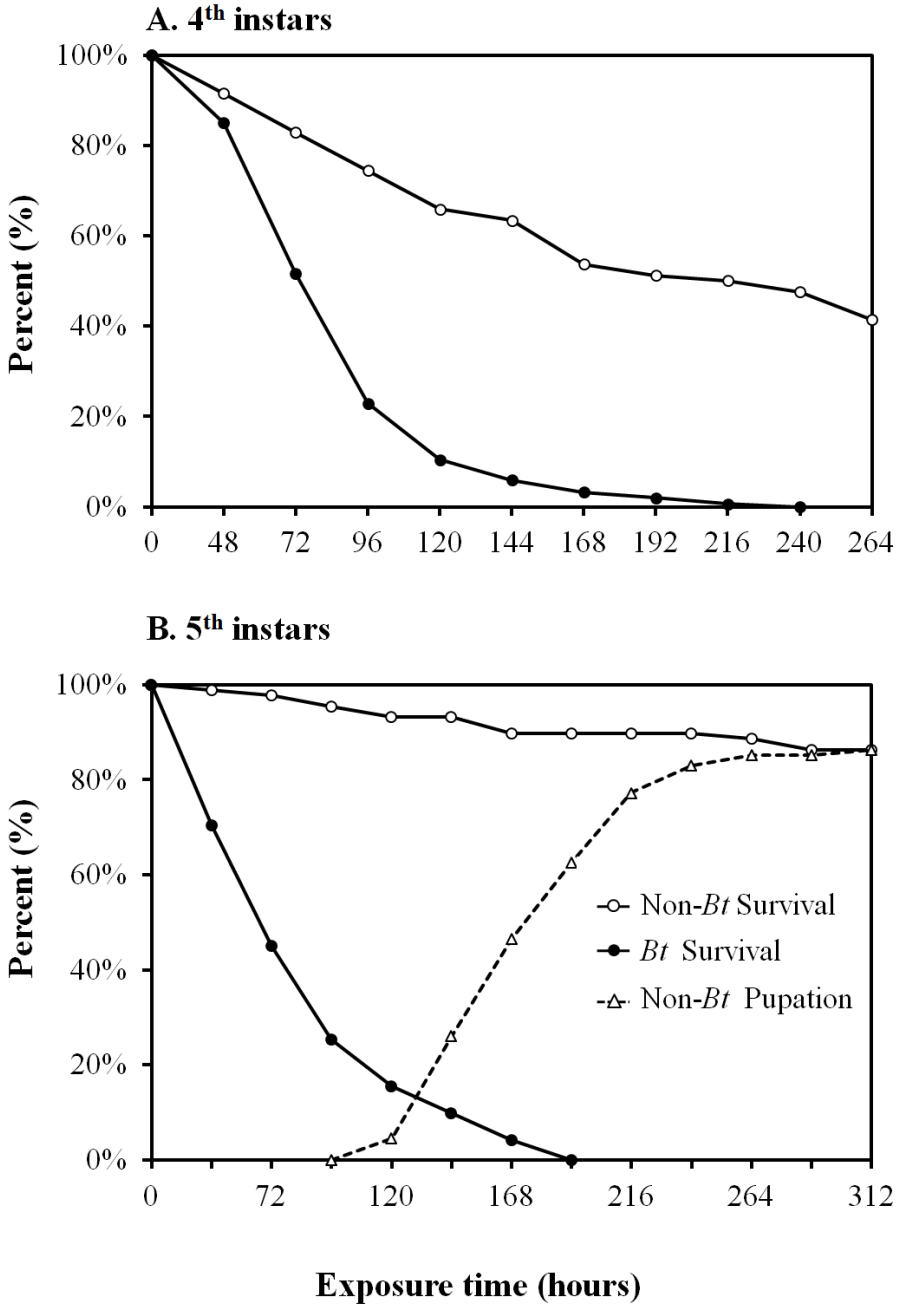

Figure 3. Survival and pupation rates of 4th and 5th instar larvae of *N. aenescens* feeding on *Bt* rice and non-*Bt* rice (n=80)

2.4.2 Effects of *Bt* rice on non-target herbivores

2.4.2.1 Effects of *Bt* rice leaf on growth of *Oxya japonica*

O. japonica is a rice pest which feeds on rice leaf and stem, inhabiting at the middle to upper part of rice plant. The total nymphal developmental period (mean±SE) of *O. japonica* was 58.1±1.5 days in non-*Bt* rice and 61.3±1.1 days in *Bt* rice and was not significantly different ($F_{1,6}$=3.04, P=0.1316). Developmental period of the 5th instar nymph was significantly longer in *Bt* rice than in non-*B*t rice rice ($F_{1,6}$=6.02, P=0.0495), but that of the 2nd instar was significantly longer in non-*Bt* rice than in *Bt* rice ($F_{1,6}$=13.07, P=0.0110) (Fig. 4).

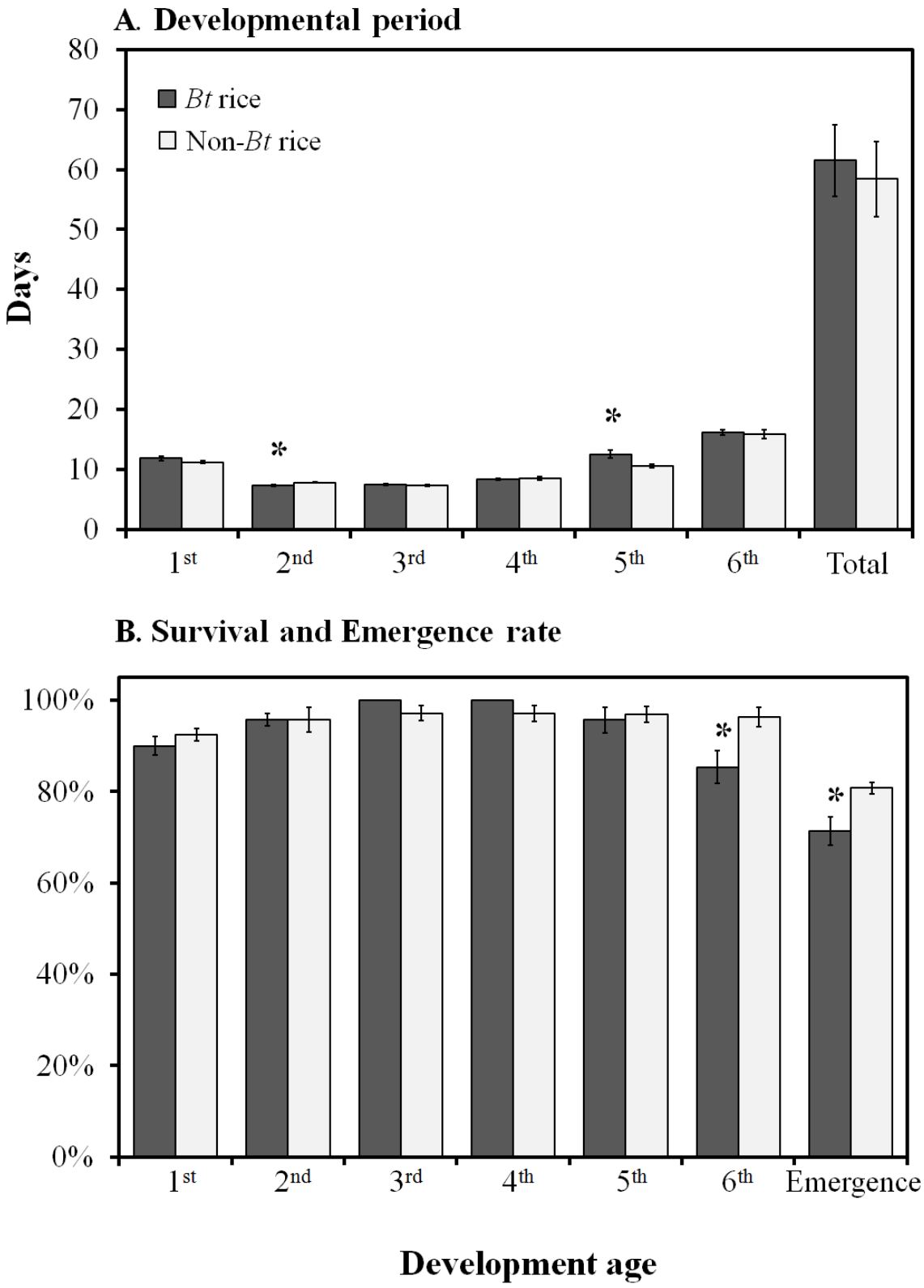

Figure 4. Developmental period (mean±SE) and survival and emergence rate (mean±SE) of *O. japonica* reared on *Bt* and non-*Bt* rice (*significantly different: One-way Anova, P<0.05, n=20, replication=4)

Survival rate of *O. japonica* was not significantly different between *Bt* and non-*Bt* rice (RM-ANOVA; $F_{1,6}$, P=0.4077), whereas that of 6th instar was significantly higher in non-*Bt* than *Bt* rice (One-way ANOVA; $F_{1,6}$=7.01, P=0.0382). Emergence of *O. japonica* began at 44 days and rapidly increased at 54 days to 74 days. *O. japonica* reared on non-*Bt* rice had shorter emergence duration than that of reared on *Bt* rice and its emergence rate was significantly higher in non-*Bt* rice than in *Bt* rice ($F_{1,6}$=8.15, P=0.0290, Fig. 5).

In terms of fitness parameter, female ratio and head width index of *O. japonica* adult were not significantly different between *Bt* rice and non- *Bt* rice (Table 2).

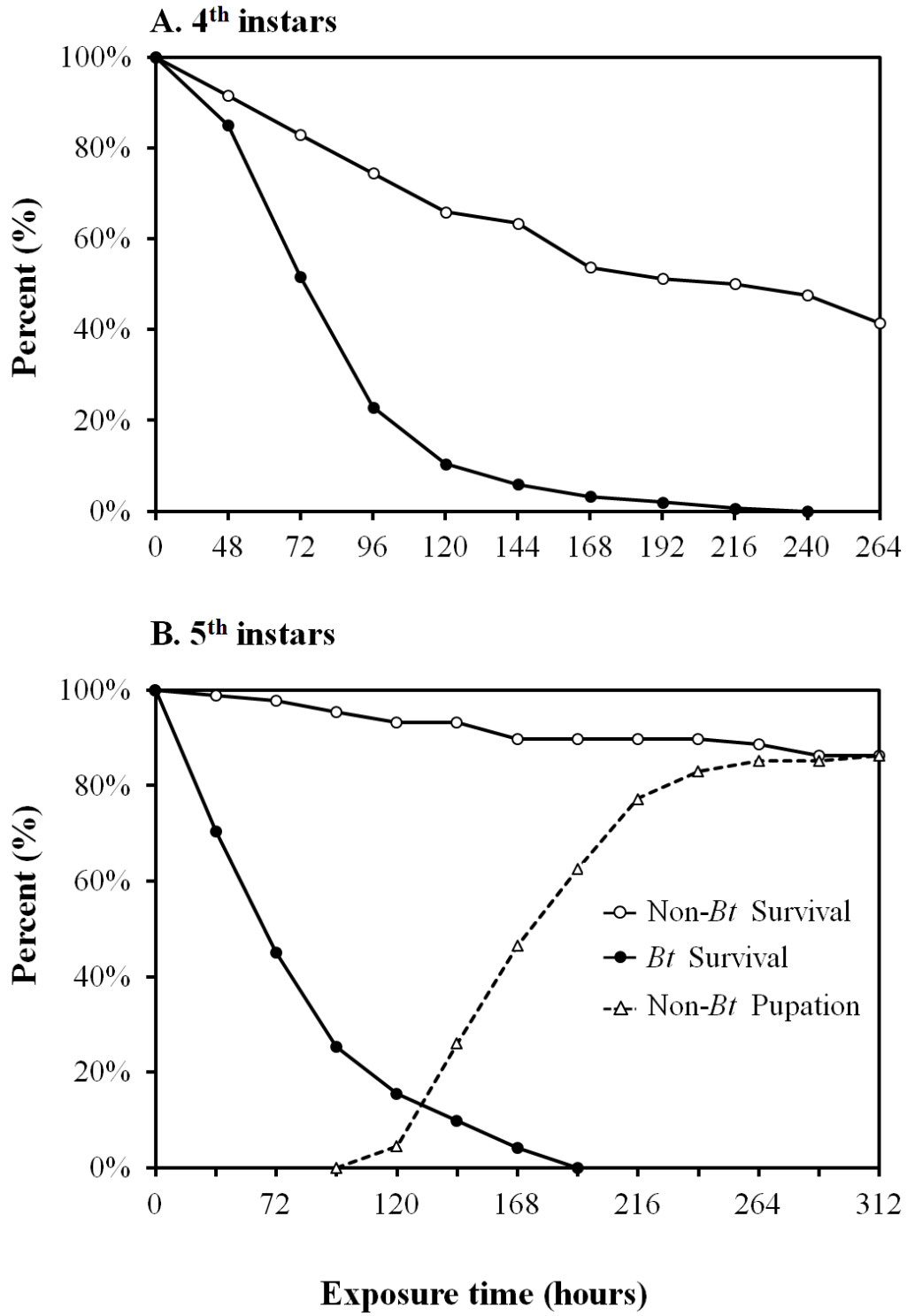

Figure 5. Survival and emergence curves of *O. japonica* reared on *Bt* and Non-*Bt* rice (n=80)

Table 2. Fitness parameters (mean±SD) of *O. japonica* adult reared on *Bt* and Non-*Bt* rice (replication=4)

Fitness parameter	Treatment		One-way ANOVA		
	Bt rice	Non- *Bt* rice	*df*	*F*	*p*
Sex ratio (female/total; %)	55.84±6.18	62.81±5.82	1	0.68	0.4396
Dry weight (mg)	80.79±21.22	79.22±16.11	1	0.04	0.8425
Head width index	1.11±0.05	1.17±0.04	1	0.01	0.9040
Body length (mm)	27.77±2.04	27.31±2.33	1	0.25	0.6208

2.4.2.2 Effects of *Bt* rice leaf on growth of *Scotinophara lurida*

S. lurida is one of the most serious rice pests which feed off leaf, stem and grain of rice plant inhabiting at the lower to upper part of rice plant. First instar of *S. lurida* become the 2nd instar feeding off their own eggshells after hatching in general without any plants, so the experiment was started with the 2nd instar. Developmental period of *S. lurida* from the 2nd instar to adult were 97.6±6.1 days in *Bt* rice and 86.5±6.6 days in non-*Bt* rice and was not significantly different between *Bt* rice and non- *Bt* rice (One-way ANOVA: $F_{1,8} = 2.26$, $P = 0.1767$). Nymphal duration of 4th instars was significantly longer in *Bt* rice than that in non-*Bt* rice (One-way ANOVA: $F_{1,8} = 5.86$, $P = 0.0418$; Fig. 6A).

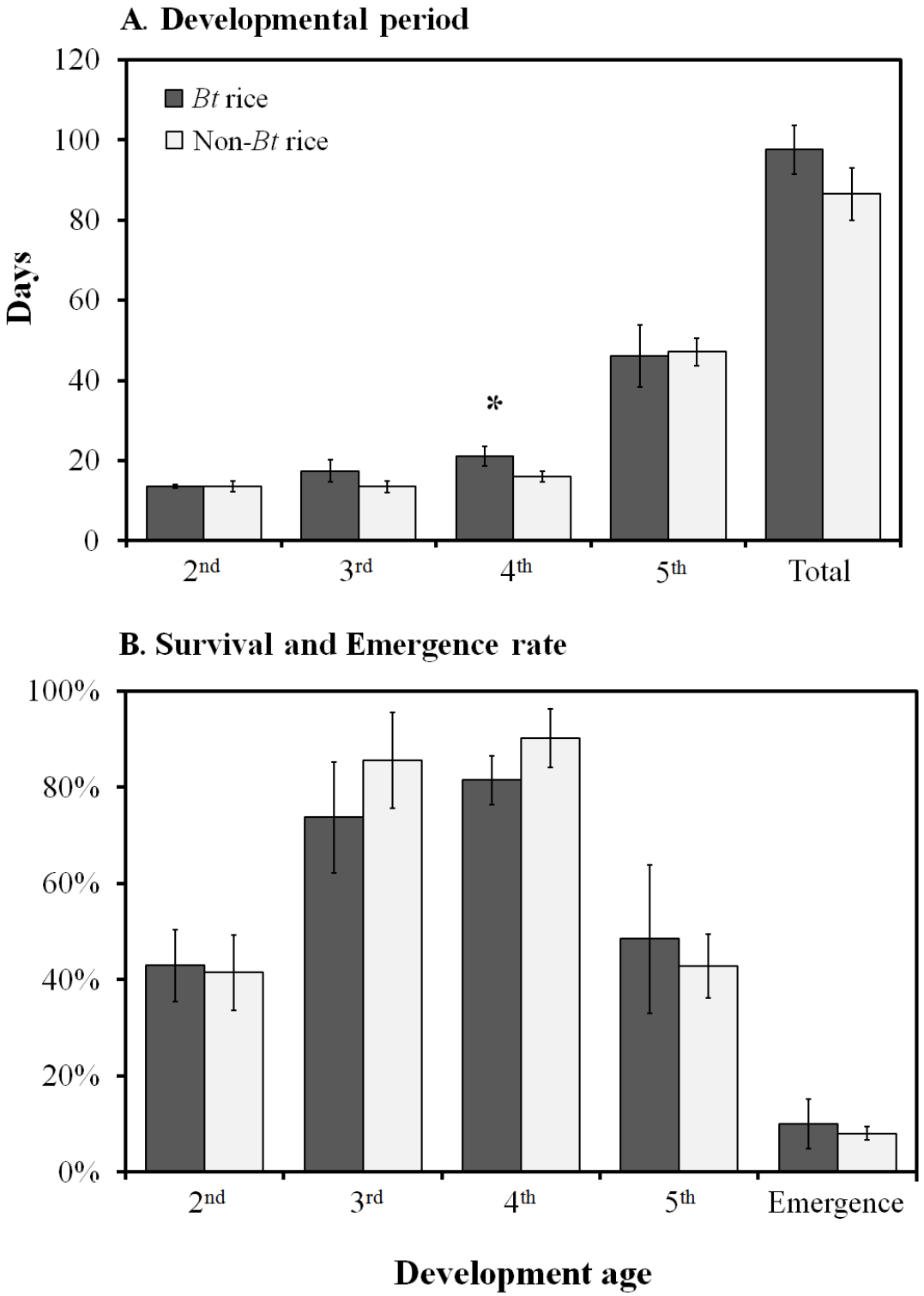

Figure 6. Developmental period (mean±SE) and survival and emergence rate (mean±SE) of *S. lurida* reared on *Bt* and Non-*Bt* rice (*significantly different: One-way Anova, P<0.05, replication=5)

Survival rate of *S. lurida* decreased in the 2nd instar after molting, nymphal survival was lowest at the 2nd instar and highest at the 4th instar in both *Bt* rice and non- *Bt* rice (Fig 6B). Survival curve of *S. lurida* showed similar pattern and was not significantly different between *Bt* rice and non-*Bt* rice (RM ANOVA: F=0.91, df=1, P=0.3669). Emergence of *S. lurida* started earlier in non-*Bt* rice, but the emergence rate was higher in *Bt* rice than non-*Bt* rice, however, there were no significant differences between *Bt* rice and non- *Bt* rice (One-way ANOVA: F=0.02, df=1, P=0.8845) (Fig. 7).

In terms of fitness parameter, dry weight, head width index and body length of *S. lurida* adult were higher in *Bt* rice, and the body length of *S. lurida* adult was significantly higher in *Bt* rice than non- *Bt* rice (One-way ANOVA: F=7.92, df=1, P=0.031) (Table 3).

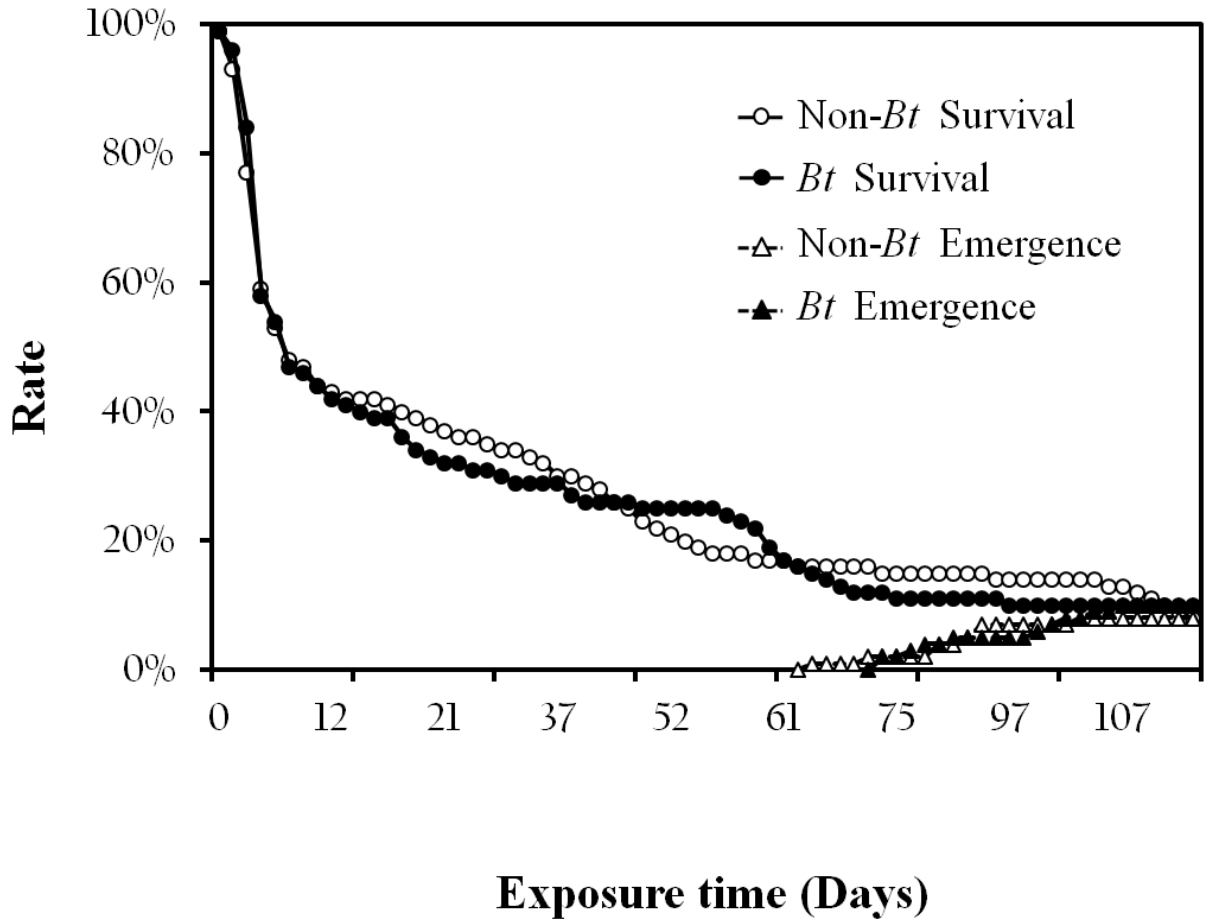

Figure 7. Survival and emergence curves of *S. lurida* reared on *Bt* and Non-*Bt* rice (n=100)

Table 3. Fitness parameters (Mean±SD) of *S. lurida* adult reared on *Bt* and Non-*Bt* rice (replication=3)

Fitness parameter	Treatment		One-way ANOVA		
	Bt rice	Non- *Bt* rice	*df*	*F*	*p*
Dry weight (mg)	5.88±1.36	4.97±0.93	1	2.33	0.1475
Head width index	1.91±0.11	1.64±0.17	1	0.83	0.3776
Body length (mm) *	8.15±1.65	7.91±1.77	1	7.92	0.0131
Body width (mm)	4.14±0.03	4.12±0.04	1	0.12	0.3708

* significantly different (One-way ANOVA, P<0.05)

2.4.2.3 Effects of *Bt* rice leaf on Growth of *Nilaparvata lugens*

N. lugens is one of the most serious rice pests which feed leaf and stem of rice plant inhabiting at lower part of rice plant, ca. 20 cm above water surface. Developmental period of *N. lugens* from the 1st instar to adult supplied with 20 day seedlings was longer in non-*B*t rice (15.5±0.9 days) than that in *Bt* rice (15.2±1.4 days), and was significantly different between *Bt* rice and non-*Bt* rice (One-way ANOVA: $F_{1.10}$=689.40, P<.0001). Developmental period of *N. lugens* from the 1st instar to adult supplied with 60 day seedlings was 17.1±1.3 days in non-*B*t rice and 17.1±1.6 days in *Bt* rice, and there was no significant different between *Bt* rice and non-*Bt* rice (One-way ANOVA: $F_{1.4}$=0.00, P=0.9873). Developmental period of *N. lugens* from the 1st instar to adult with 60 day seedlings was significantly longer than that with 20 day seedlings in both *Bt* rice and non-*Bt* rice (One-way ANOVA: $F_{1.4}$=50.93, P<0.0001, Fig. 8A). Nymphal duration of each instar were longer than in *Bt* rice except the 3rd instar supplied with 60 day *Bt* rice seedling and the 5th instar supplied with 20 and 60 days, and that of the 5th instar in 60 day seedling of *Bt* rice was significantly longer than non-*Bt* rice seedling (One-way ANOVA: $F_{1.4}$=5.01, P=0.0274). (Fig. 8A).

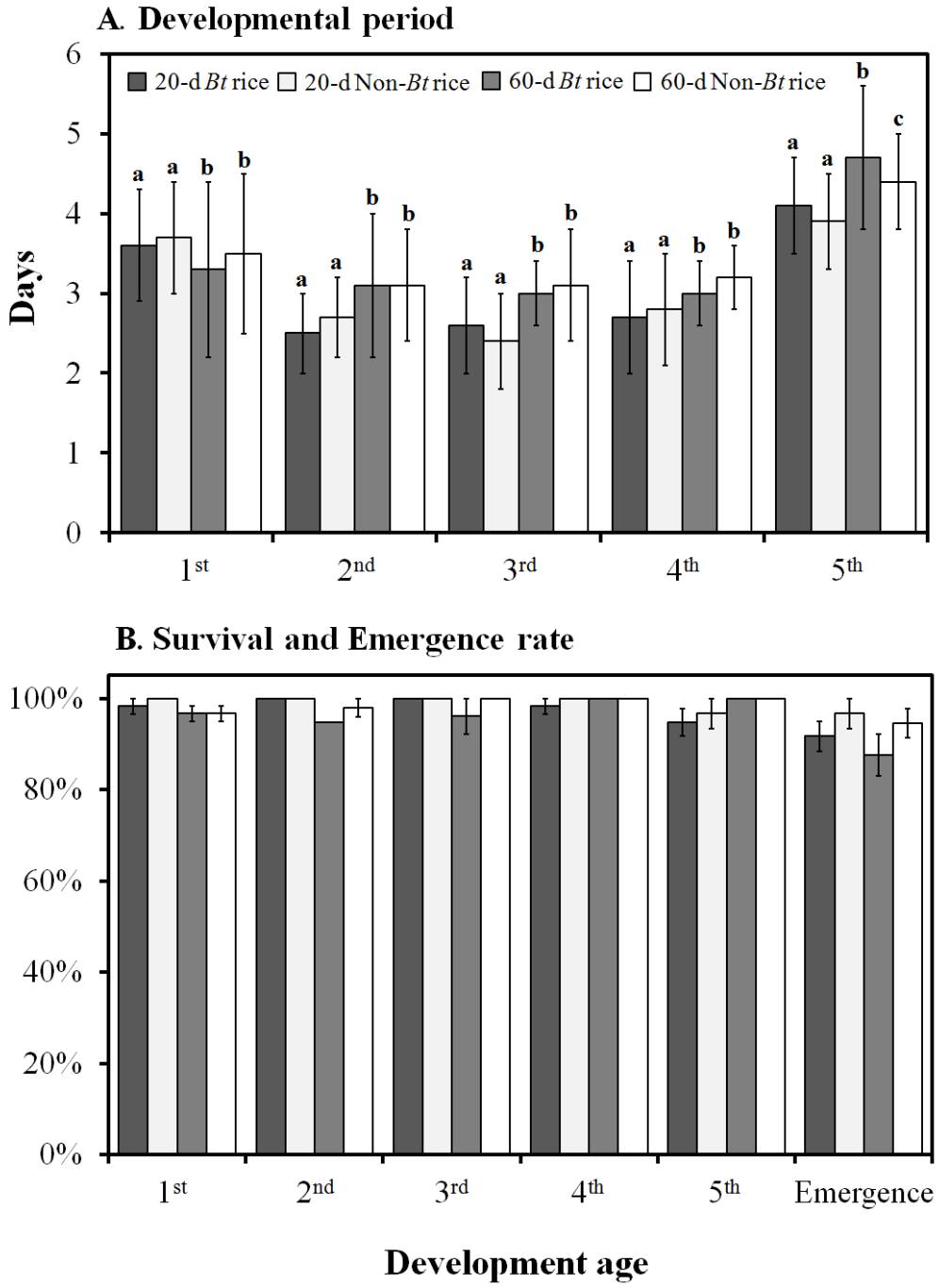

Figure 8. Developmental period (Mean±SE) and survival and emergence rate (Mean±SE) of *N. lugens* reared on *Bt* and Non-*Bt* rice (*significantly different: One-way Anova, Tukey HSD test, P<0.05, replication=3)

Survival rate of *N. lugens* was higher in 20 day seedlings than that in 60 day seedlings, but there were no significant differences between 20 day seedlings and 60 day seedlings (RM-ANOVA: $F_{1.10}$=0.99, P=0.3424). Survival curve of *N. lugens* showed similar pattern and was not significantly different between *Bt* rice and non-*Bt* rice different (RM-ANOVA: 20 day seedlings: $F_{1.4}$=2.27, P=0.2066, 60 day seedlings: $F_{1.4}$=1.49, P=0.2896). Emergence of *N. lugens* began earlier in 20 day seedlings than 60 day seedlings and *Bt* rice than non-*Bt* rice (Fig. 8B). Emergence rate of *N. lugens* were 96.7±3.3 (non-*Bt* rice) and 91.7±3.3 % (*Bt* rice) in 20 day seedlings and 94.6±3.2 (non-*Bt* rice) and 87.6±4.3 % (*Bt* rice) in 60 day seedlings rice. Emergence rate of *N. lugens* was higher in non-*Bt* rice than *Bt* rice, but that were not significantly different between *Bt* rice and non-*Bt* rice (One-way ANOVA: 20 day seedlings: $F_{1.4}$=2.09, P=0.222, 60 day seedlings: $F_{1.4}$=1.73, P=0.2590) (Fig. 9).

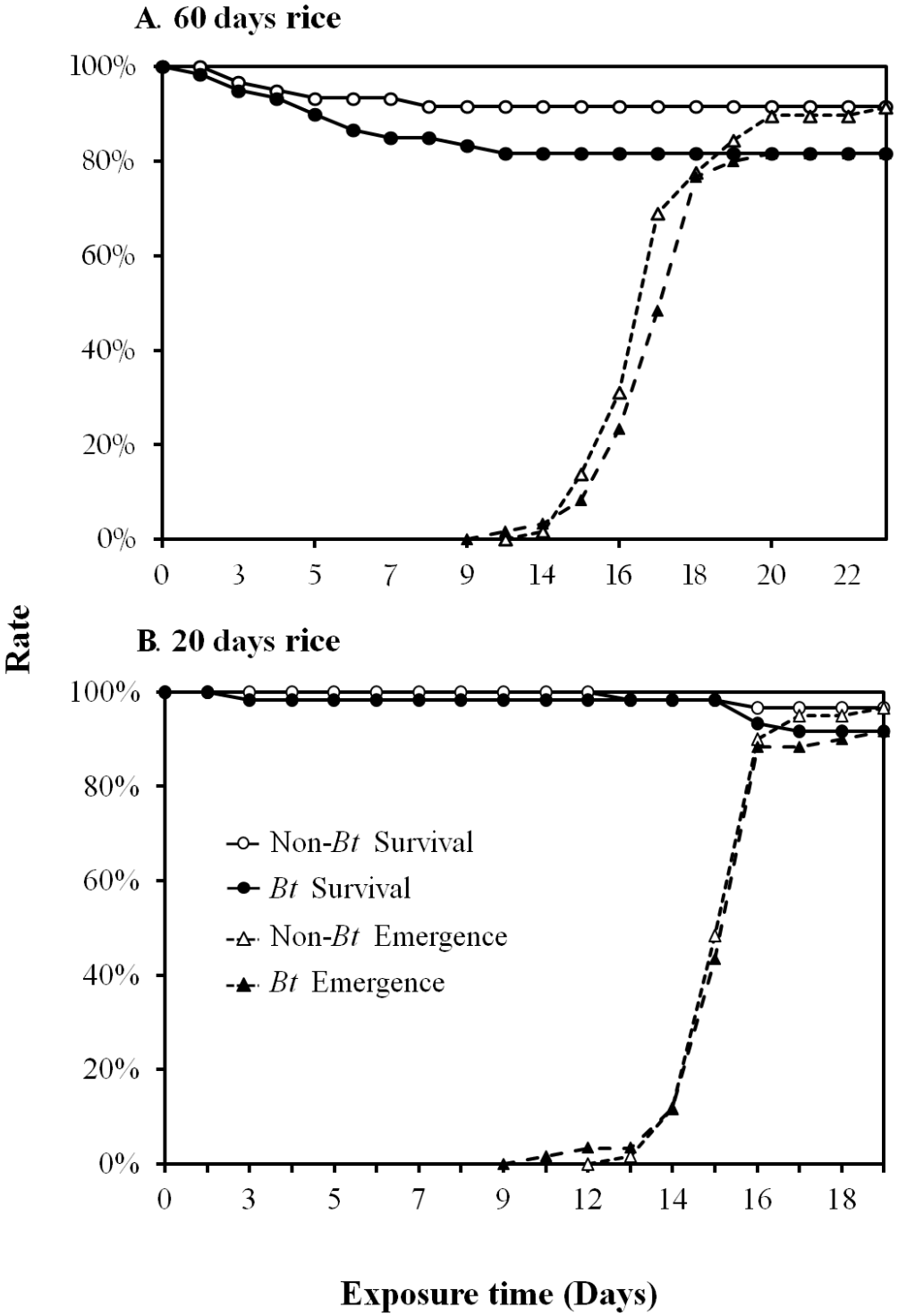

Figure 9. Survival and emergence curves of *N. lugens* reared on *Bt* and Non-*Bt* rice (n=60)

2.4.3 Effects of *Bt* rice on non-target pollen feeder

2.4.3.1 Effects of *Bt* rice pollen on Growth of *Propylea japonica*

P. japonica is one of herbivores which feed rice pollens and predators which feed planthoppers and aphids inhabiting at the upper part of rice plant. Larval duration of *P. japonica* supplied with *Bt* rice pollen and *Aphis gossypii*, non-*Bt* rice pollen and *A. gossypii* and *A. gossypii* only were 16.4±0.7 days, 14.0±0.6 days and 13.7±0.9 days, respectively. There was no significant difference among the treatments (One-way ANOVA: $F_{2.6}$=4.09, P=0.0758) except that of 1st and 2nd larva (1st: $F_{2.6}$=82.15, P<0.0001, 2nd: $F_{2.6}$=81.42, P<0.0001). Larval duration of the 2nd larva was shortest and that of the 4th larva was longest in three treatments (Fig. 10A).

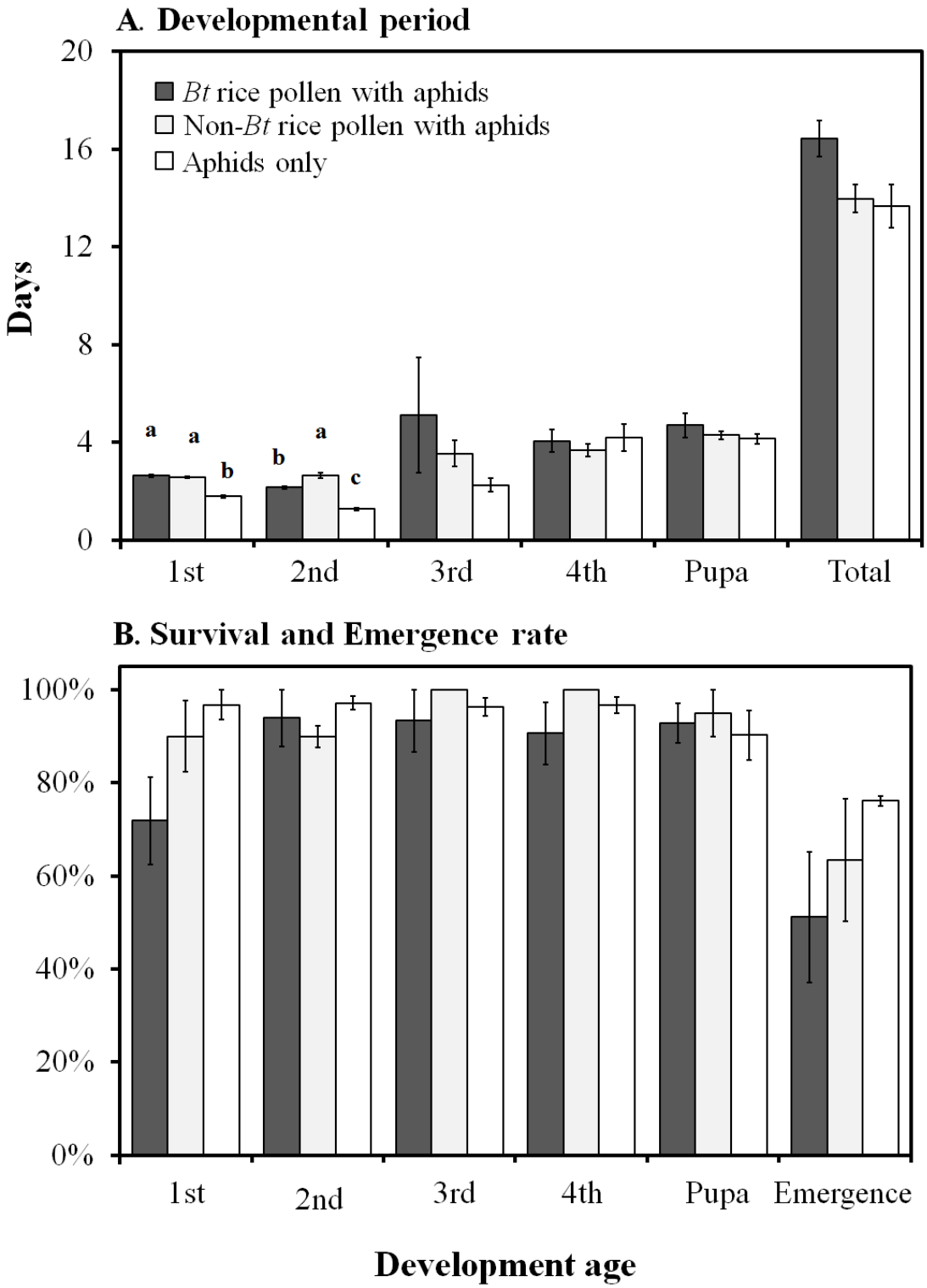

Figure 10. Developmental period (Mean±SE) and survival and emergence rate (Mean±SE) of *P. japonica* supplied with *Bt* and Non-*Bt* rice pollen with and *A. gossypii* only (*significantly different: One-way Anova, Tukey HSD test, P<0.05, replication=3)

Larval mortality of *P. japonica* supplied with rice pollens and *A. gossypii* was highest at the 1st larva in both *Bt* rice and non-*Bt* rice and at the 4th larva with *A. gossypii* only. Larval mortality of *P. japonica* was not significantly different (One-way ANOVA: $F_{2.6}$=2.47, *P*=0.1650, Fig. 10B). Survival curve of *P. japonica* supplied with rice pollens and *A. gossypii* decreased rapidly within 3days in both *Bt* rice and non-*Bt* rice and that with *A. gossypii* only decreased after 4 days (Fig. 11). Emergence rate of *P. japonica* supplied with *A. gossypii* only was highest and significantly fastest among them (One-way ANOVA: $F_{2.6}$=15.08, *P*<0.0001). Emergence rate of *P. japonica* supplied with *Bt* rice pollens and *A. gossypii* was faster than that with non-*Bt* rice pollens and *A. gossypii*, whereas emergence rate with non-*Bt* rice pollens and *A. gossypii* was higher than that with *Bt* rice pollens and *A. gossypii*. However, there were no significant difference between that with *Bt* and non-*Bt* rice pollens (RM- ANOVA: $F_{1.4}$, *P*=0.4103, Fig. 11).

In terms of fitness parameter, dry weight and elytra length of *P. japonica* adult supplied with *Bt* rice pollens and *A. gossypii* was highest among them (ta). Those of *P. japonica* adult with *A. gossypii* only were significantly lowest (Table 4).

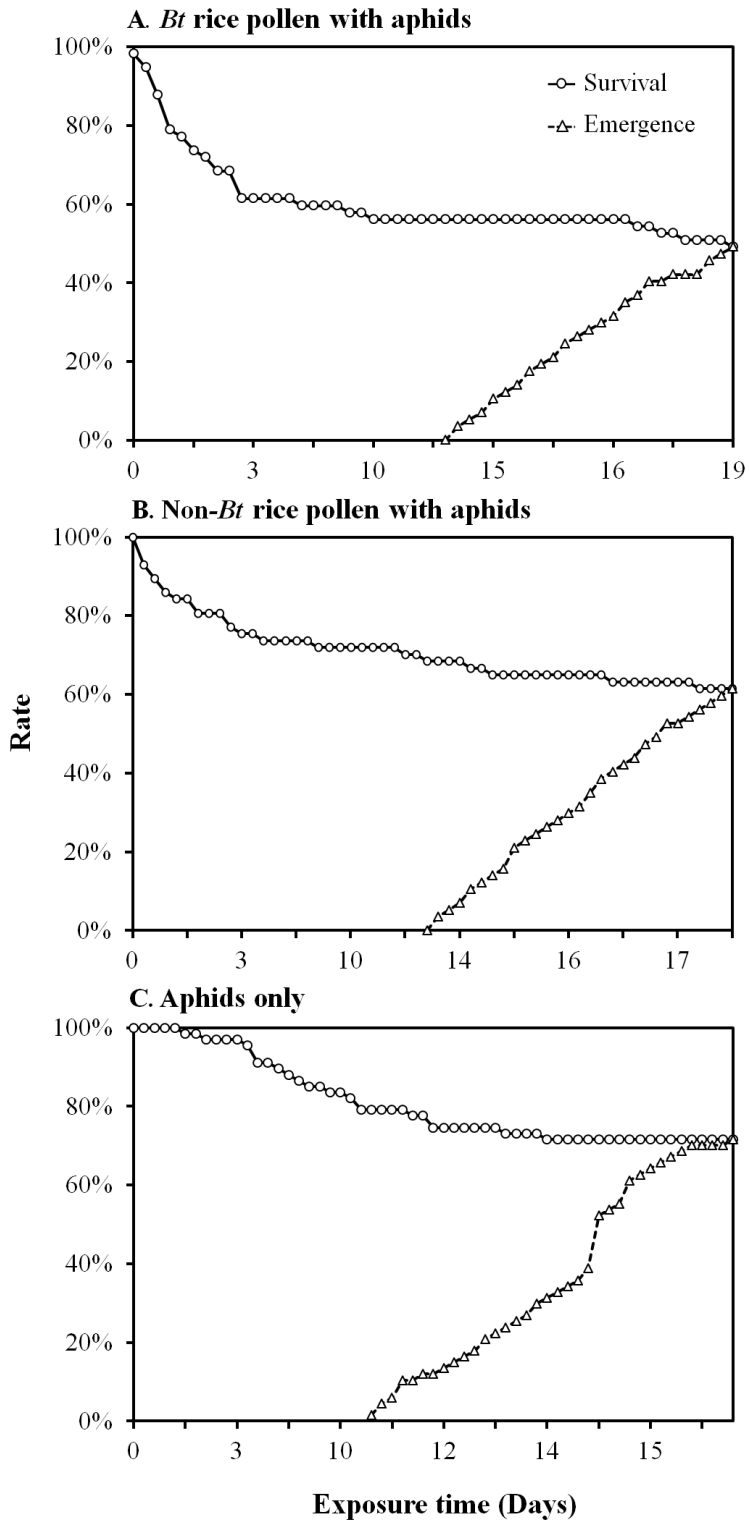

Figure 11. Survival and emergence curves of *P. japonica* supplied with *Bt* and Non-*Bt* rice pollen with and *A. gossypii* only (n=60)

Table 4. Fitness parameters (mean±SD) of *P. japonica* adult reared on *Bt* and Non-*Bt* rice pollen and *A. gossipii* only (replication=3)

Fitness parameter	Treatment			One-way ANOVA		
	Bt rice pollen	Non- *Bt* rice pollen	*A. gossipii* only	*df*	*F*	*p*
Dry weight (mg/5 individuals) *	3.95±0.31a	3.24±0.81ab	2.88±0.56b	2	4.10	0.0440
Elytra length (mm) *	2.74±0.23a	2.67±0.25ab	2.53±0.21b	2	3.52	0.0378

* Significantly different (One-way ANOVA, Tukey HSD test, $P<0.05$)

2.4.4 Effects of *Bt* rice on non-target predator

2.4.4.1 Effects of prey fed *Bt* rice on Growth of *Pirata subpiraticus*

P. subpiraticus of Lycosidae is one of the most important rice field spiders in Korea. They are wandering spiders inhabiting in the lower part of rice plant and mainly prey on the planthoppers and leafhoppers. Developmental period of *P. subpiraticus* from the 2nd spiderling to adult was 81.3±1.6 days supplied with brown planthopper (BPH) fed on *Bt* rice and 93.5±9.2 days supplied with BPH fed on non-*Bt* rice. Developmental period of *P. subpiraticus* between BPH fed on non-*Bt* rice and BPH fed on *Bt* rice was not significantly different (One-way ANOVA: $F_{1.4}$=1.65, P=0.2685). Developmental period of the 3th spiderling was longest and that of the 6th spiderling was shortest in *Bt* rice, whereas that of the 5th spiderling was longest and that of the 2nd spiderling was shortest in non-*Bt* rice (Fig. 12A).

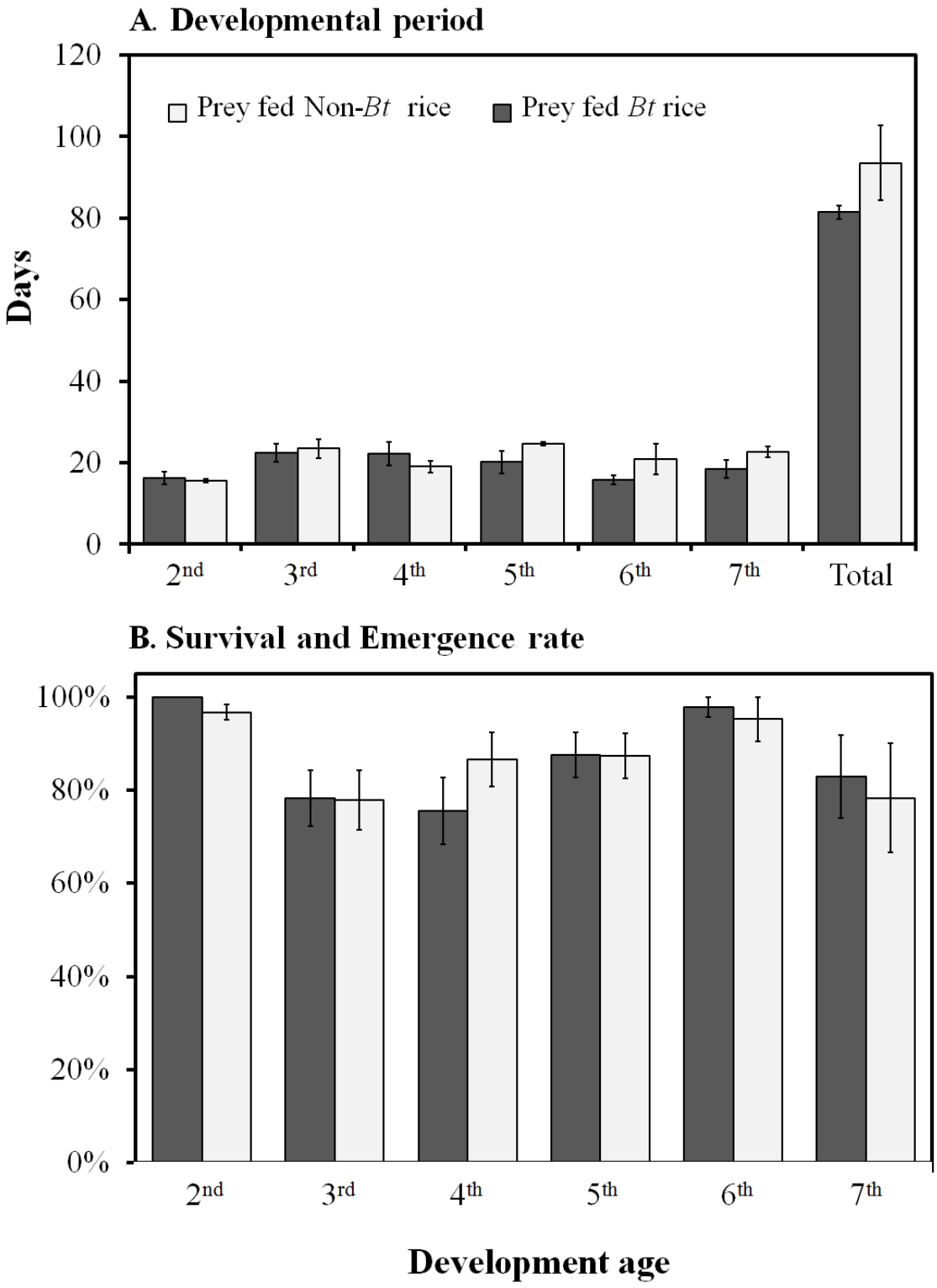

Figure 12. Developmental period (mean±SE) and survival and emergence rate (mean±SE) of *P. subpiraticus* supplied with BPH fed *Bt* and Non-*Bt* rice (replication=3)

Survival rate of *P. subpiraticus* was higher in *Bt* rice (41.7±6.7%) than that in non-*Bt* rice (37.3±4.3%), but was not significantly different (RM ANOVA: $F_{1.4}$=0.49, P=0.5232). Survival rate of each spiderling was highest at the 2nd spiderling (*Bt*: 100.0±0.0%, non-*Bt*: 96.7±1.7%) and lowest at the 3rd spiderling (*Bt*: 78.3±6.0 %, non-*Bt*: 77.8±6.4 %) in both *Bt* rice and non-*Bt* rice (Fig. 12B). Last molting (7th spiderling) of *P. subpiraticus* supplied with prey fed with *Bt* rice was faster than non-*Bt* rice, and molting rate in *Bt* rice was higher than that of non-*Bt* rice. However, there were no significant difference between that with *Bt* and non-*Bt* rice pollens (One-way ANOVA: $F_{1.4}$=0.31, P=0.6101, Fig. 13). In terms of fitness parameter, dry weight, carapace index and tibia length of *P. subpiraticus* (8th) was higher in *Bt* rice than that in non-*Bt* rice, and tibia length was significantly different between *Bt* rice and non-*Bt* rice (Table 5).

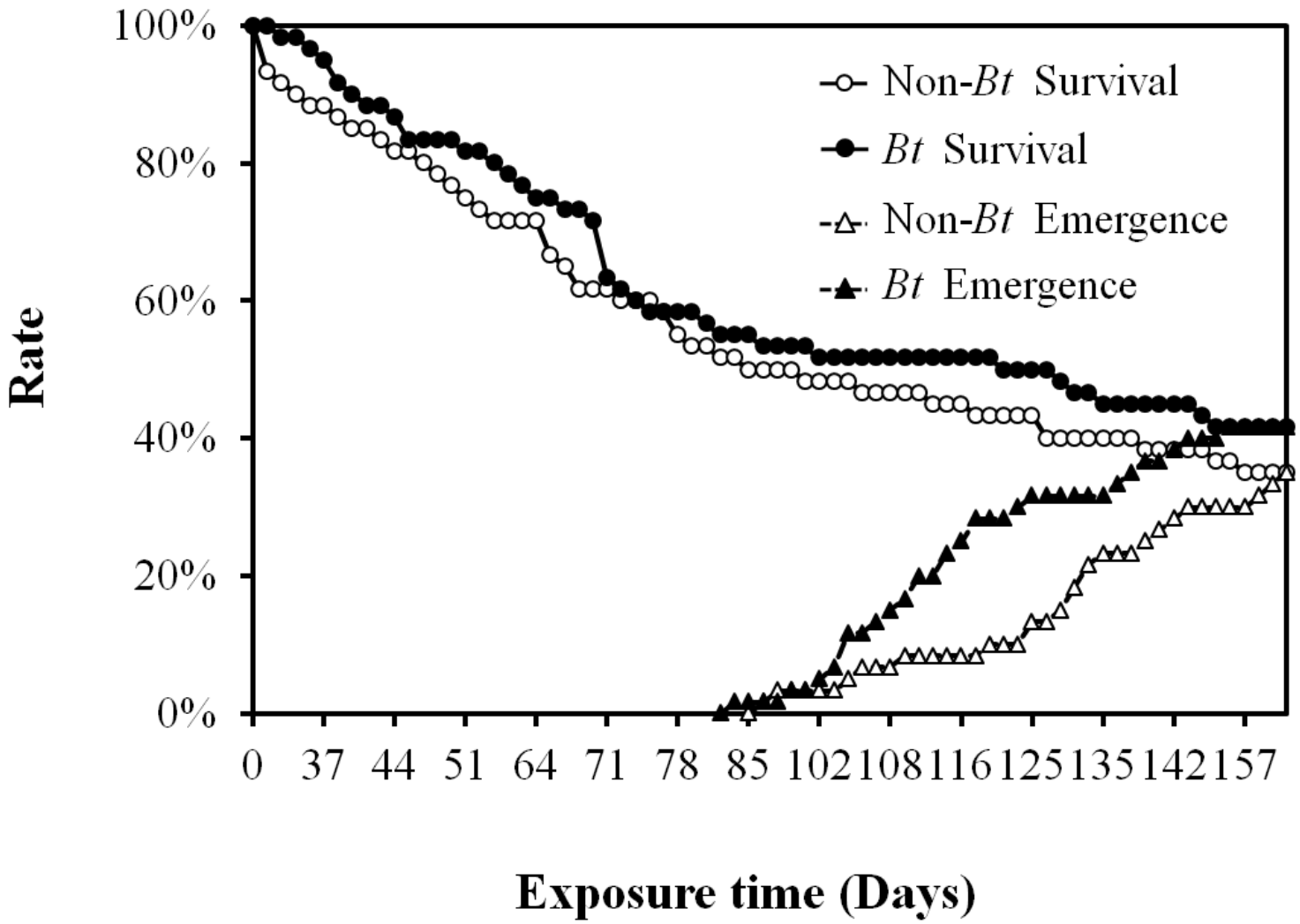

Figure 13. Survival and emergence curves of *P. subpiraticus* supplied with BPH fed *Bt* and Non-*Bt* rice (n=60)

Table 5. Fitness parameters (mean±SD) of *S P. subpiraticus* supplied with BPH fed *Bt* and Non-*Bt* rice (replication=3)

Fitness parameter	Treatment		One-way ANOVA		
	Bt rice	Non- *Bt* rice	*df*	*F*	*p*
Dry weight (mg)	2.38±0.98	1.90±0.68	1	1.82	0.1924
Carapace index	0.74±0.05	0.72±0.03	1	2.27	0.1748
Tibia length (mm) *	16.22±2.73	14.00±1.35	1	6.44	0.0196

* Significantly different (One-way ANOVA, P<0.05)

2.4.4.2 Effects of prey fed *Bt* rice on Growth of *Pachygnatha clercki*

P. clercki of Tetragnathidae is one of the most important rice field spiders in Korea. They are webbing spiders inhabiting in the middle to lower part of rice plant and mainly prey on the planthoppers, leafhoppers and Lepidopteran insect pests. Developmental period of *P. clercki* from the 2nd spiderling to adult was 44.4±1.1 days supplied with BPH fed on *Bt* rice and 45.4±2.1 days supplied with BPH fed on non-*Bt* rice. Developmental period of 2nd spiderling *P. clercki* in non-*Bt* rice was longer than that in *Bt* rice (One-way ANOVA: $F_{1,4}$=9.05, P=0.0396). However, there was no significant difference on total duration *P. subpiraticus* between supplied with BPH fed on *Bt* and non-*Bt* rice (One-way ANOVA: $F_{1,4}$=0.17, P=0.7038). Developmental period of the 6th spiderling was longest (*Bt*: 11.4±1.4 days, non-*Bt*: 14.4±1.8 days) and that of the 4th spiderling (*Bt*: 5.8±0.1 days, non-*Bt*: 6.3±0.5 days) was shortest among all spiderings in both *Bt* rice and non-*Bt* rice (Fig. 14A).

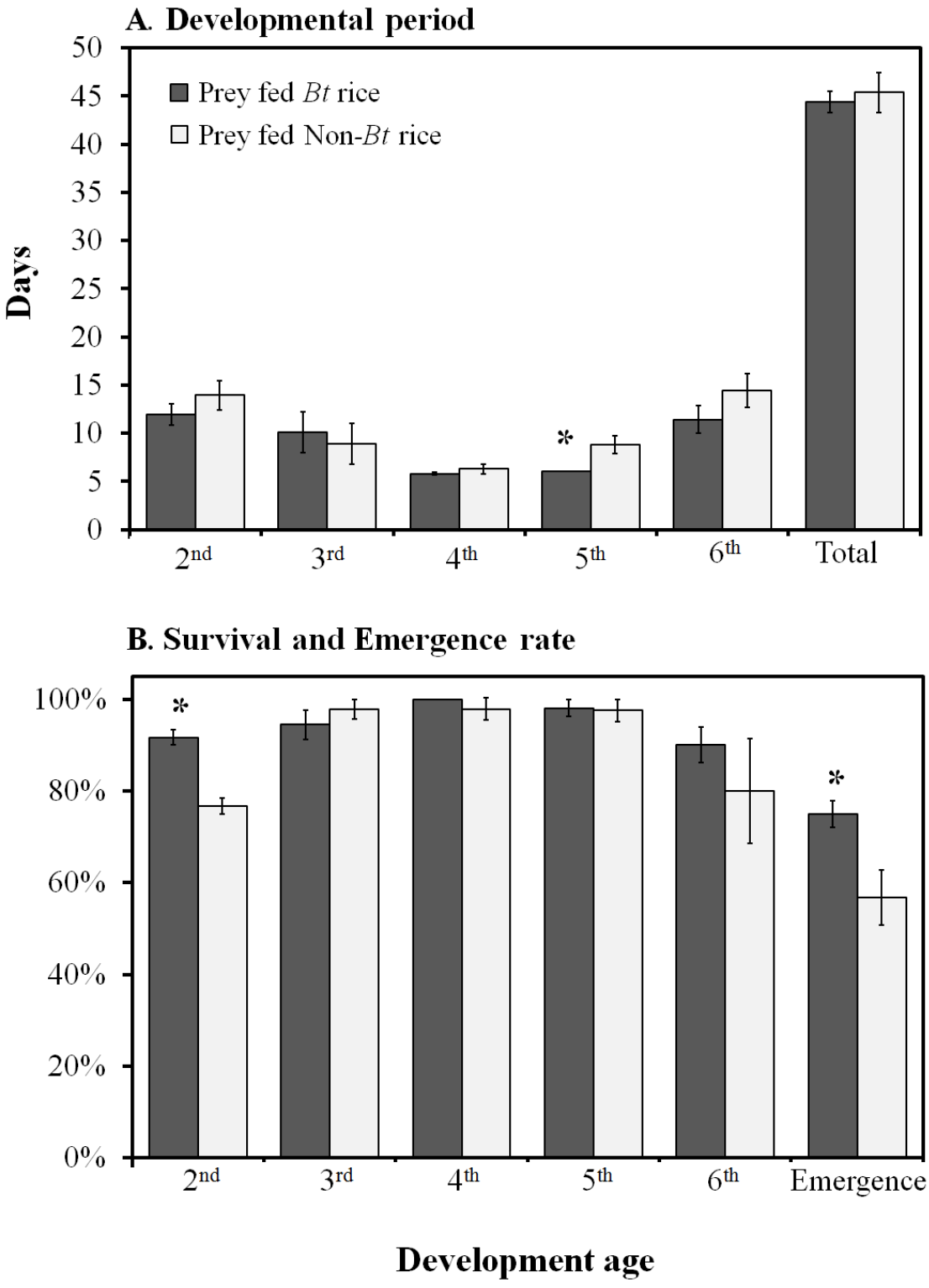

Figure 14. Developmental period (mean±SE) and survival and emergence rate (mean±SE) of *P. clerki* supplied with BPH fed *Bt* and Non-*Bt* rice (replication=3)

Survival rate of *P. clercki* supplied with BPH fed on *Bt* rice was higher than that in non-Bt rice. Survival rate of each spiderling was highest at the 5th spiderling (*Bt*: 100.0±0.0%, non-*Bt*: 97.6±2.4%) and lowest at the 2nd spiderling (*Bt*: 91.7±1.%, non-*Bt*: 76.7±1.7%) in both *Bt* rice and non-*Bt* rice. In particular, mortality of the 2nd spiderling which could not proceed normal molting was higher in non-*Bt* rice than that in *Bt* rice and significantly different (One-way ANOVA: $F_{1,4}$=32.04, P=0.0048). Overall mortality of *P. clercki*, however, was not significantly different (RM ANOVA: $F_{1,4}$=0.79, P=0.2520, Fig. 14B). Emergence and its rate of *P. clercki* was faster and significantly higher in *Bt* rice than that in non-*Bt* rice (One-way ANOVA: $F_{1,4}$=7.94, P=0.0479), respectively (Fig. 15).

In terms of fitness parameter, carapace index of *P. clercki* adult was higher in those supplied with BPH fed on non-*Bt* rice than that in *Bt* rice. Dry weight and tibia length, however, were higher in *Bt* rice than that in non-*Bt* rice, and tibia length was significantly different between *Bt* rice and non-*Bt* rice (Table 6).

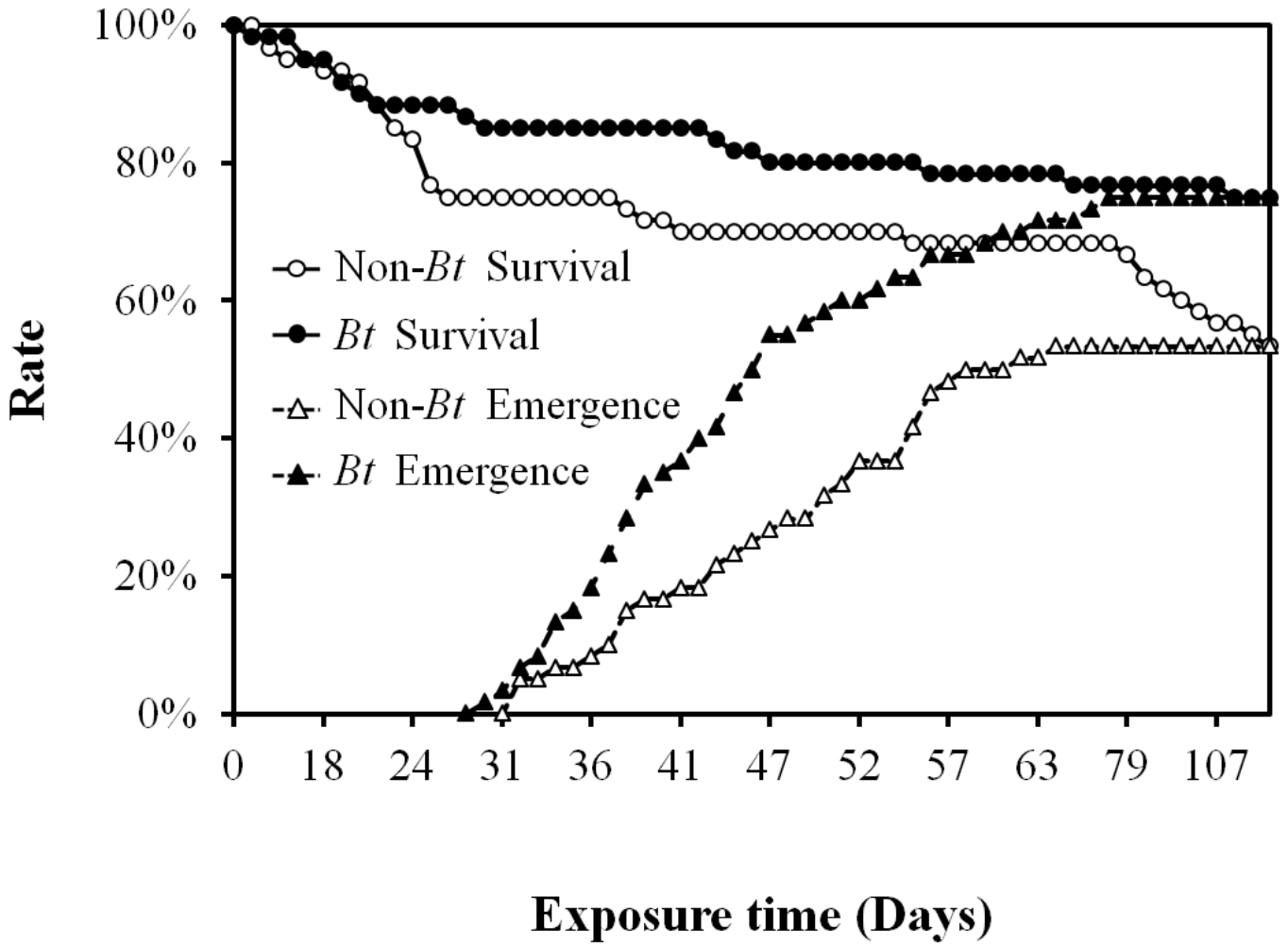

Figure 15. Survival and emergence curves of *P. clercki* supplied with BPH fed *Bt* and Non-*Bt* rice (n=60)

Table 6. Fitness parameters (mean±SD) of *S P. clercki* supplied with BPH fed *Bt* and Non-*Bt* rice (replication=3)

Fitness parameter	Treatment		One-way ANOVA		
	Prey fed *Bt* rice	Prey fed Non- *Bt* rice	*df*	*F*	*p*
Dry weight (mg)	2.48±0.69	2.24±0.74	1	1.42	0.2389
Carapace index	0.85±0.05	0.86±0.04	1	1.82	0.1924
Tibia length (mm) *	17.60±1.48	16.54±1.22	1	6.44	0.0196

* Significantly different (One-way ANOVA, P<0.05)

2. 5. Discussion

Development and commercialization of *Bt* crops have innovative insect pest management (Shelton et al. 2002). Since commercialization in 1996, the rate of adoption has been unprecedented in agriculture and in 2011 *Bt* crops were grown on 160 million ha worldwide (James 2011). However, potential impact of *Bt* crops on non-target organisms, especially natural enemies, continues to be the focus of considerable debate (Romeis et al. 2006, Marvier et al. 2007). Although many field studies to date have shown negligible or no effect on non-target organisms (Romeis et al. 2006, Marvier et al. 2007, Wolfenbarger et al. 2008, Peterson et al. 2011), some laboratory studies have shown negative effects (Ferry et al. 2003). So far, there are no clear universal guidelines for assessing the effects of *Bt* plants on selected non-target arthropods. A test base tiered system that has been adapted to assess the effect of GM crop on non-target arthropods and that includes a selection of suitable test organism at first tier conducted under laboratory conditions (Romeis et al. 2008, 2011). For a risk assessment of GM crops, it is impossible to test all species that are potentially present in the receiving environment and exposed to *Bt*, therefore testing organisms should be selected that represent different habitats (e.g., soil- or plant-dwelling arthropods) or different ecosystem services such as ecological functions (e.g., predator, parasitoid or decomposer), taxonomic groups, the availability of the test organism or the likelihood of exposure to GM crops (Jepson et al. 1994, Dutton et al. 2003, Rose 2007, Romeis et al. 2008, 2011, Hilbeck et al. 2011). Especially, on selecting non-target herbivore, it is considered the test organism's mode of feeding and the site and the time of protein expression in the *Bt* crop (Dutton et al. 2002, Romeis et al. 2008). Our study selected six non-target arthropods considering expression sites of insecticidal protein in plant tissue, exposure possibility to insecticidal expression sites,

inhabiting sites on rice plant, ecologically functional role, relative importance on rice, easiness and control for tests and feeding mode of test species (Figure 2-5). The results of non-target herbivore showed that there were no significant differences in development, survival and emergence of *O. japonica, S. lurida* and *N. lugens* feeding on *Bt* and non-*Bt* rice leaf tissue. Despite the longer development, higher mortality and lower emergence in a few larval stage of *O. japonica* and *S. lurida*, those adults which did survive on a *Bt* rice diet reached the same or larger size as adults from the non *Bt* rice and had even longer body length of *S. lurida* adult. Chen et al. (2012) reported that the population density of brown planthopper (BPH) nymphs was significantly lower in cry1Ab *Bt* rice, but the temporal pattern of population dynamics of BPH adults was similar between the Bt and non-*Bt* rice and had no distinctive negative effects on the survival and developmental duration of BPH nymphs in laboratory study. In other studies, no marked effects on non-target rice herbivore were detected. Fu et al. (2003) reported that none of the development and reproduction parameters were differed when measured in the BPH, *N. lugens*, and the white-backed planthopper, *S. furcifera* reared on *Bt* rice expressing a fusion protein of Cry1Ab/CpTI and non-*Bt* rice. Similarly, there was no difference in any of the five fittness parameters, survival to the adult stage, male and female weight, and male and female developmental time, of *N. lugens* reared on *Bt* rice and non-*Bt* rice (Bernal et al. 2002). Tan et al. (2006) reported that *Bt* rice had no significant difference on either oviposition behavior or fecundity of the white-backed planthopper comparing to non-*Bt* rice. Also, the result of *N. lugens* with 2 different rice seedlings show that similar pattern on development, survival and emergency but that of 20 days seedling showed shorter development, lower mortality and higher and faster emergence than that of 60 days seedling. It seems that 20 days seedling may be more suitable

to detect effect of *Bt* itself reducing the stress related to host or prey if the amount of *Bt* toxin expression is almost the same.

The results of non-target predaotors showed that there were no significant differences in development and survival of *P. subpiraticus* and *P. clercki* feeding *N. lugens* reared on *Bt* and non-*Bt* rice although *P. subpiraticus* and *P. clercki* with *N. lugens* fed *Bt* rice showed shorter development, lower mortality and heavier and larger size as adults (8^{th} spiderling *in P.subpiraticus*) and tibia length of these species in *Bt* rice was significantly longer than in non-*Bt* rice. This is consistent with other laboratory studies that assessed the potential effects of *Bt* toxin on spiders. Tian et al (2012) reported that *Bt* rice expressing Cry1Ab (KMD1 and KMD2) had positive effects did not affect survival, developmental time and fecundity of *Pardosa pseudoannulata* (Araneae: Lycosidae) via its prey *N. lugens*. According to Chen et al. (2009), survivorship and fecundity of *P. subpiraticus* preying on *Bt* rice-fed *C. medinalis* were not significantly affected although its developmental time was significantly longer. Tian et al. (2010) also reported that, although *Ummeliata insecticeps* (Araneae: Linyphiidae) ingested measurable amounts of Cry1Ab protein when it was supplied with Bt rice-fed *N. lugens*, Bt rice did not have negative effects on the developmental time and fecundity of *U. insecticeps*. Liu et al. (2006) founded that no negative prey-mediated effects on two spiders, *Hylyphantes graminicola* (Araneae: Linyphiidae) and *Coleosoma octomaculatum* (Araneae: Theridiidae) were observed when they fed on *Bt* cotton (expressing Cry1Ac protein)-fed prey. Similarly, Meissle and Romeis (2009) reported *Bt* maize expressing Cry3Bb1 had no adverse impact on mortality, weight development or offspring production of the web-building spider, *Theridion impressum* (Araneae: Theridiidae).

The pollen feeding result showed no adverse effect on the development, survival and fitness of adult *P. japonica* after ingestion of *Bt* rice pollen expressing Cry1Ac when

compared with pollen from the corresponding non-transformed rice plant. The results were consistent with a previous study by Bai et al. (2006) reported that no effects were found on development, survival and reproduction indices of *P. japonica* adults, when fed on Cry1Ab-containing pollen from *Bt* rice lines KMD1 and KMD2. Bai et al. (2005) also reported that pollen of *Bt rice* lines KMD1 and KMD2 did not harm adults of *Chrysoperla sinica* (Neuroptera: Chrysopidae). Similarly no effects were found on the survival, fecundity and fertility of *C. carnea* adults, when fed on *Bt* maize pollen from Event 176 expressing Cry1Ab or Mon88017 expressing Cry3Bb1 (Li et al. 2008). In addition, the impact of *Bt* maize pollen on another Chysopid, *Chrysoperla plorabunda* was also tested (Mason et al. 2008). Wang et al. (2012) reported the pollen feeding bioassay showed no adverse effect on the fitness of adult *C. sinica* after ingestion of *Bt* rice pollen expressing Cry2Aa when compared with pollen from the corresponding non-transformed rice plant. The result of target species test show that *N. aenescens* larvae, closed to target species, are significantly negatively affected by cry1Ac in terms of developmental time and survival. This is not surprising as many lepidopteren species have been shown to be susceptible to Cry1A (Escriche et al. 1998, Glare and O'Callaghan 2000, Vojtech et al. 2005). We observed the highest mortality in the 1st and 2nd larval stage, where 100% of larvae died the first 48 hours on Bt rice. Other studies confirm that susceptibility of lepidoptera to *Bt* toxins is highest in the first larval stages and then decreases with progressed larval development (Sneh et al. 1981, Vojtech et al. 2005). While some studies were conducted only with the 1st larval stage when investigated effects of *Bt* crop on lepidopteran pest (Dutton et al. 2002), our study was conducted all larval stages of *N. aenescens*. As herbivores in a *Bt* rice field are likely to ingest toxic leaf material for their whole larval life rather than a few days only, long term exposure of hosts is necessary to obtain biologically relevant data when assessing the risk to natural enemies. In spite of the

difference of *N. aenescens* survival, our data showed that *C. medinalis* could find correct hosts but there was no fecundity preference *C. medinalis*, target herbivore, between *Bt* rice and non-*Bt* rice. This result revealed that *Bt* rice is not likely to affect fecundity of target species.

In this study, we assessed effects of two plant parts of *Bt* rice, leaf and pollen, on non-target arthropods, considering 1) ecological function, herbivore, pollen-feeder and predator, 2) various taxonomic group, Araneae, Coleoptera, Heteroptera, Lepidoptera and Othoptera, 3) test organism's mode of feeding, Chewing, pierce sucking, hunting and web-building, 4) the site and the time of protein expression in the *Bt* rice, pollen and leaf of 20 days and 60 days rice seedling. And *Bt* rice expressing cry1Ac had no significant difference on development, survival and fitness parameter of these species adults except *N. lugens* closed to target species.

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Chapter 3.

Effects of *Bt* rice on arthropods in Field conditions

3.1. Abstract

To assess the potential adverse effects of a *Bt*-rice line (Japonica rice cultivar Nakdong) expressing a synthetic Cry1Ac1 gene, C7-1-9-1-B, which was highly active against all larval stages of *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae), we investigated the community structure of insects and spiders in *Bt* and non-*Bt* rice fields during the rice growing season in 2007 and 2008 in Chungcheongnam-do, Korea. Insects and spiders were surveyed with a sweep net and suction device and rarefaction curves were constructed to confirm the sample size was appropriate. A total 43 familes in 10 orders were identified from 64,099 collected insects and classified four guilds, Herbivore, Predator, Parasitoid, and Detritivore. Family richness, abundance and Shannon's index of insects were very similar between *Bt* and non-*Bt* rice. However, significantly higher abundance was observed in the non-*Bt* rice in the herbivore in 2007, and predator, and Coenagrionidae, in 2008. A total 29 species in 23 genera and 9 families were identified from 4,937 collected spiders and both *Bt* and non- *Bt* rice fields showed a typical Korean spider assemblage. Species richness, abundance and Shannon's index of spiders were very similar between *Bt* and non- *Bt* rice, although in 2008 significant difference was observed in the abundance of *P. oculiprominens, T. maxillosa and P. clercki, P. oculiprominens* and *T. maxillosa* were higher in non-*Bt* rice and *P. clercki* was higher in *Bt* rice. The cause of differences in these species seems to be that interspecific competition of spiders and accidental early immigration of some spiders affect the overall spider abundance during the rice growing season rather than by the *Bt* construct itself. Overall, insect and spider community structure including diversity, dominant family and species, and abundance were not significantly different between *Bt* and non- *Bt* rice. The results indicated that the transgenic Cry1Ac rice lines tested in this study had no significant

adverse effects on the rice insect and spider community structure.

3.2. Introduction

Bt crops require environmental risk assessment (ERA) to determine any effects on the ecological community through *Bt* toxicity and gene flow. The toxins are produced in *Bt* plants throughout the entire crop growth stage and whole plant tissue (Wu et al. 2002, Saxena et al. 2004). Thus, target and nontarget arthropods have various chances to contact *Bt* toxins, by feeding on plant parts themselves, through feeding on target or non-target herbivorous insects, or via the environment (Hillbeck et al. 1999, Dutton et al. 2002, Groot and Dicke 2002, Zwahlen et al. 2003). This has concern about the potential impacts of releasing genetically engineered organisms into the environment (Tiedje et al. 1989, Poppy 2000). Various studies showed that *Bt* crops did not have any significant effects on not only non-target arthropods (Head et al. 2001, Raps et al. 2001, Bernal et al. 2002, Chen et al. 2005) but also natural enemies (Head 2005, Poza et al. 2005, Toschki et al 2007, Meissle and Romeis 2009, Tian et al. 2010, Alvarez-Alfageme et al. 2011), but still concerns remain, and require further studies. Natural enemies, especially generalist arthropod predators, have been the focus of many ERA studies of *Bt* crops because of their importance in insect pest control, and likelihood of exposure to *Bt* toxin directly while they feed on prey (Nyffeler 1999, Romeis et al. 2006, 2009) and indirectly via prey feeding on *Bt* crops (Dutton et al. 2003). Harwood et al (2005) reported that some predatory arthropods, such as Coccinellidae, Nabidae and Araneae, contained significant quantities of *Bt* toxin through a food web in the field. Prey-mediated effects of *Bt* crops on higher trophic levels are well documented in the laboratory study (Hilbeck et al. 1998, Bernal et al. 2002, Dutton et al. 2002, Romeis et al. 2004, 2006, Lövei and Arpaia 2005, Hilbeck and Schmidt 2006, Torres and Ruberson 2006, 2008, Chen et al. 2009, Naranjo 2009).

Spiders, one of the most important predator groups in many agricultural ecosystems, are also likely to be exposed to *Bt* toxins through the consumption of a wide variety of prey which may have been exposed to *Bt* toxins through their diet (Dutton et al 2002). Additionally, secondary predation of spiders on smaller arthropod predators that contain *Bt* toxins may occur (Jiang et al. 2004, Chen et al. 2005, 2009, Tian 2010). Another potential route of *Bt* toxin movement to spiders is ingestion of soil-dwelling arthropods via root exudates and plant biomass (Peterson et al. 2011). Saxena et al (2004) reported that *Bt*- corn, potato, and rice release transgenic protein in root exudates during plant growth. Furthermore, spiders are likely to be exposed to *Bt* toxins through pollen of *Bt* crops especially during anthesis in the fields. Spiders may ingest pollen when recycling their webs or when their prey has collected or consumed pollen or is dusted with it (Ludy and Lang 2006b). Spiders rapidly colonize a crop field and are more likely to remain during periods of low prey abundance (Wise 1993, Sunderland et al. 1997, Greenstone 1999). Thus, spiders have a positive effect on reducing pest populations in a crop field and study of *Bt*-crop effects on spiders is necessary.

Bt rice has been engineered to express cry1Ac and/or cry1Ab for the control of several lepidopteran pests, including the striped stem borer (*Chilo suppressalis*: Crambidae), yellow stem borer (*Scirpophaga incertulas*: Pyralidae), and the rice leaffolder (*Cnaphalocrocis medinalis*: Crambidae) (High et al. 2004, Wang and Johnston 2007). In Korea, *Bt*-rice line expressing cry1Ac1 for control of *C. medinalis*, an important rice insect pest in Asia (Wada et al. 1980, Bautista et al. 1984, Dale 1994) was developed (Shin et al. 2009). *C. medinalis* is difficult to control by insecticides because larvae roll rice leaves and stay inside. *Bt* rice can be an alternative control option for achieving yield increase and less insecticide application. To date, a few field tests of potential effects of *Bt* rice on spiders were conducted on non-

target arthropods and most of the studies have focused on a limited number of species in the field (Chen et al. 2005, 2009, Toschki et al. 2007, Akhtar et al. 2010, Bai et al 2010, Tian et al. 2010). Especially, comprehensive field study of its effects on the overall spider community is not yet conducted. We conducted a 2-year study to determine potential impacts of *Bt* rice on the spider and insect community in the rice field.

3.3. Material and Methods

Plot design and rice planting

This study was conducted in an isolated rice field in the Chungcheongnam-do Agricultural Research and Extension Services (CARES), Korea in 2007 and 2008. The mean monthly air temperature ranged between 21^{o}C and 26^{o}C from June to September in both years. The mean monthly rainfall ranged from 106.7mm to 470.6mm, and 89.3mm to 287.2mm in 2007 and 2008, respectively. The size of the field was 3467.25m^2 in 2007 and 4,125m^2 in 2008. A field was divided into fifteen 22.5 m × 12.0 m plots and twelve 22.5 m × 12.0 m plots in 2007 and 2008, respectively (Fig. 1). Of them, three plots were randomly selected for each *Bt* and non-*Bt* rice.

Transgenic *Bt* rice line with a synthetic cry1Ac1 gene, C7-1-9-1-B was used with its non-*Bt* isoline Japonica rice cultivar, Nakdong. The C7-1-9-1-B line was developed to express insecticidal action derived from *Bacillus thuringiensis* Berliner. This *Bt* rice line is highly active against all larval stages of *C. medinalis* in the bioassay (Shin et al. 2009). Both rice seedlings were transplanted in a 15 cm × 30 cm spacing on 25 May in 2007 and on 10 June in 2008. The field was managed according to the standard rice cultural practices, but insecticides and herbicides were not treated.

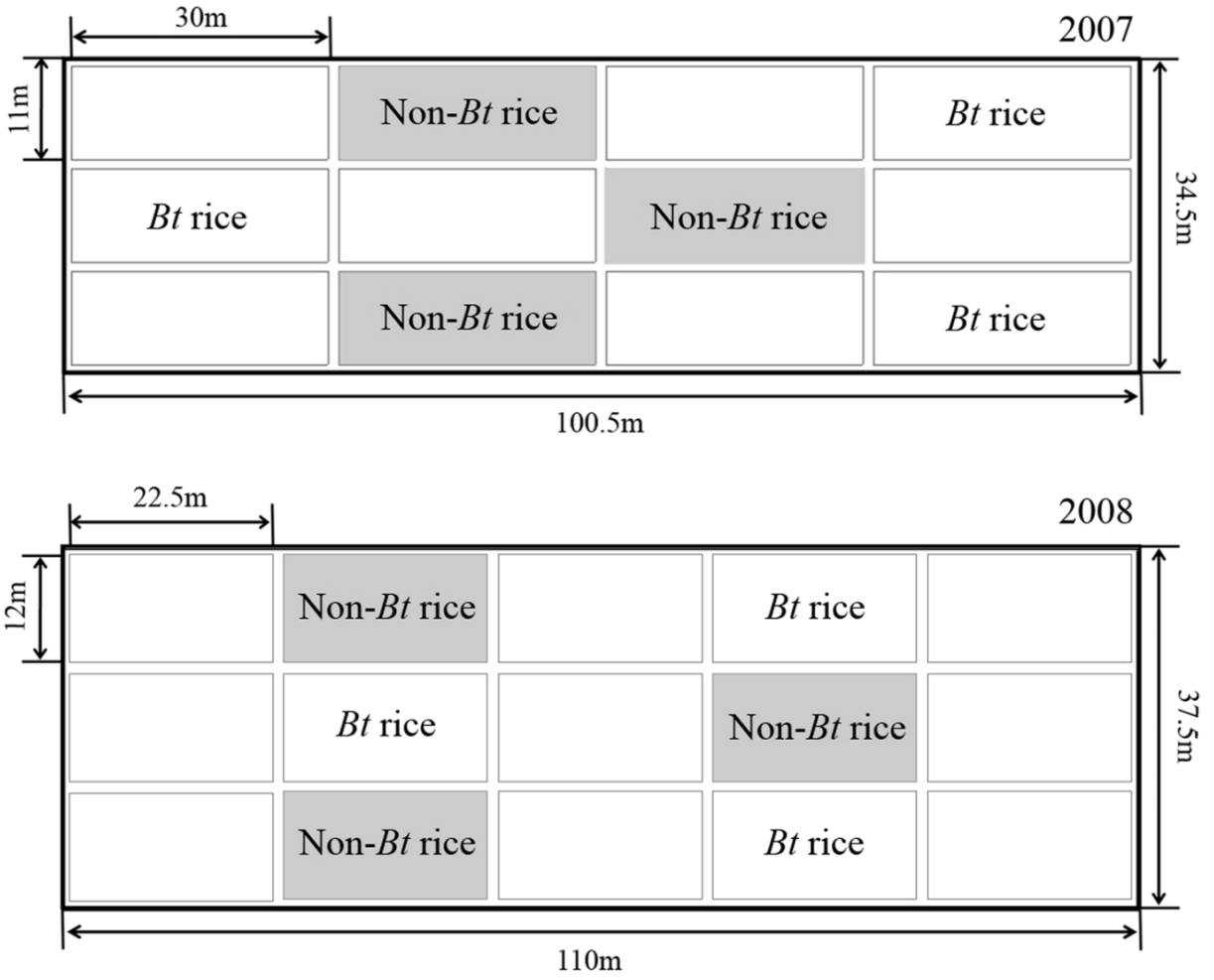

Figure 1. Plot layout and allocation in 2007 and 2008.

Spider and insect sampling

To dectect *Bt* effects, spider and insect sampling were conducted from 28 June to 4 October in 2007, from 7 July to 25 September in 2008. Sampling was conducted at one week intervals in 2007 and at two weeks intervals in 2008. Thus a total of 15 and 7 sampling occasions were made in 2007 and 2008, respectively. To detect the effects of sampling plot size, spider sampling was conducted at one week intervals in 2009. A battery-powered suction device (DC 12V, Bioquip Co., Rancho Dominguez, CA, USA) was used to collect spiders and insects inhabiting the lower and middle parts of the rice plant. Four rice hills were sucked for one suction sample. Also, a sweep net (39cm in diameter) was used to collect spiders and insects inhabiting the upper and top parts of the rice plant. Sweeping was made at an angle of 180^{o} and repeated three times per a sweeping sample. Suction and sweeping samples were taken 5 times each on every sampling date. Also, sampling was made by keeping a certain distance (3m) cross-diagonally while avoiding interference of each sampling. The direction of the sampling was changed on each sampling date. Sampling was not made on the board (1 m) of each plot.

Spider identification

Collected spiders were fixed in 85% ethanol and identified to species level under a dissecting microscope according to Namkung (2003), Chikuni (2008) and Ono (2009). Species names followed Namkung et al. (2010) and order of families followed taxonomic orders of Platnick's catalogue ver. 12.5 (2012), and adopted the latest taxonomical transformation. Spider guilds were identified according to Young and Edwards (1990). Specimens were deposited in Laboratory of Insect Ecology, College of Agriculture and Life Sciences, Seoul National University.

Insect identification

All collected insects were fixed in 85% ethanol and identified to family level except Psocoptera. The insects were separated into four guilds: 1) herbivores, 2) predators, 3) parasitoids, 4) detritivores, considering the ecological function of insects. Specimens were deposited in Laboratory of Insect Ecology, College of Agriculture and Life Sciences, Seoul National University.

Data analysis

Abundance of spiders and insects was transformed by log (n+1) and guild proportion was transformed by arcsine for statistical analysis. A repeated measurement ANOVA (Proc RM ANOVA) in SAS 9.2 (SAS Institute 2004) was used to analyze the effect of "*Bt* status" (i.e. *Bt*-rice and non-*Bt* rice) on number of individuals, number of species, Shannon's diversity and guild proportion. To evaluate sample size adequacy and compare species richness between Bt and non-Bt rice plots, we constructed rarefaction curves (Gotelli and Colwell 2001) for each treatment using Species Diversity and Richness v3.0 computer program (Henderson and Seaby 2002). Samples were randomly reordered 50 times and standardized to the number of individuals caught to avoid the problem that the samples are listed can have a large impact on the result when calculating species richness or fitting a species accumulation curve. Also, diversity analysis was conducted for comparison of the spider community structures (species richness, species diversity, and similarity) between *Bt* and non-*Bt* rice using PRIMER-5 (Clacrke and Warwick 2001). Shannon's -diversity, H', (Shannon and Weaver 1949) is:

$$H' = -\sum p_i \ln p_i$$

where p_i is the proportion of the *i*th species in the total sample.

Similarity of the spider communities is measured by considering variation of species found in them, and in our study this was determined by means of the Sorenson quantitative coefficient (Magurran 2004). The equation for the Sørenson quantitative coefficient (C_N) is:

$$C_N = 2jN/(N_a + N_b)$$

where N_a = the total number of individuals in the first treatment, N_b = the total number of individuals in the second treatment, and $2jN$ = the sum of the lower of the two abundances for species found in both treatments. The value of the index is 1 in the case of complete similarity and 0 when the samples compared have no common species. This index was calculated for each year.

Cluster analysis, using the paired group method and Bray-Curtis similarity measures, was used to check the similarity of the predefined groups and to depict similarity of spider assemblages from all plot sizes.

3.4. Results

3.4.1 Effects of *Bt* rice on insect community

3.4.1.1 Overall insect community of rice fields

A Total of 43 families in 10 orders were identified from 31,765 and 32,334 collected insects in 2007 and 2008, respectively (Table 1). Hemiptera was most dominant order on family richness and abundance accounting for 31.8% and 59.6% and Diptera followed Hemiptera accounting for 11.4% and 26.6% for two years in the rice fields, respectively (Fig. 3). At the family level, five families, Aphididae, Cicadellidae and Delphacidae in Hemiptera, Chironomidae in Diptera and Tomoceridae in Collembola were dominated on abundance ranged from 8.8% to 35.2% of collected total insects for two years in the rice fields (Fig. 8). Of the 44 families collected, 30 were represented by <0.1% of abundance for two years, 10 families in Hemiptera, six families in Coleoptera and Hymenoptera, two families in Diptera and Odonata, a family in Neuroptera, Orthoptera and Thysanoptera and Psocoptera, occupied 68.2% of total number of families and 0.6% of total number of individuals (Table 1). Five of these families, Alydidae and Coreidae (Hemiptera), Diapriidae and Scelionidae (Hymenoptera) and Libellulidae (Odonata) were represented by only a single individual for two years accounting for 11.4% of total number of families and <0.01% of total number of individuals (Table 1). At the ecological functional guilds, 17 families in 4 orders with 38,487 individuals were herbivore, 3 families in 3 orders with 22,573 individuals were detritivore, 15 families in 6 orders with 2,238 individuals were predator and 7 families in 1 order with 801 individuals were parasitoid (Table 1). The most dominant ecological guild was herbivore occupying 39% of family richness and 60% of insect abundance (Table 1, Fig 5). Most

abundant families by ecological functional guilds were Cicadellidae (Hemiptera) occupying 58.7% of total herbivores, Chironomidae (Diptera) occupying 74.2% of total detritivores, Coenagrionidae (Odonata) occupying 87.1% of total predators and Braconidae and Drynidae (Hymenoptera) occupying 86.9% of total parasitoids (Fig. 9).

Table 1. Insects recorded in *Bt* and non-*Bt* rice throughout the rice growing season for 2 yeras

Guild	Order	Family	Sampling method	2007		2008	
				Bt	Non-*Bt*	*Bt*	Non-*Bt*
Herbivore	Orthoptera	Acrididae	Suction/Sweeping	22	15	13	21
		Pyrgomorphidae	Suction/Sweeping	2	-	0	1
	Thysanoptera	Thripidae	Suction/Sweeping	6	7	2	1
	Hemiptera	Alydidae	Sweeping	-	1	-	-
		Aphididae	Suction/Sweeping	1,210	1,840	1,974	1,154
		Berytidae	Sweeping	1	1	-	-
		Cicadellidae	Suction/Sweeping	2,341	3,373	8,705	8,165
		Coreidae	Sweeping	1	-	-	-
		Delphacidae	Suction/Sweeping	2,133	2,889	2,168	2,022
		Lygaeidae	Suction/Sweeping	9	2	1	-
		Miridae	Suction/Sweeping	41	31	46	40
		Pentatomidae	Suction/Sweeping	-	-	4	1
		Rhopalidae	Sweeping	1	1	-	-

	Coleoptera	Apionidae	Suction/Sweeping	4	6	7	8
		Chrysomelidae	Suction/Sweeping	4	4	-	1
		Curculionidae	Suction/Sweeping	72	35	50	49
		Elateridae	Suction	2	-	-	-
Predator	Odonata	Aeshnidae	Sweeping	4	1	-	-
		Coenagrionidae	Suction/Sweeping	1,050	803	61	36
		Libellulidae	Sweeping	1	-	-	-
	Hemiptera	Anthocoridae	Suction/Sweeping	2	4	2	1
		Gerridae	Suction/Sweeping	-	-	-	4
		Hydrometridae	Suction	-	3	-	-
		Veliidae	Suction/Sweeping	12	8	14	2
	Neuroptera	Chrysopidae	Suction/Sweeping	1	2	5	3
	Coleoptera	Carabidae	Suction	6	2	4	4
		Coccinellidae	Suction/Sweeping	20	26	9	11
		Dytiscidae	Suction/Sweeping	-	1	2	-
		Staphylinidae	Suction/Sweeping	6	10	7	2
	Hymenoptera	Formicidae	Suction	1	-	-	2

	Diptera	Sciomyzidae	Suction/Sweeping	17	12	27	16
		Syrphidae	Sweeping	18	15	1	-
Parasitoid	Hymenoptera	Braconidae	Suction/Sweeping	118	138	49	28
		Chalcididae	Suction/Sweeping	5	3	5	2
		Diapriidae	Sweeping	-	-	1	-
		Drynidae	Suction/Sweeping	142	212	4	5
		Ichneumonidae	Suction/Sweeping	14	11	3	9
		Mymaridae	Suction/Sweeping	20	18	5	8
		Scelionidae	Sweeping	-	-	1	-
Detritivore	Collembola	Tomoceridae	Suction/Sweeping	21	55	3,086	2,436
	Psocoptera	Psocoptera	Suction/Sweeping	8	1	-	-
	Diptera	Chironomidae	Suction/Sweeping	6,351	8,517	1,021	853
	Diptera	Culicidae	Suction/Sweeping	16	5	96	69
		Tipulidae	Suction/Sweeping	21	10	4	3

3.4.1.2 Effects of *Bt* rice on biodiversity

A Total of 41 families in 10 orders from 31,080 individuals and 39 families in 10 orders from 33,019 individuals were identified in *Bt* rice and non-*B* rice fields, respectively (Table 1). At the ecological functional guilds, 4 orders from 17 families with 18,819 individuals and 16 families with 19,688 individuals were herbivore, 6 orders from 13 families with 1,270 individuals and 14 families with 968 individuals were predator, an order from 7 families with 367 individuals and 5 families with 434 individuals were parasitoid and 3 orders from 5 families in with 10,624 individuals and 19,668 individuals were detritivore in *Bt* rice and non-*Bt* rice fields, respectively (Table 1). Overall, the insect family richness, abundance and Shannon's index were not significantly different between *Bt* rice and non-*Bt* rice fields and no significant interaction between year and treatment (Table 2).

Table 2. Biodiversity (mean±SE, n = 3) of insect community per plot in *Bt* and non-*Bt* rice throughout the rice growing season

Year	Treatment		RM-ANOVA *F*-ratio (*P*-value)		
	Bt	Non-*Bt*	Treatment (*T*)	Year (*Y*)	Interaction (*T*x*Y*)
Families richness					
2007	28.7±1.3	27.3±0.3	$6.38_{1,4}(0.065)$	$0.31_{1,8}(0.134)$	$0.29_{1,8}(0.604)$
2008	25.7±0.7	22.3±0.7	$1.42_{1,4}(0.300)$		
Abundance					
2007	4567.7±415.3	6020.7±380.7	$5.08_{1,4}(0.087)$	$0.04_{1,8}(0.841)$	$4.09_{1,8}(0.078)$
2008	5792.3±638.2	4985.7±794.4	$0.41_{1,4}(0.557)$		
Shannon's index					
2007	1.61±0.05	1.52±0.05	$3.83_{1,4}(0.122)$	$0.98_{1,8}(0.351)$	$0.03_{1,8}(0.866)$
2008	1.48±0.04	1.40±0.17	$0.16_{1,4}(0.711)$		

Seasonal change of family richness and abundance of insects oscillated in the season, but showed very similar serrated seasonality between *Bt* rice and non-*Bt* rice fields (Fig. 2). Though there were some significant differences between *Bt* rice and non-*Bt* rice fields in family richness of 35 ($F_{1,4}$=12.29, P=0.0248) and 105 ($F_{1,4}$=8.16, P=0.0461) days after transplanting (DAT) in 2007, abundance of 28 ($F_{1,4}$=8.29, P=0.0451) and 84 ($F_{1,4}$=8.21, P=0.0457) DAT in 2007 and in the Shannon's index at 49 ($F_{1,4}$=12.79, P=0.0232) and 84 ($F_{1,4}$=8.14, P=0.00462) DAT in 2007, there was no significant difference in *Bt* rice fields compared to non-*Bt* overall (Table 2). Family richness and abundance of insect families were not differently affected in *Bt* rice fields compared to non-*Bt*, but abundance of Hemiptera was lower in *Bt* rice fields compare to non-*Bt* in 2007 ($F_{1,4}$=10.11, P=0.0335, Fig. 3). Family rarefaction curves reached asymptote as sample size and insect individual numbers increased, indicating total sample size for this study is proper. Also, these curves were almost the same between *Bt* and non-*Bt* rice in 2008, although more insect families were captured in *Bt* rice and more individuals were captured in non-*Bt* rice (Fig. 4)

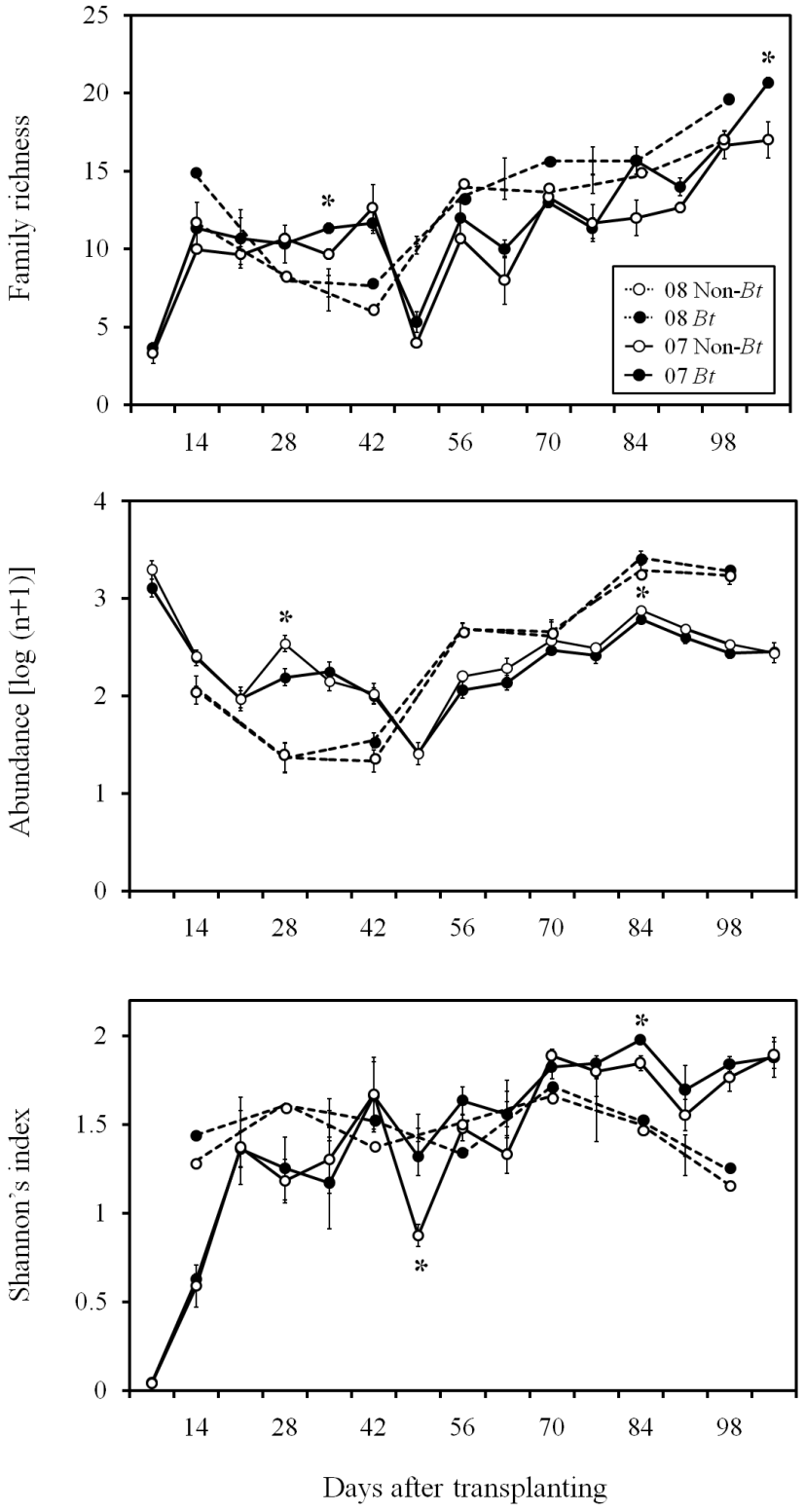

Figure 2. Seasonal dynamics of insects (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season. Abundance was individual numbers of 5 samples and a sample consisted of one suction sampling and five sweepings. Refer to the main text for details on sampling.

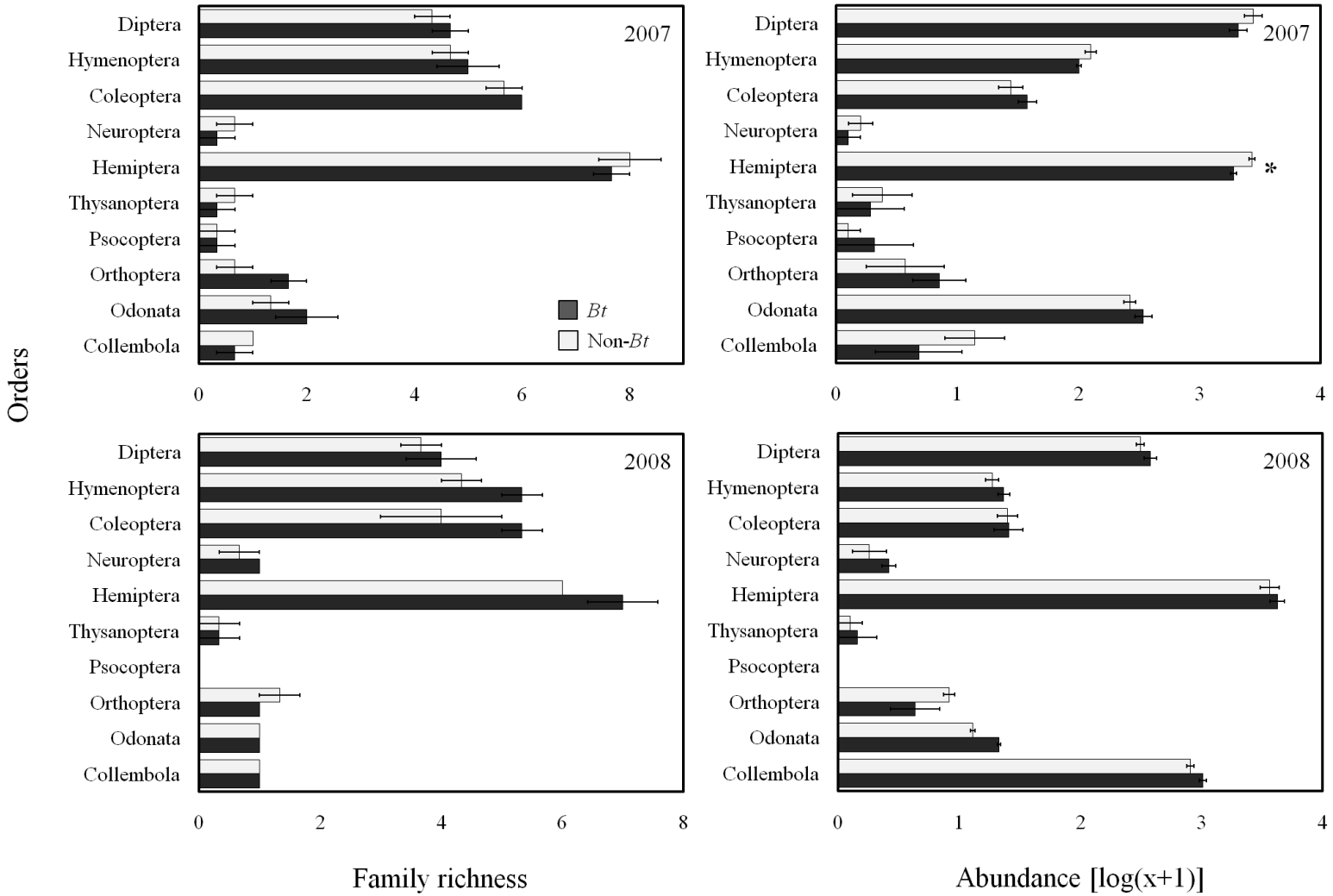

Figure 3. Comparison of the family richness (mean±SE) and abundance (mean±SE) of insects by order in *Bt* and non-*Bt* rice in 2007 and 2008 (n=3)

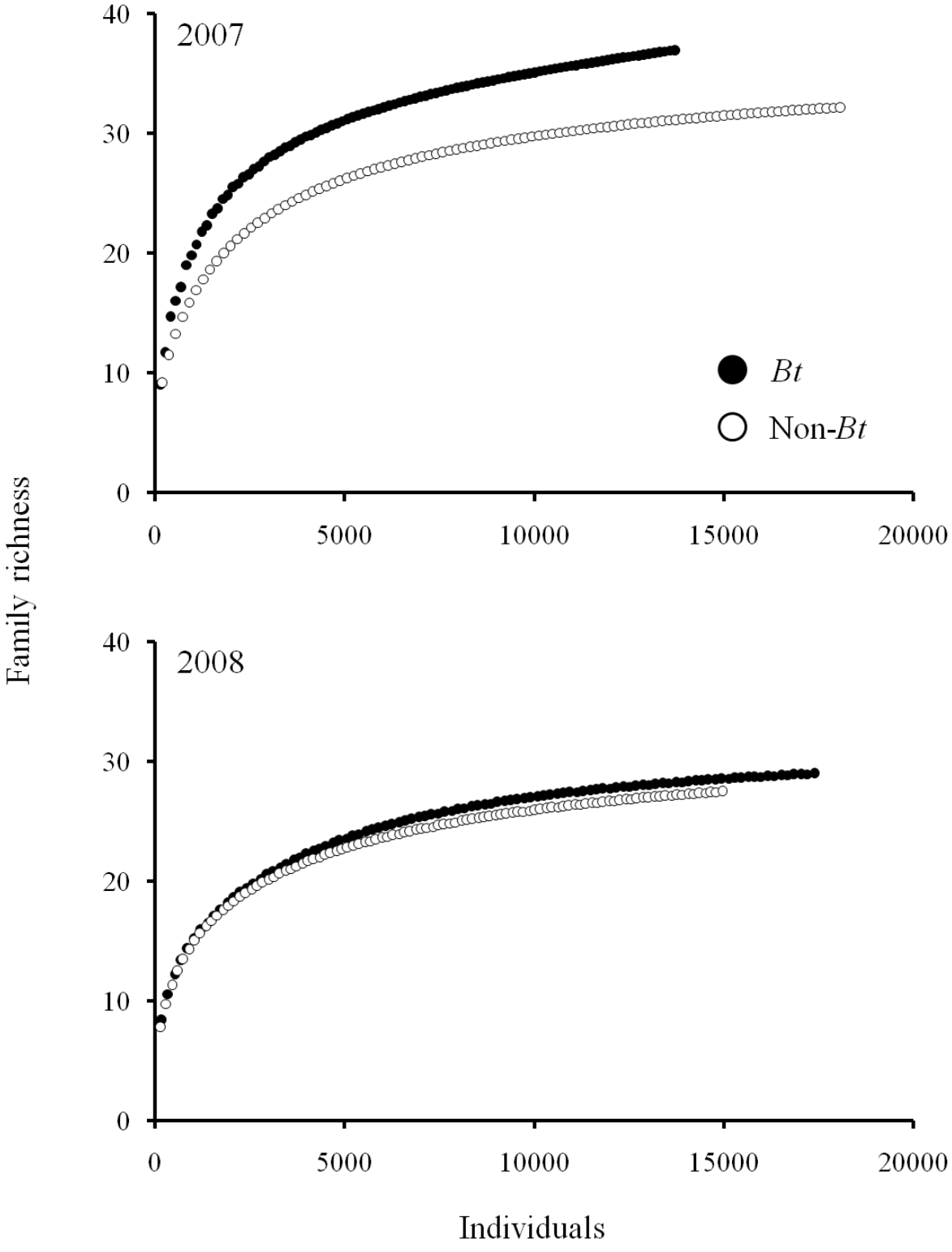

Figure 4. Comparison of family rarefaction curves of insects in *Bt* rice and non-*Bt* rice fields throughout the rice growing season based on number of individuals.

3.4.1.3 Effects of *Bt* rice on insect guild

The insect ecological guilds comprised of 60.4±2.3% herbivore, 34.3±2.0% detritivore, 4.1±0.3% predator and 1.2±0.1% parasitoids in *Bt* rice fields. The corresponding values of non-*Bt* rice fields were 59.2±2.9%, 36.6±2.9%, 2.9±0.23% and 1.3±0.1%, respectively (Fig. 5). Herbivores were the most abundant and parasitoids were the least in *Bt* rice and non-*Bt* rice fields (Fig. 5). Abundance of ecological guilds was higher in non-*Bt* rice fields than *Bt* except predators in 2007 vice versa in 2008. Especially, herbivore was significantly higher in non-*Bt* rice fields than *Bt* rice fields in 2007 ($F_{1,4}$=8.21, P=0.0457) and predator was significantly higher in *Bt* rice fields than non-*Bt* rice fields in 2008 ($F_{1,4}$=10.26, P=0.0328, Fig. 6).

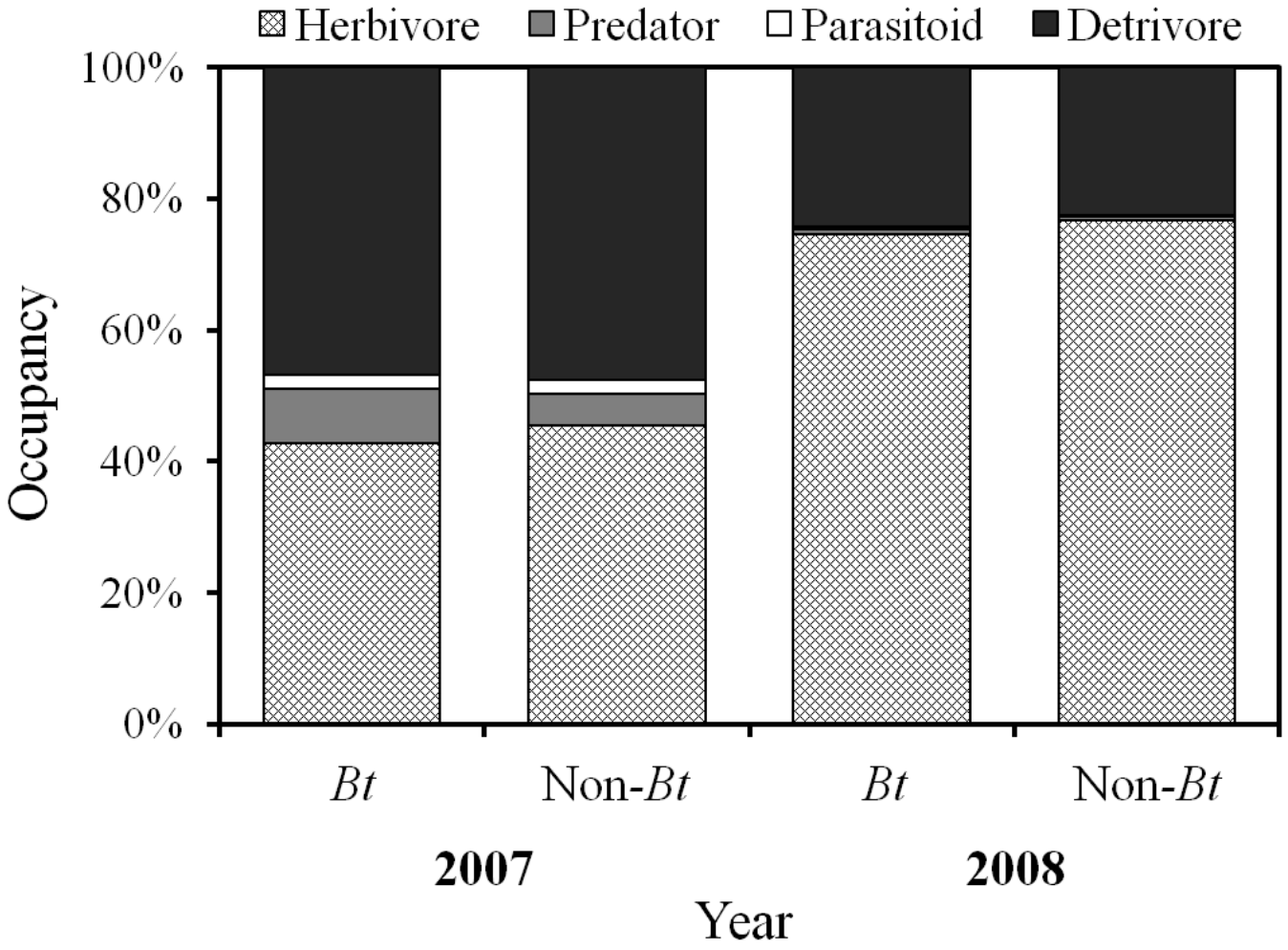

Figure 5. Proportion of insect guilds (mean±SE) in the *Bt* rice and non-*Bt* rice fields throughout the rice growing season

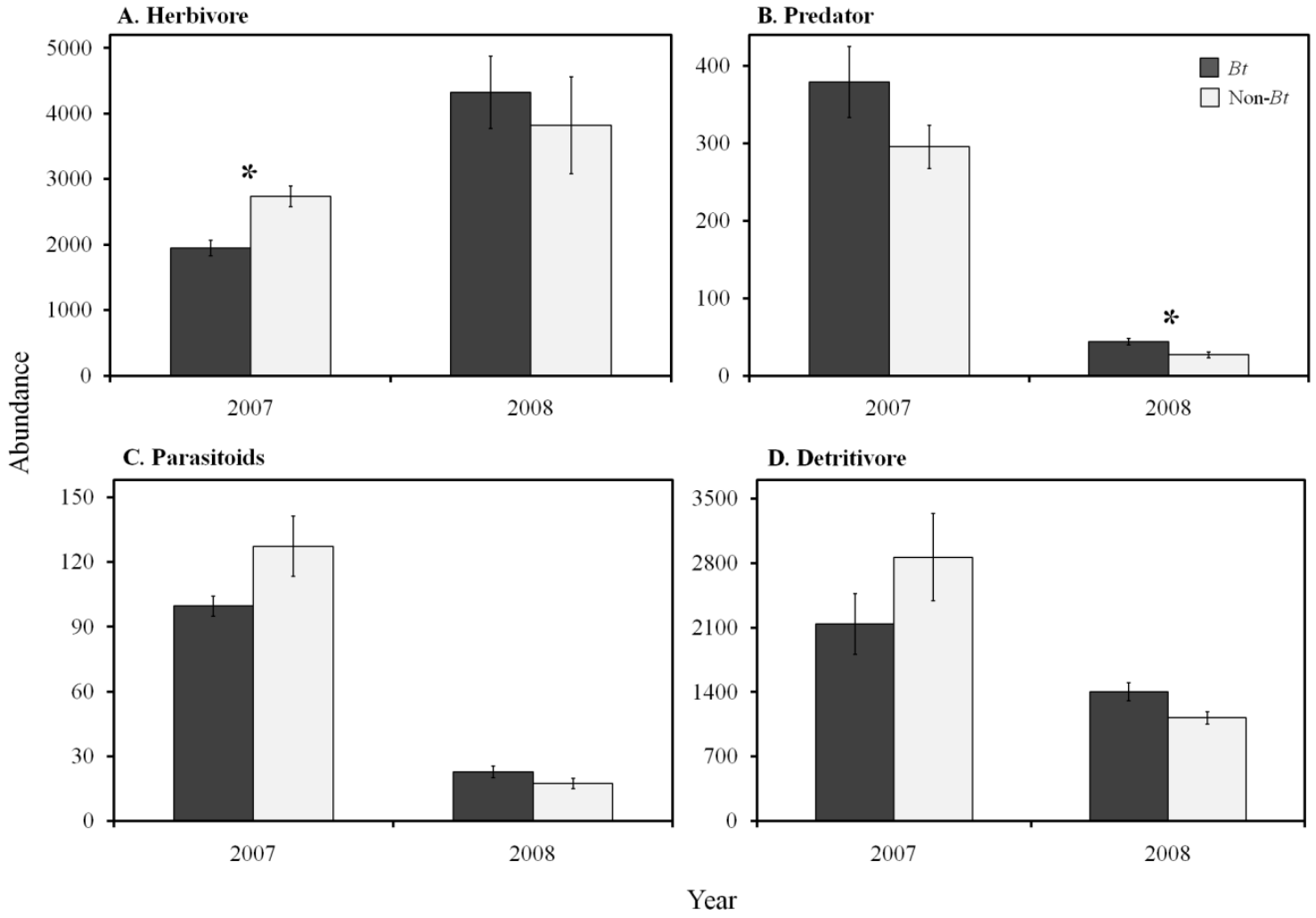

Figure 6. Comparison of insect guild in *Bt* and non-*Bt* rice throughout the rice growing season for 2 years (n=3)

Seasonal change of abundance of insect guilds oscillated in the season and year, but showed very similar serrated seasonality between *Bt* rice and non-*Bt* rice fields (Fig. 7). Though there were some significant differences of abundance between *Bt* rice and non-*Bt* rice fields in herbivore of 56 days ($F_{1,4}$=17.89, P=0.0134), 70 days ($F_{1,4}$=19.77, P=0.0353) and 84 days ($F_{1,4}$=14.88, P=0.0182) after transplanting in 2007 and predator of 14 days ($F_{1,4}$=12.76, P=0.0233), parasitoids of 98 days ($F_{1,4}$=8.25, P=0.0454) and detritivore of 42 days ($F_{1,4}$=8.92, P=0.0454), 56 days ($F_{1,4}$=8.25, P=0.0454) and 98 days ($F_{1,4}$=10.12, P=0.0335) after transplanting in 2008, there was no significant difference in *Bt* rice fields compared to non-*Bt* overall (Table 2).

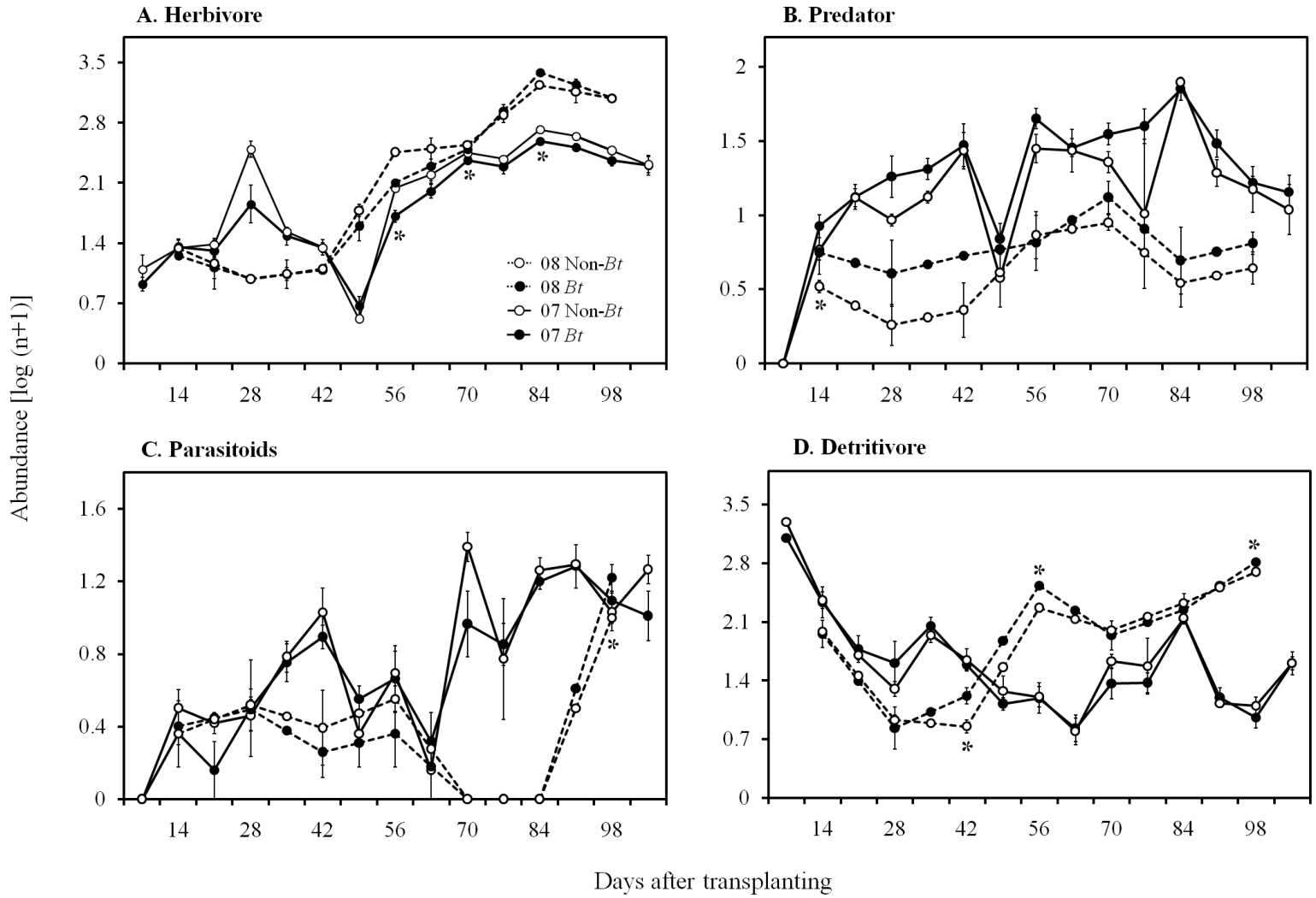

Figure 7. Seasonal dynamics of insect guilds (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season. Abundance was individual numbers of 5 samples and a sample consisted of one suction sampling and five sweepings.

3.4.1.4 Effects of *Bt* rice on dominant families

Abundant families by ecological functional guilds were Cicadellidae occupying 58.2±2.5% and Delphacidae 23.0±1.0% of total herbivores, Chironomidae occupying 68.9±2.6% and Tomoceridae occupying 39.7±3.2% of total detritivores, Coenagrionidae occupying 86.6±4.2% of total predators and Braconidae occupying 45.6±1.8% and Drynidae occupying 39.7±3.2% of total parasitoids in *Bt* rice fields. The corresponding values of non-*Bt* rice fields were 56.6±8.3% and 26.2±5.6% of total herbivores, 77.7±3.5%, 21.5±3.5% of total detritivores, 86.3±2.2% of total predators and 38.7±2.0% and 49.9±2.4% of total parasitoids, respectively and the occupancy rate of each dominant families were not significantly different between *Bt* rice and non-*Bt* rice fields (Fig. 8).

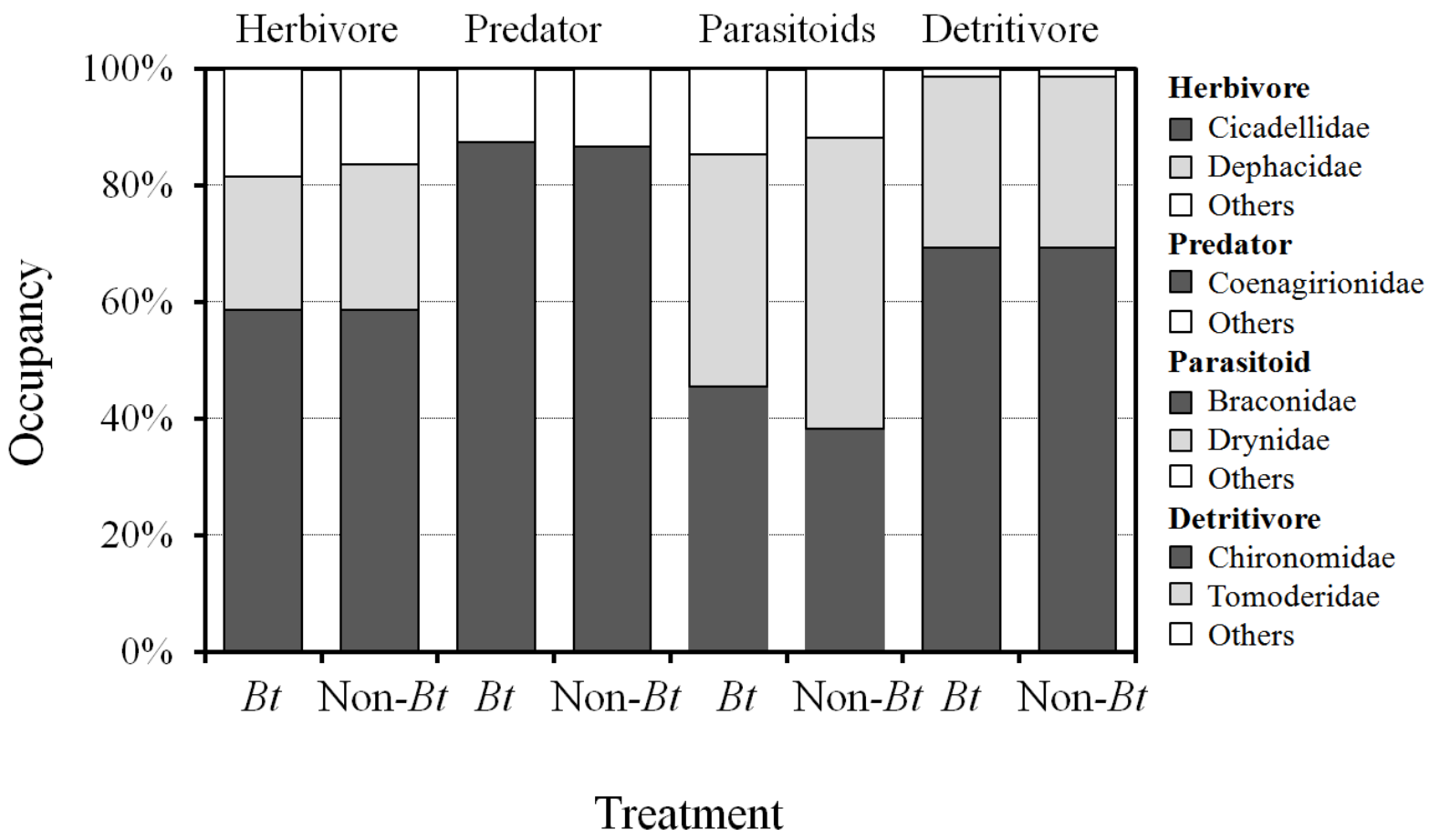

Figure 8. Proportion of dominant insect family (mean±SE) according to insect guilds in the *Bt* rice and non-*Bt* rice fields throughout the rice growing season

The most dominant family was Chironomidae in 2007 (46.8% of total number of individuals) and Cicadellidae in 2008 (52.2% of total number of individuals) and no significant differences were observed for these families with the other 5 dominant families when comparing *Bt* rice fields to non-*Bt* for 2 years (Fig. 9-1~4). Moreover, there were no significant diffrernces on seasonality of 5 dominant families between *Bt* rice and non-*Bt* rice fields showing similar pattern (Fig. 9-1~4). According to Sørenson quantitative coefficient, similarity of insect ecological guilds between *Bt* rice and non-*Bt* rice fields was high ranging between 0.800 to 1.000 and that of detritivore was higher than the other guilds (Table 3).

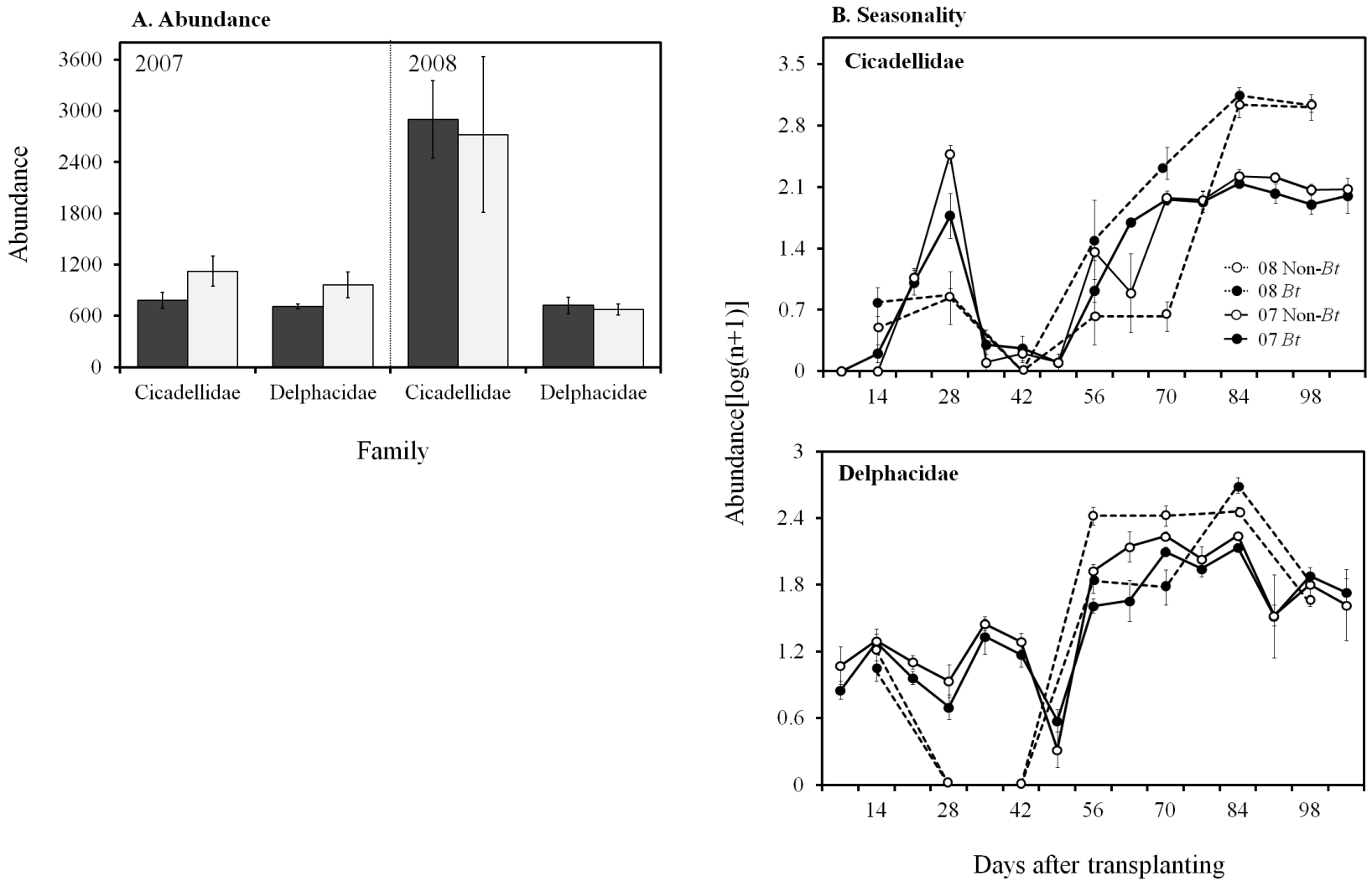

Figure 9-1. Comparison of dominant herbivore family (mean±SE) in *Bt* and non-*Bt* rice throughout the rice growing season.

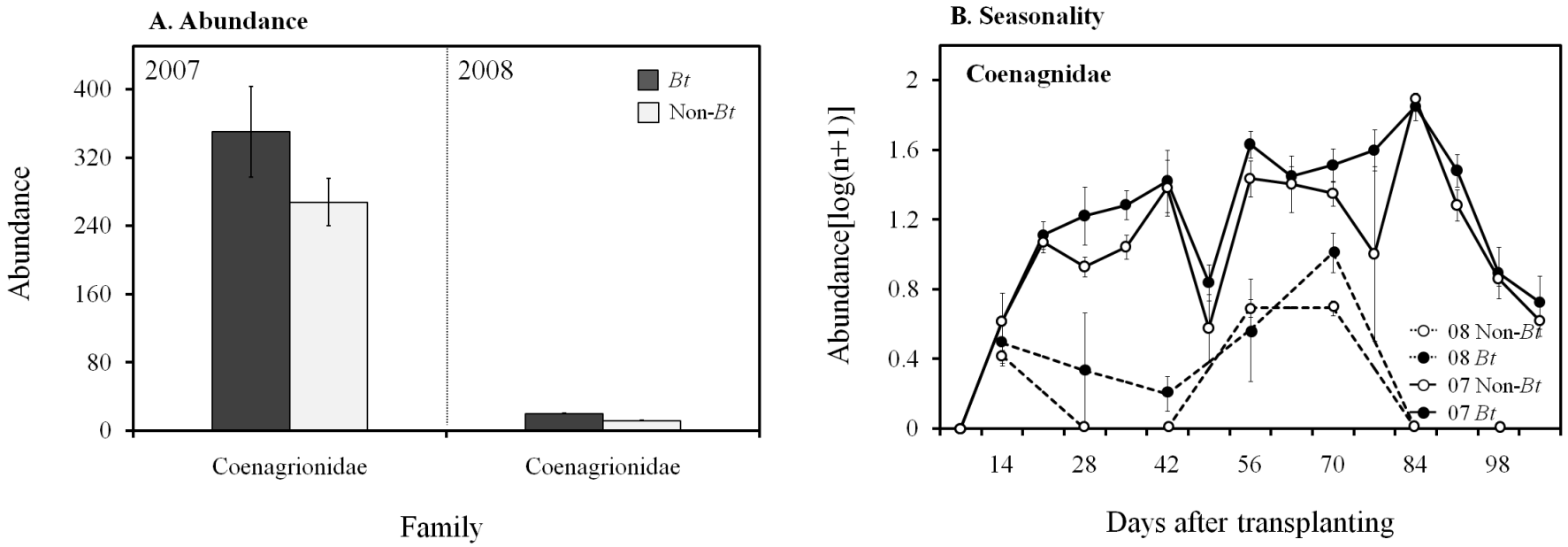

Figure 9-2. Comparison of dominant predator family (mean±SE) in *Bt* and non-*Bt* rice throughout the rice growing season.

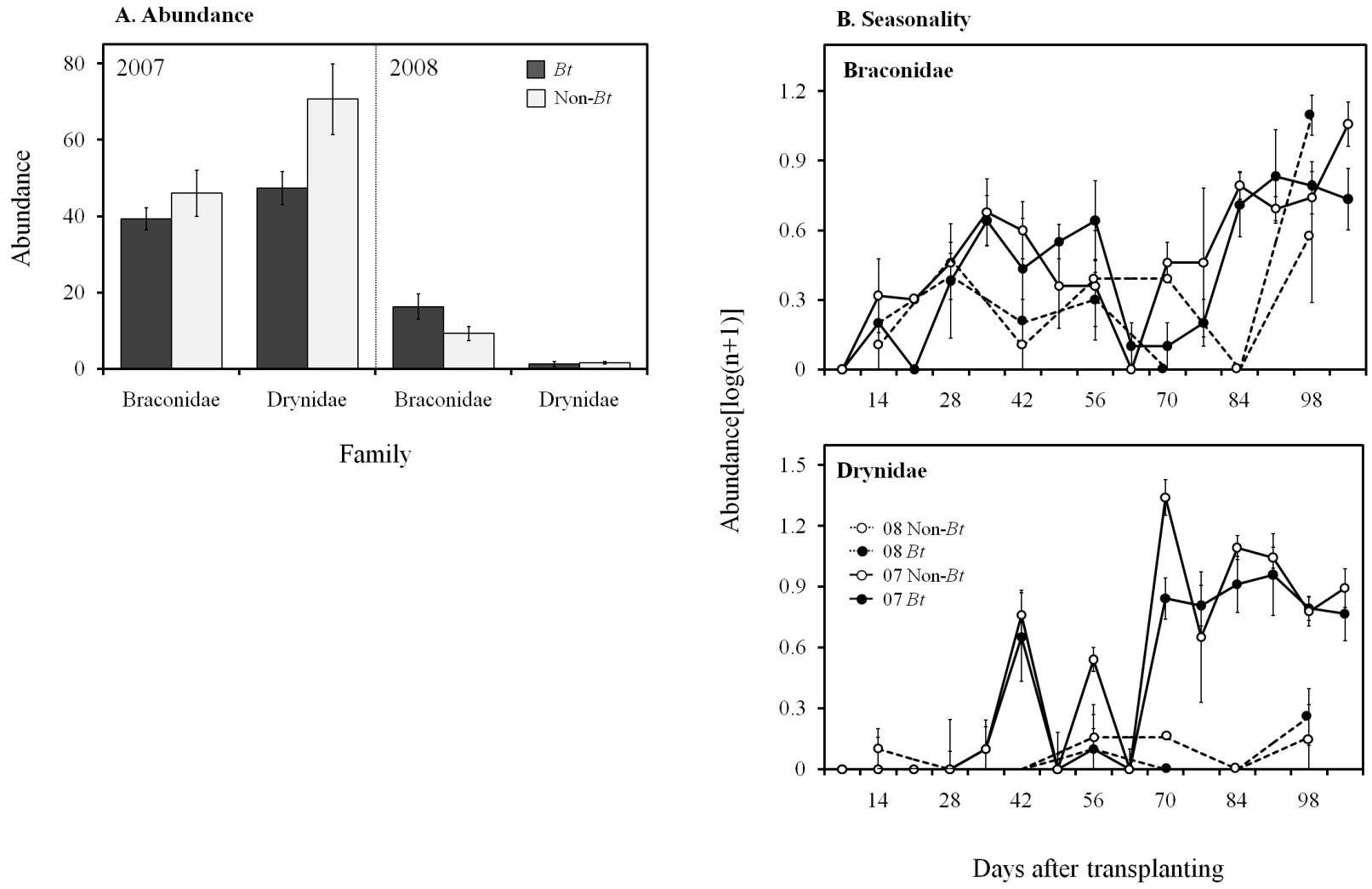

Figure 9-3. Comparison of dominant parasitoid family (mean±SE) in *Bt* and non-*Bt* rice throughout the rice growing season.

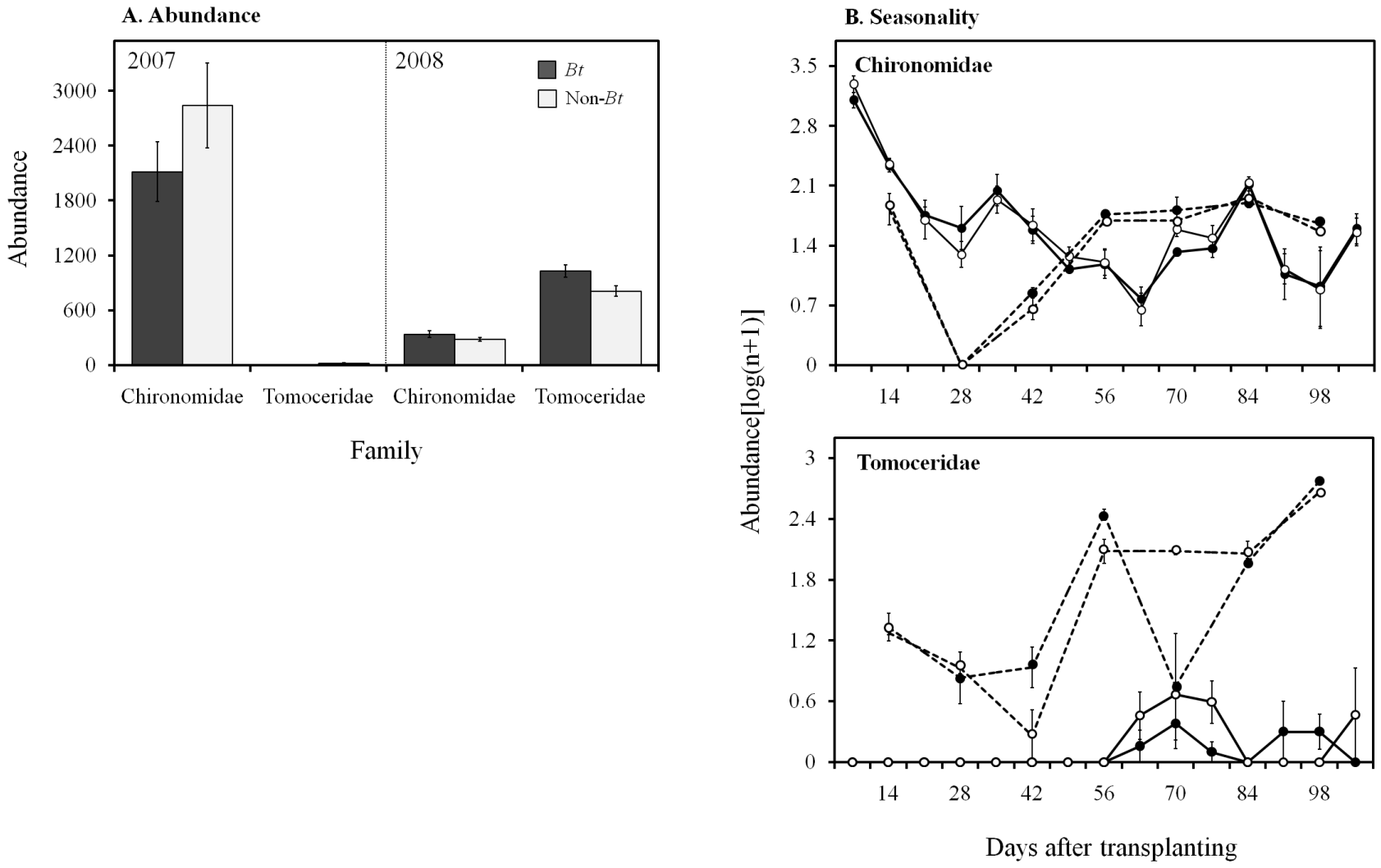

Figure 9-4. Comparison of dominant detritivore family (mean±SE) in *Bt* and non-*Bt* rice throughout the rice growing season.

Table 3. Similarity of insect communities between *Bt* rice and non-*Bt* rice fields throughout the rice growing season according to Sørenson quantitative coefficient

Functional group	Surveyed year	
	2007	2008
Total	0.917 (33/72)	0.852 (26/61)
Herbivores	0.857 (12/28)	0.857(9/21)
Predators	0.833 (10/24)	0.800 (8/20)
Parasitoids	1.000 (5/10)	0.833 (5/12)
Detritivores	1.000 (5/10)	1.000 (4/8)

Numbers in parentheses are shared species/total species

3.4.2 Effects of *Bt* rice on spider community

3.4.2.1 Overall spider community of rice fields

A Total of 29 species in 23 genera and 9 families were identified from 2,372 and 2,565 collected spiders in 2007 and 2008, respectively (Table 4). At the family level, four families (Lycosidae, Linyphiidae, Theridiidae and Tetragnathidae) were dominant on species richness and abundance, occupying 72.4% and 92.4%, respectively for 2 years (Fig. 14). Six dominant spider species, ranging from 4.2% to 36.9%, were *Gnathonarium dentatum* and *Ummeliata insecticeps* (Linyphiidae), *Pachygnatha clercki* and *Tetragnatha maxillosa* (Tetragnathidae), *Pirata subpiraticus* (Lycosidae), and *Clubiona kurilensis* (Clubionidae). They accounted for 84.1% and 79.8% of all spiders collected in the rice fields in 2007 and 2008, respectively. The most abundant species was *P. subpiraticus* (36.9% of total number of individuals), followed by *U. insecticeps* (18.4%). Of the 29 species collected, 10 species were represented by <10 individuals, *Nesticella mogera* (Nestidae), *Parasteatoda angulithorax* (Theridiidae), *Erigone koshiensis*, and *Ummeliata feminea* (Linyphiidae), *Pachygnatha tenera* and *Tetragnatha extensa* (Tetragnathidae), *Argiope bruennichi* and *Larinioides cornutus* (Araneidae), *Arctosa ebicha* (Lycosidae), and *Ebrechtella tricuspidata* (Thomisidae), occupying 34% of total number of species and 0.6% of total number of individuals (Table 4). Five of these species, *N. mogera*, *E. koshiensis*, *P. tenera*, *T. extensa*, and *A. ebicha*, were represented by only a single individual. In guild structure, 7 species in 3 families (Lycosidae, Pisauridae and Clubionidae) with 2,231 individuals were wandering-active spiders, 5 species in 1 family (Lynyphiidae) with 1,147 individuals were web-sheet spiders, 9 species in 2 families (Araneidae and Tetragnathidae) with 864 individuals were web-orb spiders, 6 species in 2 families (Nesticidae and Theridiidae) with 661 individuals were web-matrix

spiders and 2 species in 1 family (Thomisidae) with 34 individuals were wandering-ambush spiders . The most dominant guild was the wandering-active spider, occupying 24% of total number of species and 45% of total number of individuals (Table 4).

Table 4. Spiders recorded in *Bt* and non-*Bt* rice throughout the rice growing season.

Family	Guild[a]	Scientific name	Sampling Method[b]	2007		2008	
				Bt	Non-*Bt*	*Bt*	Non-*Bt*
Nesticidae	W/M	*Nesticella mogera* (Yaginuma 1972)	Su	-	1	-	1
Theridiidae	W/M	*Chrysso octomaculata* (Bösenberg et Strand 1906)	Su/Sw	7	9	38	49
		Enoplognathaabrupta (Karsch 1879)	Su/Sw	51	55	21	31
		Paidiscura subpallens (Bösenberg et Strand 1906)	Su	17	15	3	6
		Parasteatoda angulithorax (Bösenberg et Strand 1906)	Sw	-	-	1	-
		Parasteatoda oculiprominens (Saitō 1939)	Su/Swg	3	13	130	210
Linyphiidae	W/S	*Bathyphantes gracilis* (Blackwall 1841)	Su/Sw	1	-	16	7
		Erigone koshiensis Oi 1960	Su	-	-	1	-
		Gnathonarium dentatum (Wider 1834)	Su/Sw	45	76	48	37
		Ummeliata feminea (Bösenberg et Strand 1906)	Su	1	7	-	-
		Ummeliata insecticeps (Bösenberg et Strand 1906)	Su/Sw	25	20	423	440
Tetragnathidae	W/O	*Pachygnatha clercki* Sundevall 1823	Su/Sw	75	65	102	75
		Pachygnatha tenera Karsch 1879	Su	-	-	-	1
		Tetragnatha extensa (Linnaeus 1758)	Sw	-	-	1	-

		Tetragnatha maxillosa Thorell 1895	Su/Sw	152	163	16	40
		Tetragnatha pinicola L. Koch 1870	Su/Sw	15	14	-	-
		Tetragnatha vermiformis Emerton 1884	Su/Sw	-	-	32	53
Araneidae	W/O	*Argiope bruennichi* (Scopoli 1772)	Sw	-	1	-	1
		Larinioides cornutus (Clerck 1757)	Su/Sw	3	1	2	-
		Neoscona adianta (Walckenaer 1802)	Su/Sw	10	24	9	9
Lycosidae	W/Ac	*Arctosa ebicha* Yaginuma 1960	Su/Sw	-	-	-	1
		Arctosa stigmosa (Thorell 1879)	Su/Sw	34	50	10	7
		Pardosa laura Karsch 1879	Su/Sw	3	1	4	2
		Pirata subpiraticus (Bösenberg et Strand 1906)	Su/Sw	634	596	331	263
		Trochosa ruricola (De Geer 1778)	Su/Sw	9	7	1	1
Pisauridae	W/Ac	*Dolomedes sulfureus* L. Koch 1877	Su/Sw	1	5	4	4
Clubionidae	W/Ac	*Clubiona kurilensis* Bösenberg et Strand 1906	Su/Sw	86	59	55	63
Thomisidae	W/Am	*Ebrechtella tricuspidata* (Fabricius 1775)	Sw	2	5	-	1
		Xysticus hedini Schenkel 1936	Su/Sw	7	4	9	6

[a] Guild of spiders: **W/Ac**, wand-active, **W/Am**, Wand-ambush, **W/M**, web-matrix, **W/O**, web-orb, **W/S**, web-sheet.

[b] ASampling metod: **Su**,Suction, **Sw**, Sweeping

3.4.2.2 Effects of *Bt* rice on biodiversity

Twenty five species in 21 genera and 8 families with 2,438 individuals and 26 species in 21 genera and 9 families with 2,499 individuals were identified in *Bt* and non-*Bt* rice, respectively (Table 4). Species richness, abundance and Shannon's index of spiders during the season were higher in 2008 than in 2007, but were very similar between *Bt* and non-*Bt* rice with no significant differences (Table 5).

Table 5. Biodiversity (mean±SE, n = 3) of spider community per plot in *Bt* and non-*Bt* rice throughout the rice growing season

Year	Treatment		RM-ANOVA *F*-ratio (*P*-value)		
	Bt	Non-*Bt*	Treatment (*T*)	Year (*Y*)	Interaction (*T*x*Y*)
Species richness					
2007	16.33±1.20	18.33±0.67	$1.68_{1,4}$(0.265)	$53.33_{1,8}$ (<.0001)	$0.49_{1,8}$ (0.504)
2008	18.00±1.00	17.33±0.67	$0.61_{1,4}$(0.477)		
Abundance					
2007	393.67±16.56	396.67±28.85	$0.77_{1,4}$ (0.431)	$34.90_{1,8}$ (<.0001)	$1.68_{1,8}$ (0.265)
2008	419.00±16.46	435.67±25.37	$7.49_{1,4}$ (0.052)		
Shannon's index					
2007	1.71±0.13	1.83±0.04	$4.32_{1,4}$(0.173)	$34.90_{1,8}$ (<.0001)	$1.68_{1,8}$ (0.265)
2008	1.96±0.05	2.01±0.06	$0.13_{1,4}$(0.732)		

Although significant differences were found in species richness at 7 ($F_{1,4}$=13.08, P=0.0224), 56 ($F_{1,4}$=20.58, P=0.0105) and 105 ($F_{1,4}$=13.75, P=0.0207) DAT in 2007 and at 56 ($F_{1,4}$=13.92, P=0.0203) DAT in 2008, and in the Shannon's index at 56 days ($F_{1,4}$=7.93, P=0.0480) DAT in 2008 (Fig. 10), overall, they were not significantly different between *Bt* and non-*Bt* rice and interaction between treatment and year (Table 5).

Species richness and abundance of spider families were also not significantly different between *Bt* and non-*Bt* rice except for the abundance of Theridiidae which was lower in *Bt* rice in 2008 ($F_{1,4}$=14.11, P=0.019, Fig. 11) due to the *Parasteatoda oculiprominens* occupying 69.5% of Theridiidae in 2008 . Species rarefaction curves reached asymptote as sample size and insect individual numbers increased, indicating total sample size for this study was proper Also, these curves were almost the same between *Bt* and non-*Bt* rice for 2 years (Fig. 12)

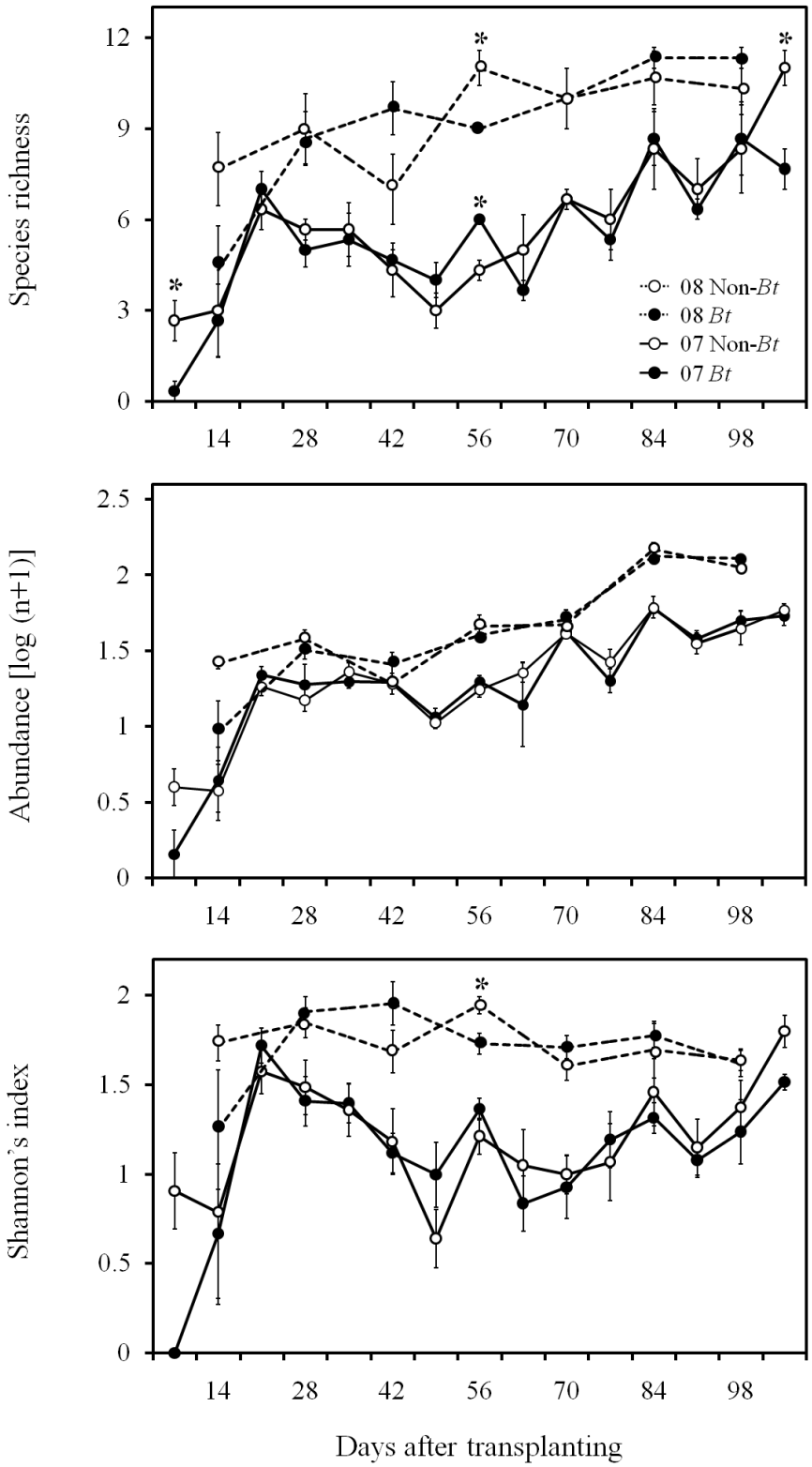

Figure 10. Seasonal dynamics of spiders (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season. Abundance was individual numbers of 5 samples and a sample consisted of one suction sampling and five sweepings.

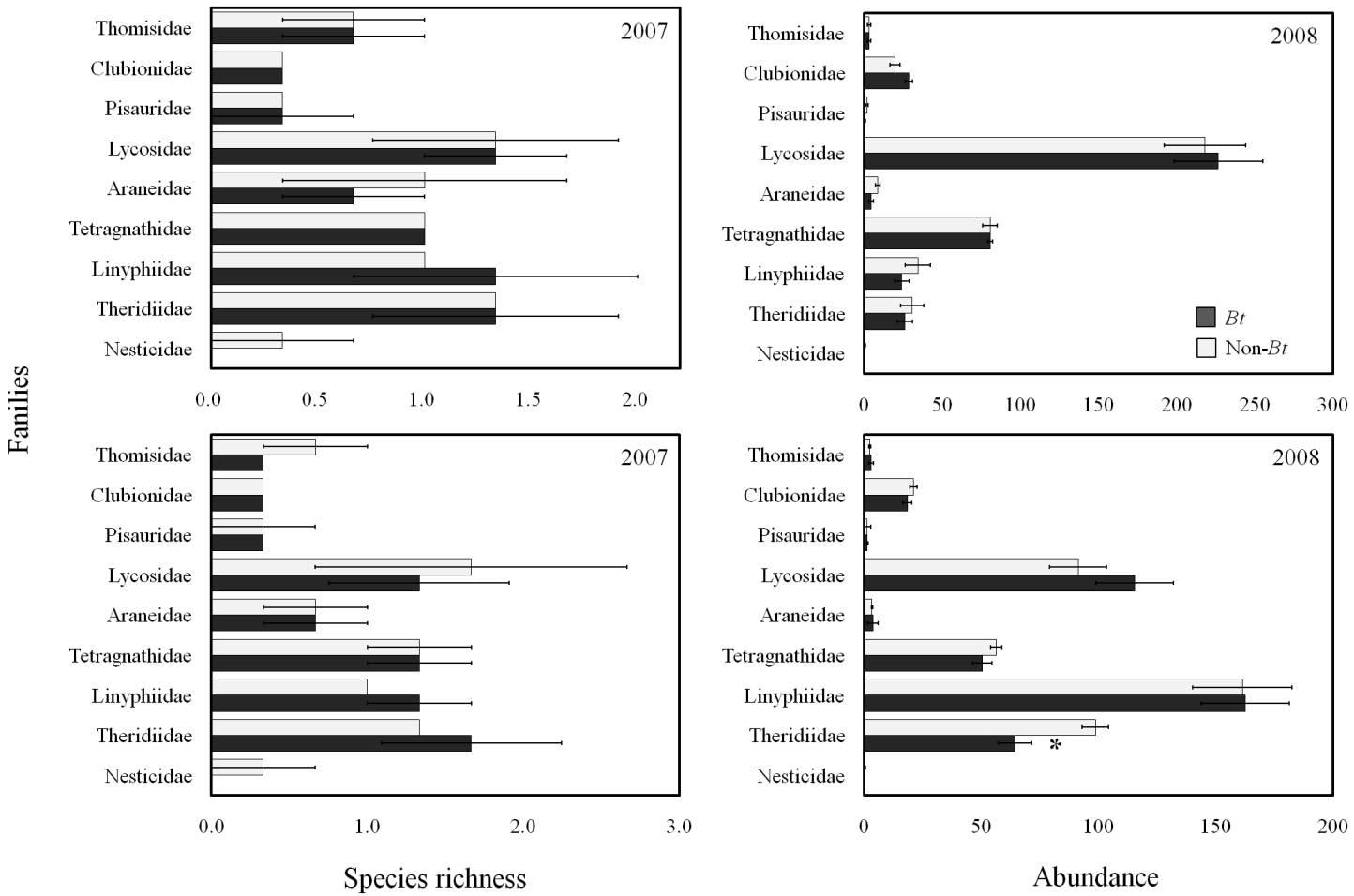

Figure 11. Comparison of the species richness (mean±SE) and abundance (mean±SE) of spiders by family in *Bt* and non-*Bt* rice in 2007 and 2008 (n=3)

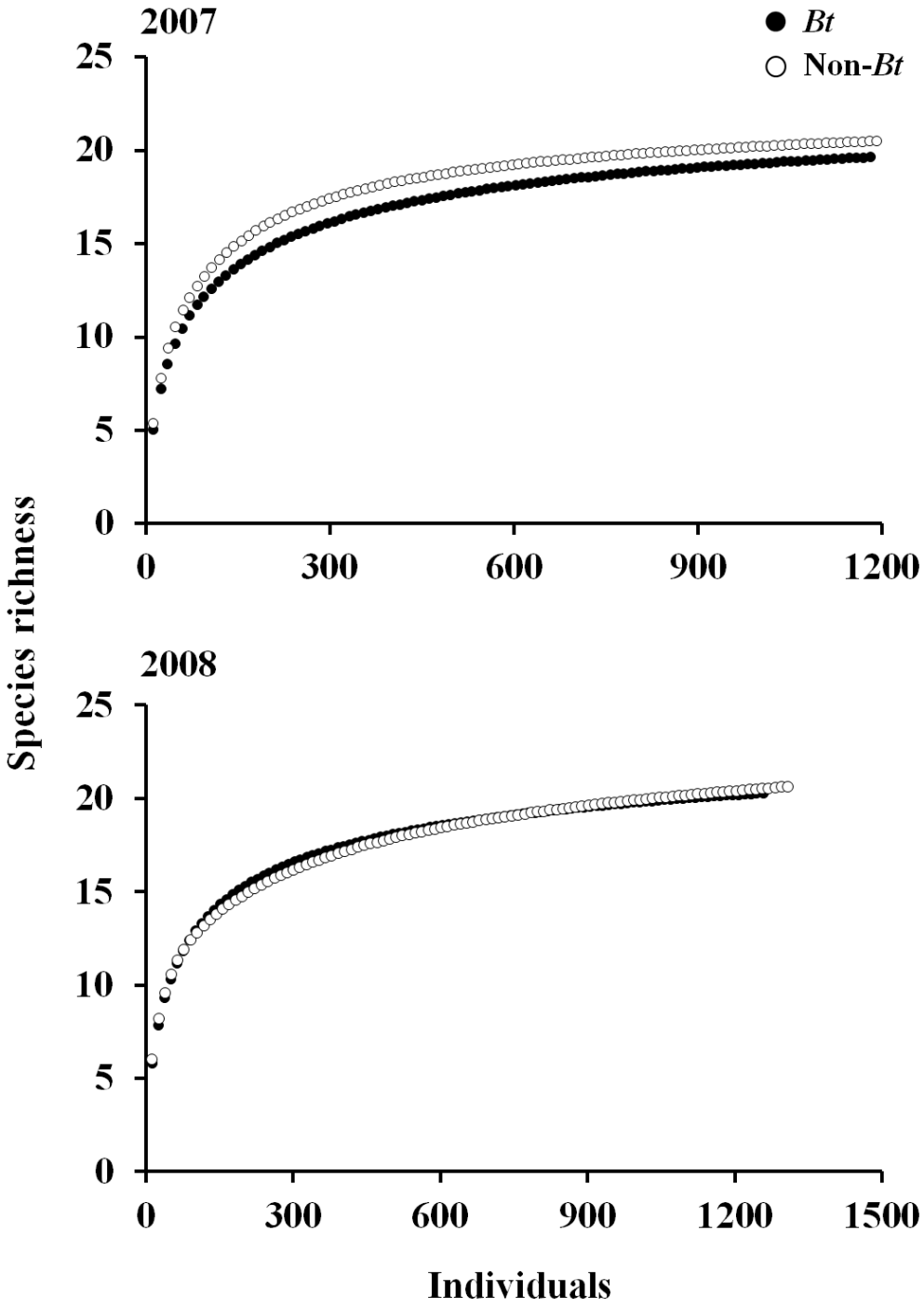

Figure 12. Comparison of species rarefaction curves of spiders in *Bt* and non-*Bt* rice based on number of individuals.

3.4.2.3 Effects of *Bt* rice on dominant species

The spider community was dominated by six species, occupying 81.7±2.2% (Mean±SE) in *Bt* rice and 76.2±2.7% in non-*Bt* rice (Fig. 13). The most dominant species was *P. subpiraticus* in 2007 (51.8%) and *U. insecticeps* in 2008 (33.6%). Abundance of dominant spiders during the season were higher in 2007 than in 2008 except for the *U. insecticeps* and *P. clercki*, but were very similar between *Bt* and non-*Bt* rice (Fig. 14.-2, 5). Although significant differences were found on *C. kurilensis* at 105 ($F_{1,4}$=9.98, P=0.0342) DAT and *P. clercki* at 7 ($F_{1,4}$=37.56, P=0.0036) DAT in 2007 and at 42 ($F_{1,4}$=10.69, P=0.0308) DAT in 2008, overall, no significant differences were observed for these 6 dominant species between *Bt* and non-*Bt* rice except for *P. clercki* ($F_{1,4}$=7.81, P=0.0491) and *T. maxillosa* ($F_{1,4}$=7.77, P=0.0494) in 2008 (Fig. 14-1~6).

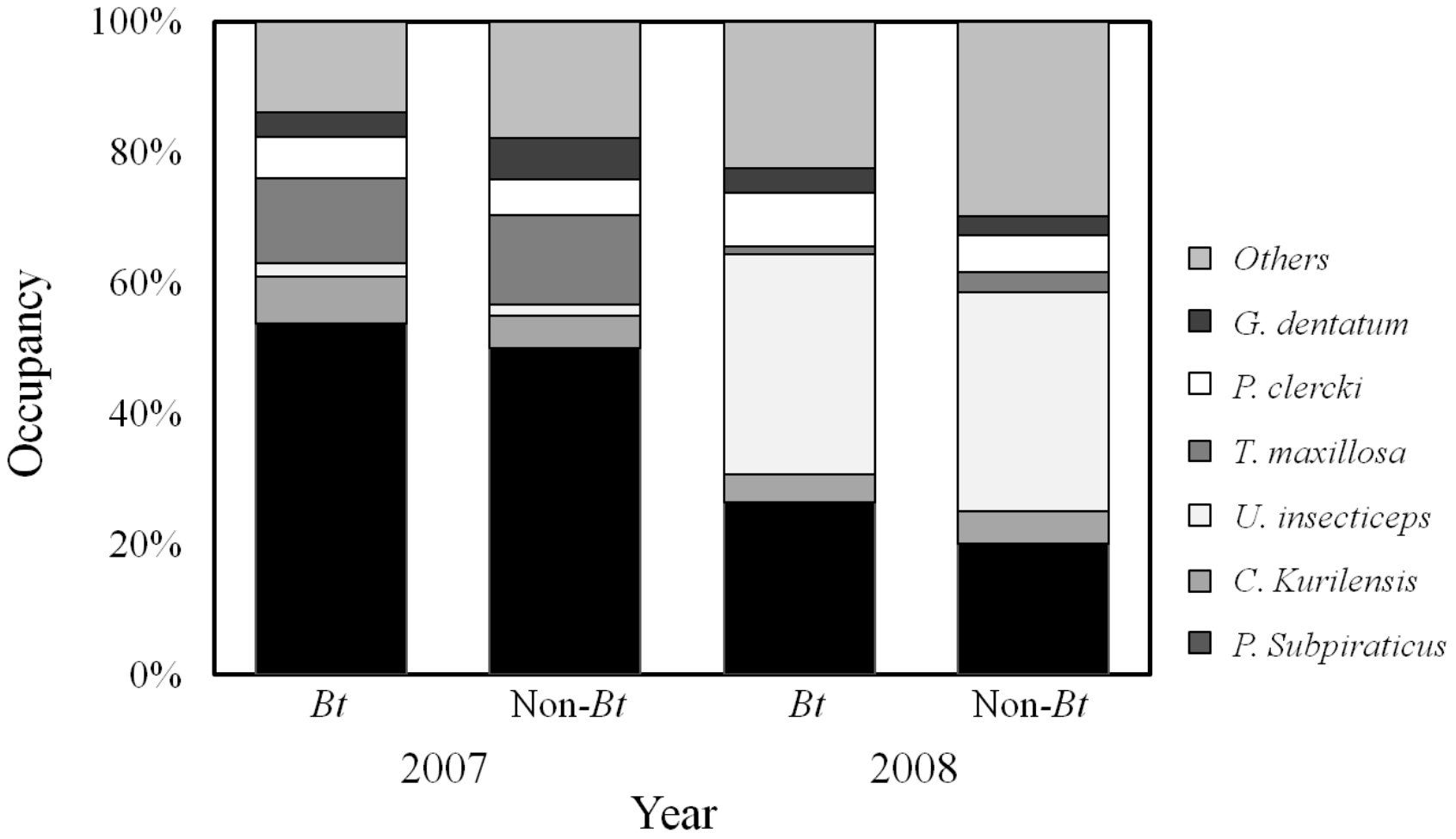

Figure 13. Comparison of dominant spiders in *Bt* and non-*Bt* rice throughout the rice growing season

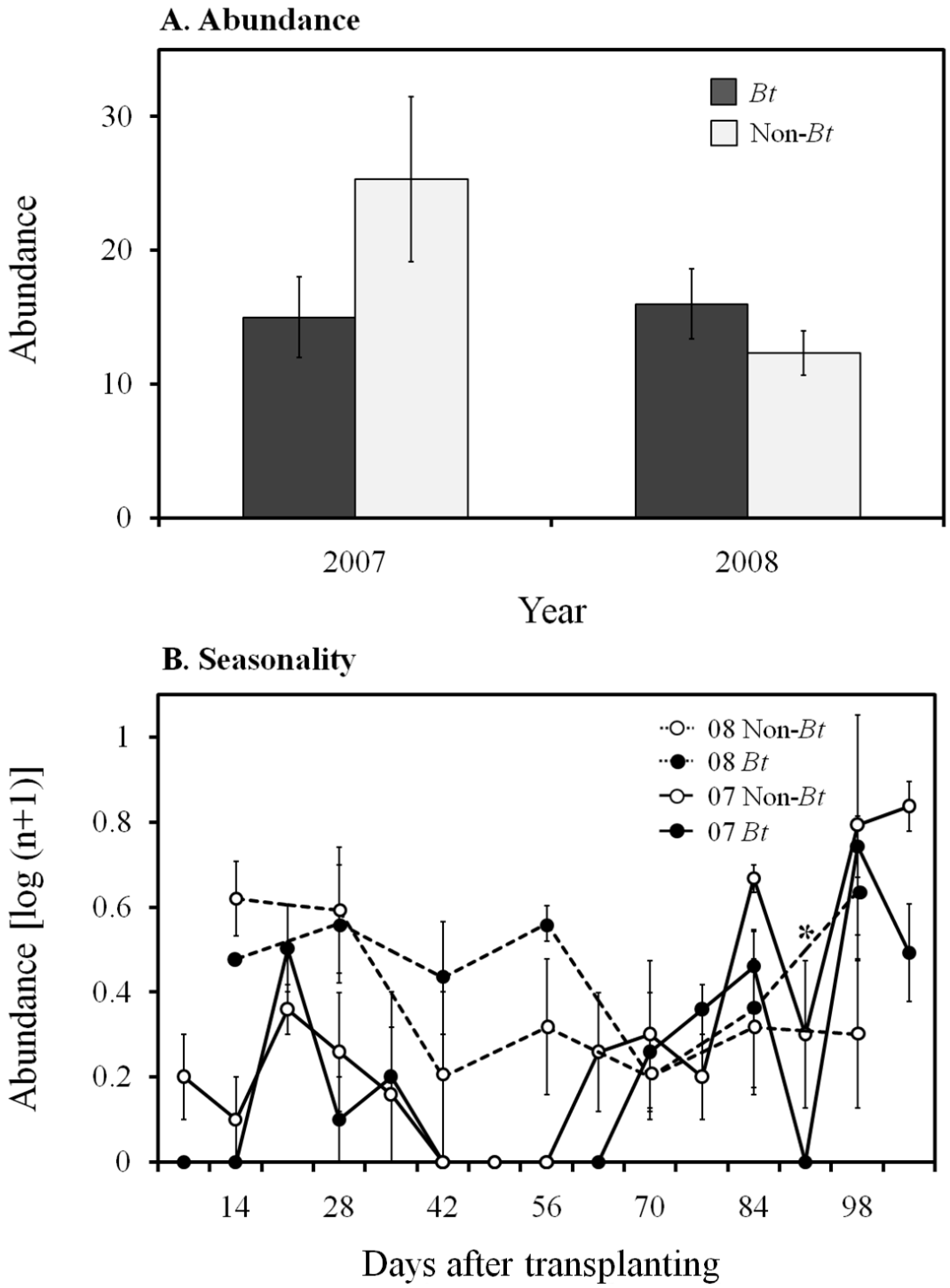

Figure 14-1. Abundance and seasonal dynamics of *G.dentatum* (mean±SE, n = 3) per plot *Bt* and non-*Bt* rice throughout the rice growing season. Abundance was individual numbers of 5 samples and a sample consisted of one suction sampling and five sweepings.

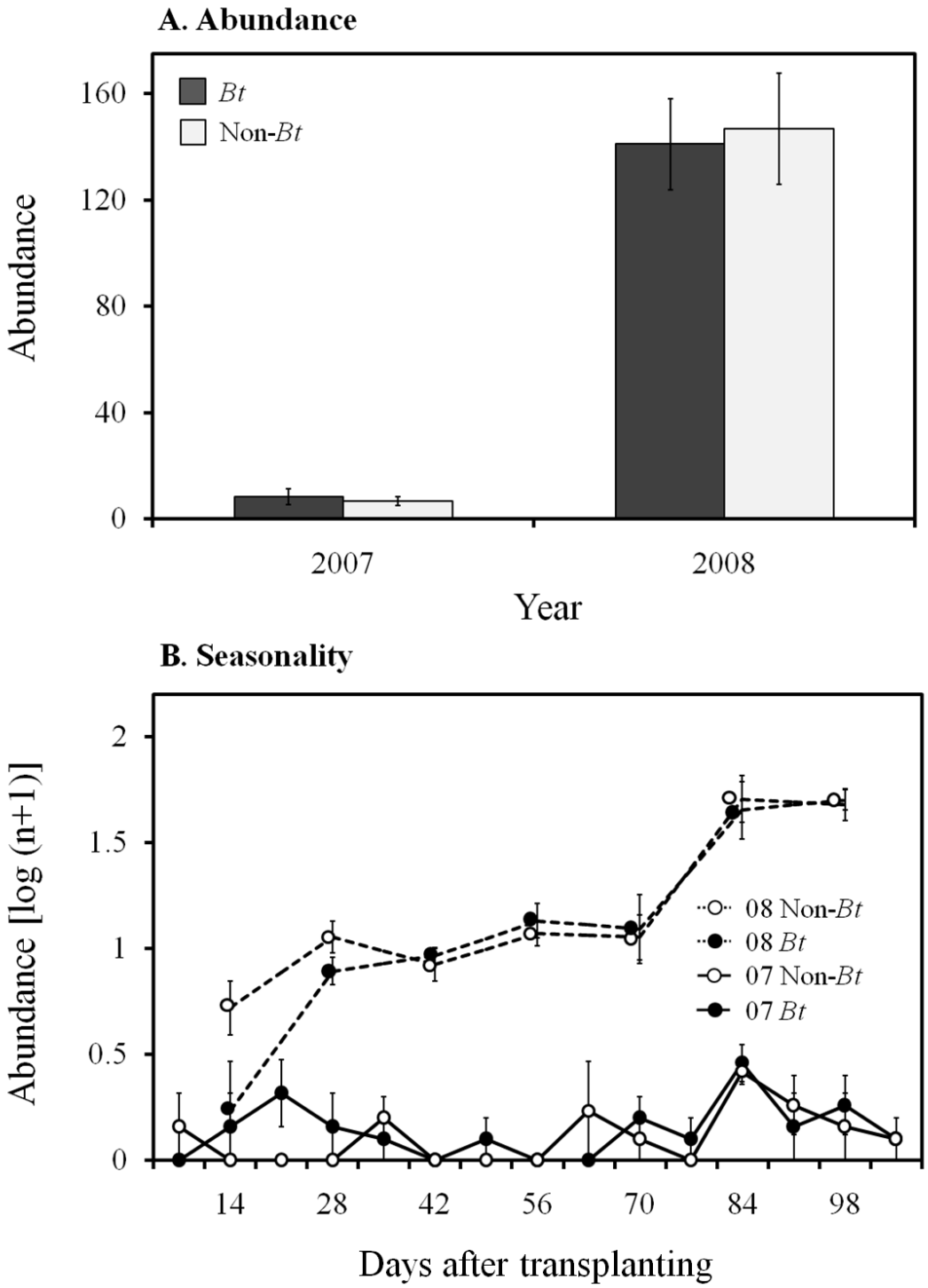

Figure 14-2. Abundance and seasonal dynamics of *U. insecticeps* (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season.

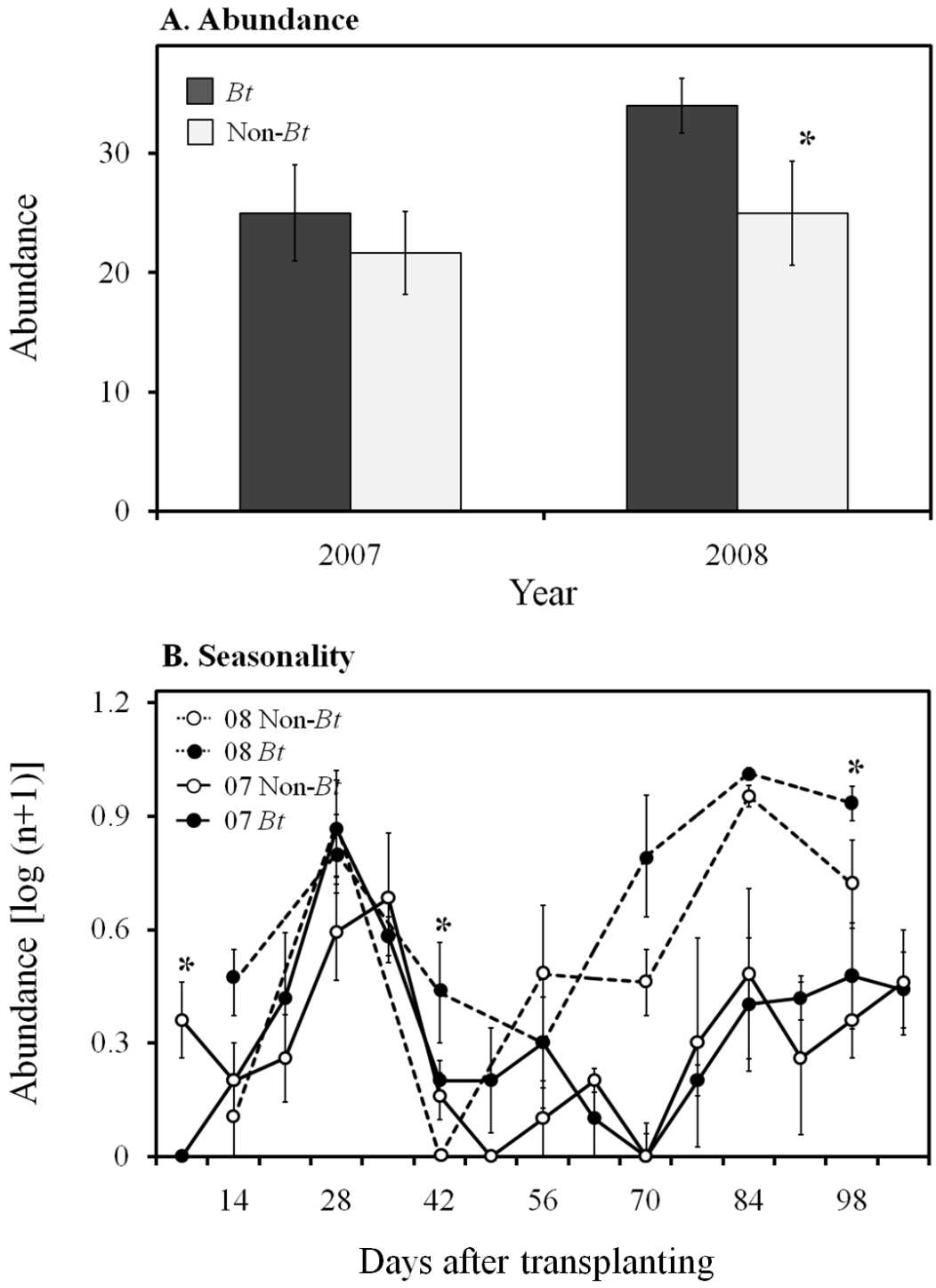

Figure 14-3. Abundance and seasonal dynamics of *P. clercki* (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season.

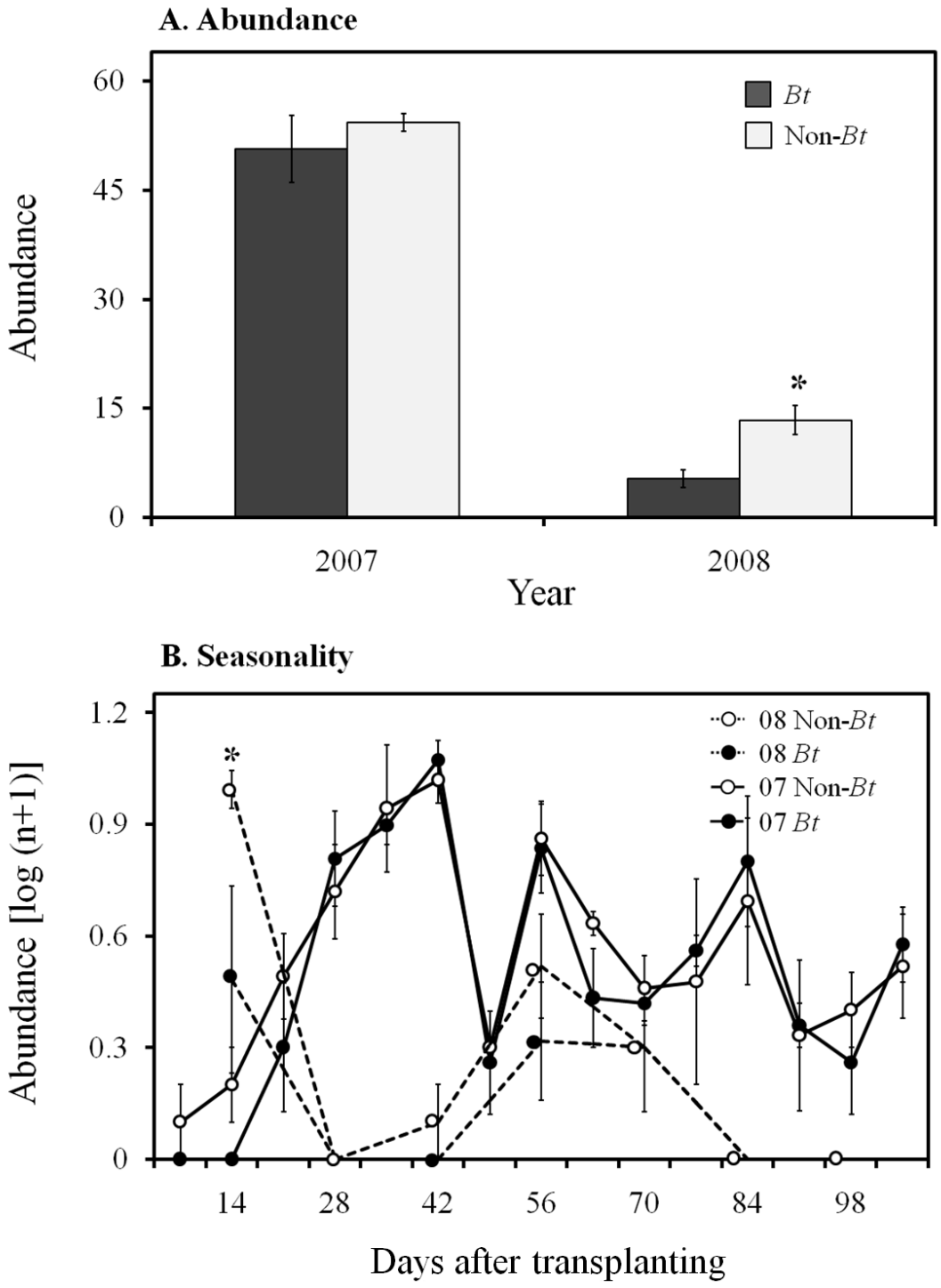

Figure 14-.4. Abundance and seasonal dynamics of *T. maxillosa* (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season.

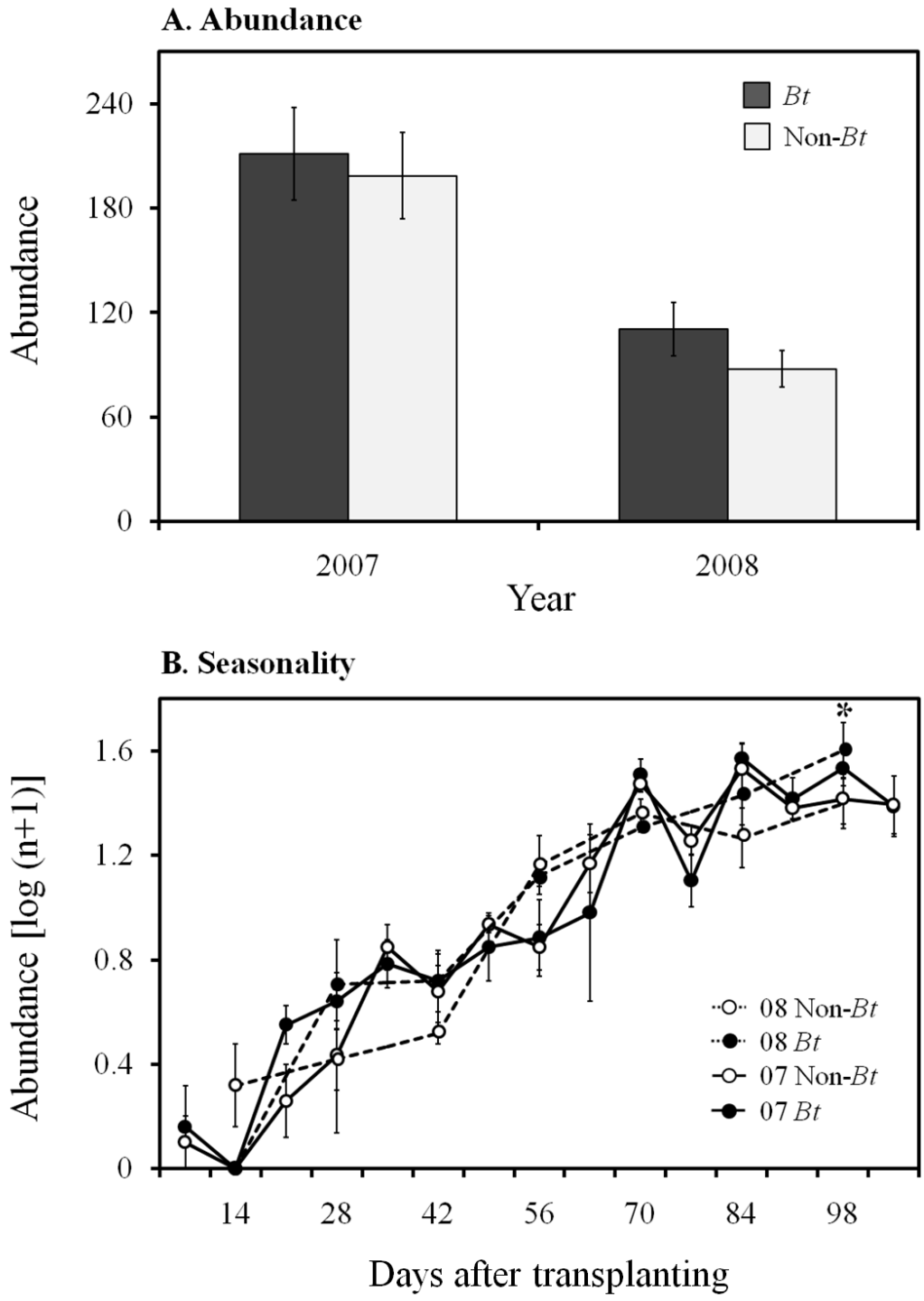

Figure 14-.5 Seasonal dynamics of *P. subpiraticus* (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season.

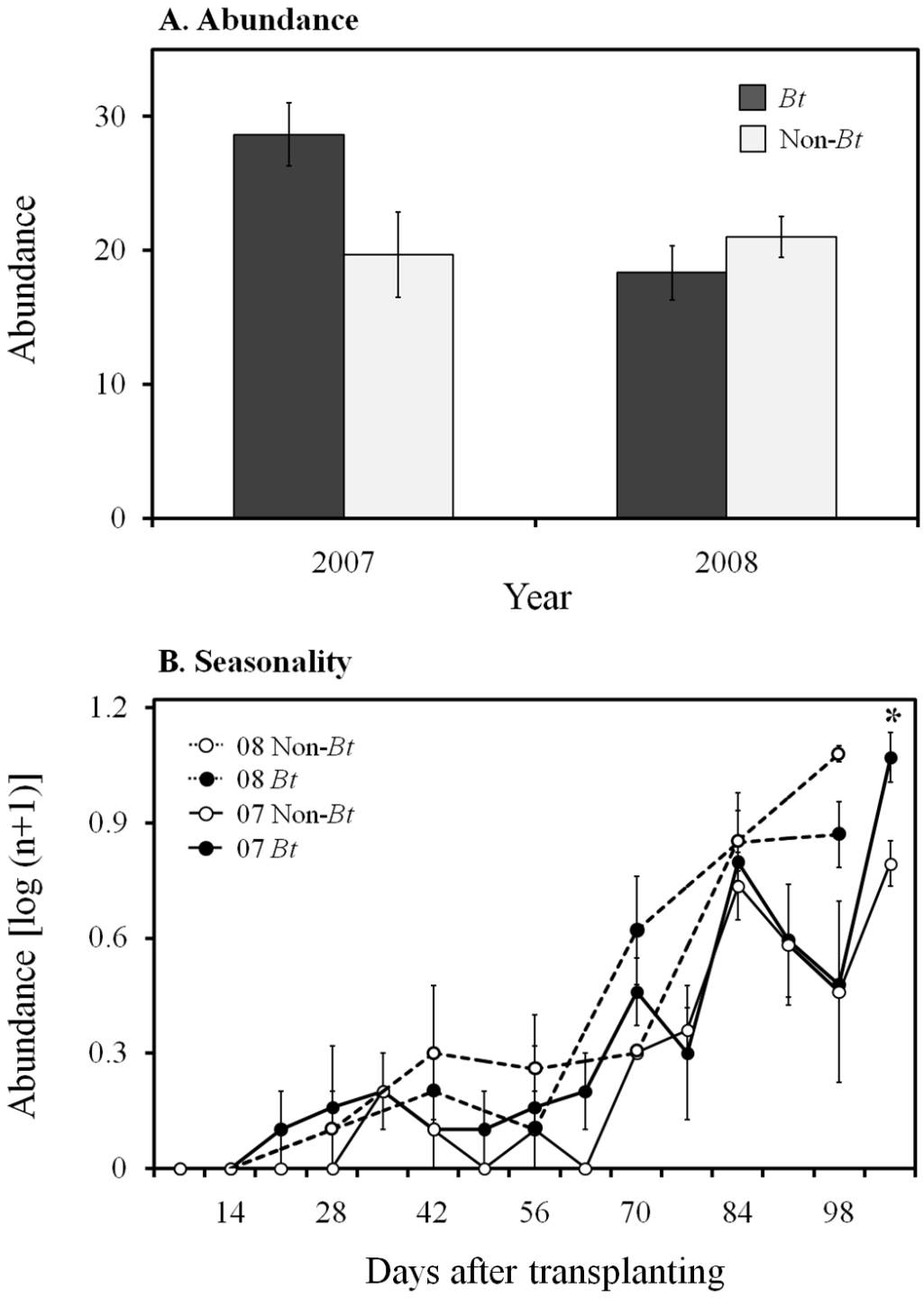

Figure 14-.6 Seasonal dynamics of *C. kurilensis* (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season.

3.4.2.4 Effects of *Bt* rice on spider guild

The spider guild comprised of wandering-active spiders (46.6±7.6%), web-sheet spiders (25.2±7.5%), web-orb spiders (16.0±2.0%), web-matrix spiders (11.5±2.3%), and wandering-ambush spiders (0.7±0.2%) in *Bt* rice. The corresponding values in non-*Bt* rice were 40.3±7.8%, 24.9±6.5%, 18.1±2.4%, 16.1±3.4%, and 0.7±0.1%, respectively (Fig. 15). Although significant differences were found on web-matrix at 105 ($F_{1,4}$=12.43, P=0.0243), web-orb at 7 ($F_{1,4}$=16.00, P=0.0161) and web-sheet at 21 ($F_{1,4}$=13.15, P=0.0222) DAT in 2007 and wand-ambush at 56 ($F_{1,4}$=Infty, P<.0001) DAT and web-sheet at 14 ($F_{1,4}$=7.86, P=0.0487) DAT in 2008, there was no significant difference in spider guilds between *Bt* and non-*Bt* rice except for web-matrix spiders in 2008 ($F_{1,4}$=11.74, P=0.0266) due to Theriididae occupying 99.8% of total web-matrix spiders (Fig 16-1~5). Similarity of spider guilds between *Bt* and non-*Bt* rice was high ranging from 0.667 to 1.000 and that of wandering-active spiders was higher than other guilds (Table 6).

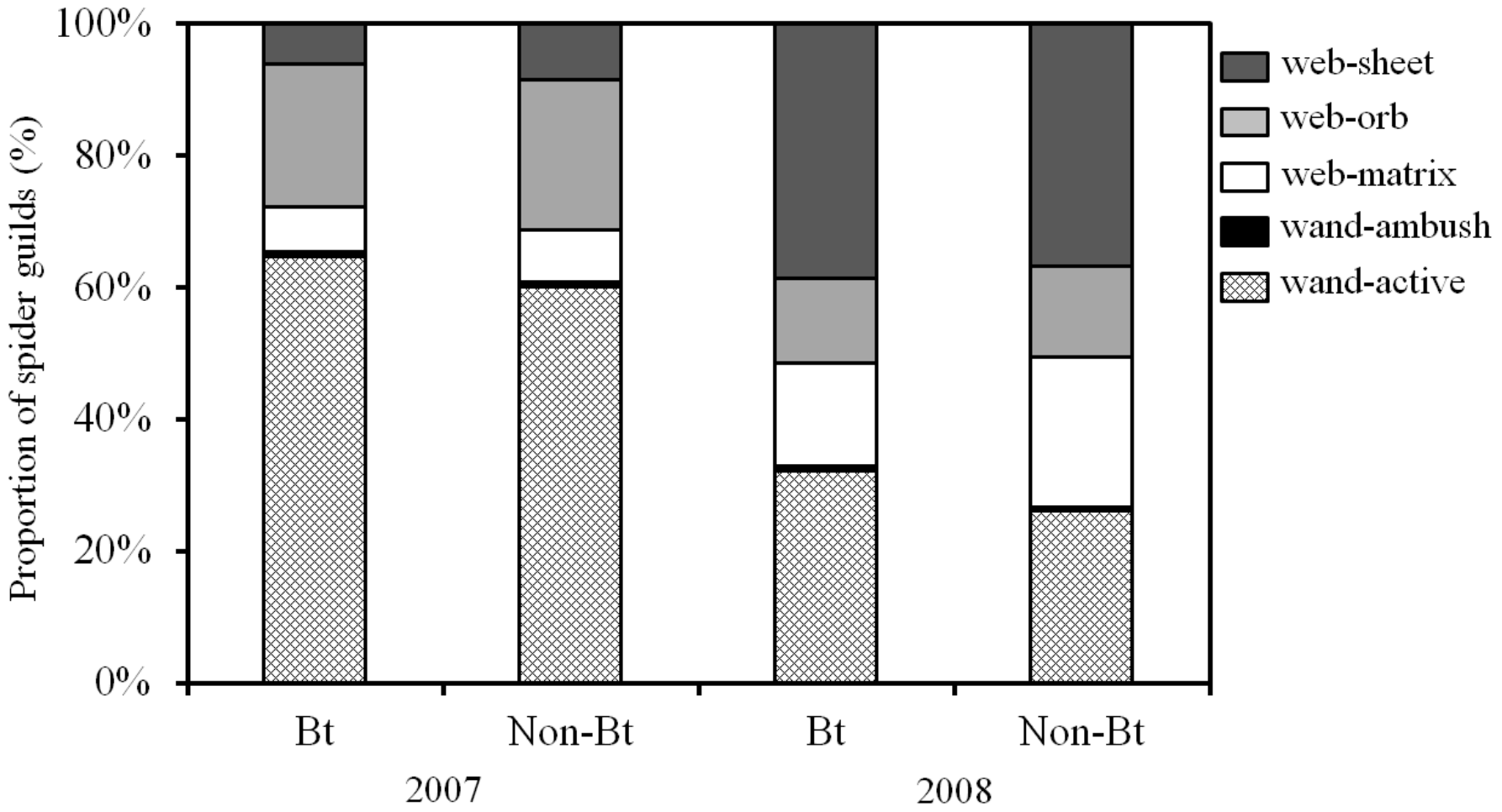

Figure 15. Proportion of spider guilds (mean±SE) in *Bt* and non-*Bt* rice throughout the rice growing season

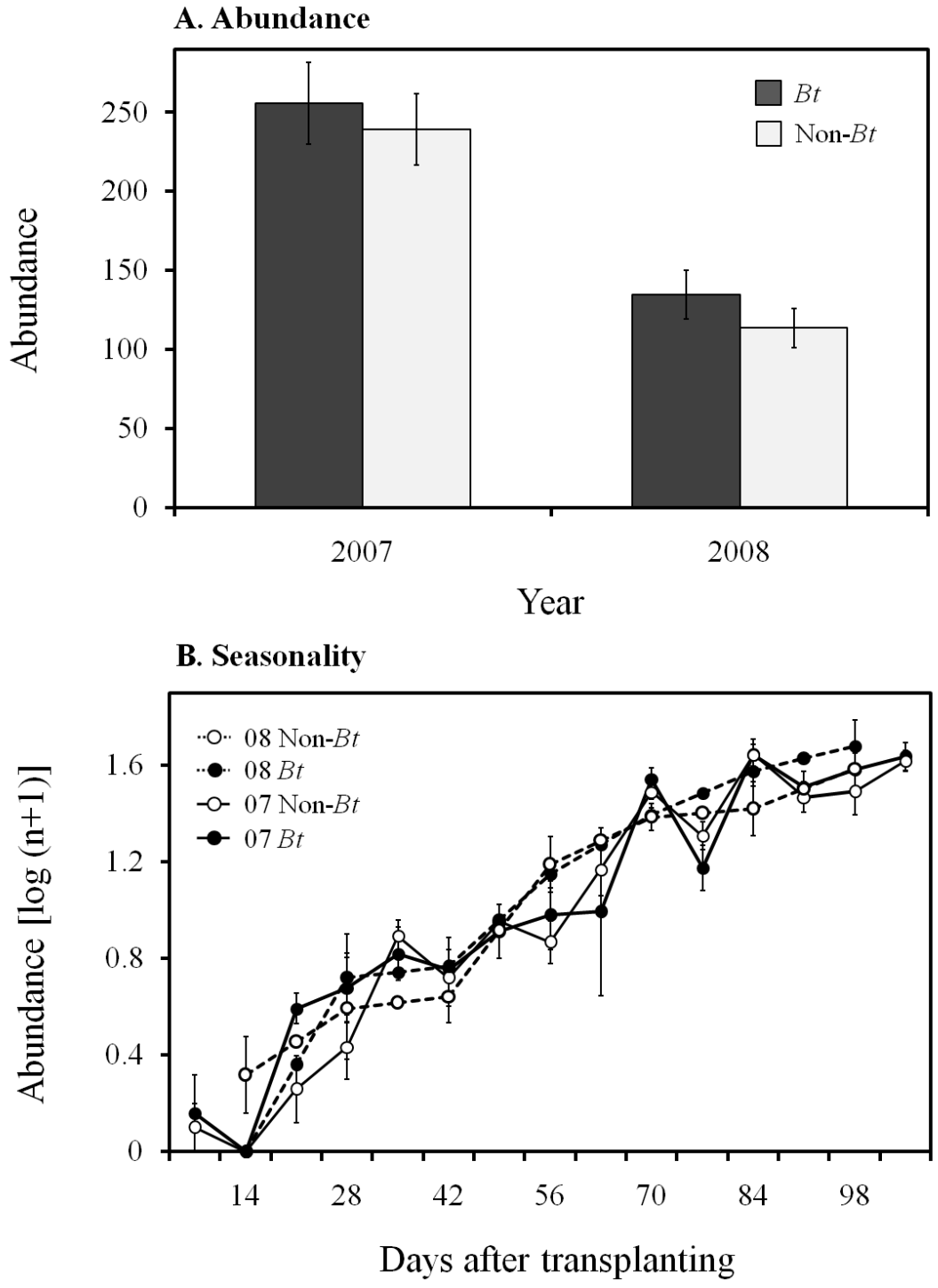

Figure 16-1. Abundance and seasonal dynamics of wander-active spiders (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season. Abundance was individual numbers of 5 samples and a sample consisted of one suction sampling and five sweepings. Refer to the main text for details on sampling.

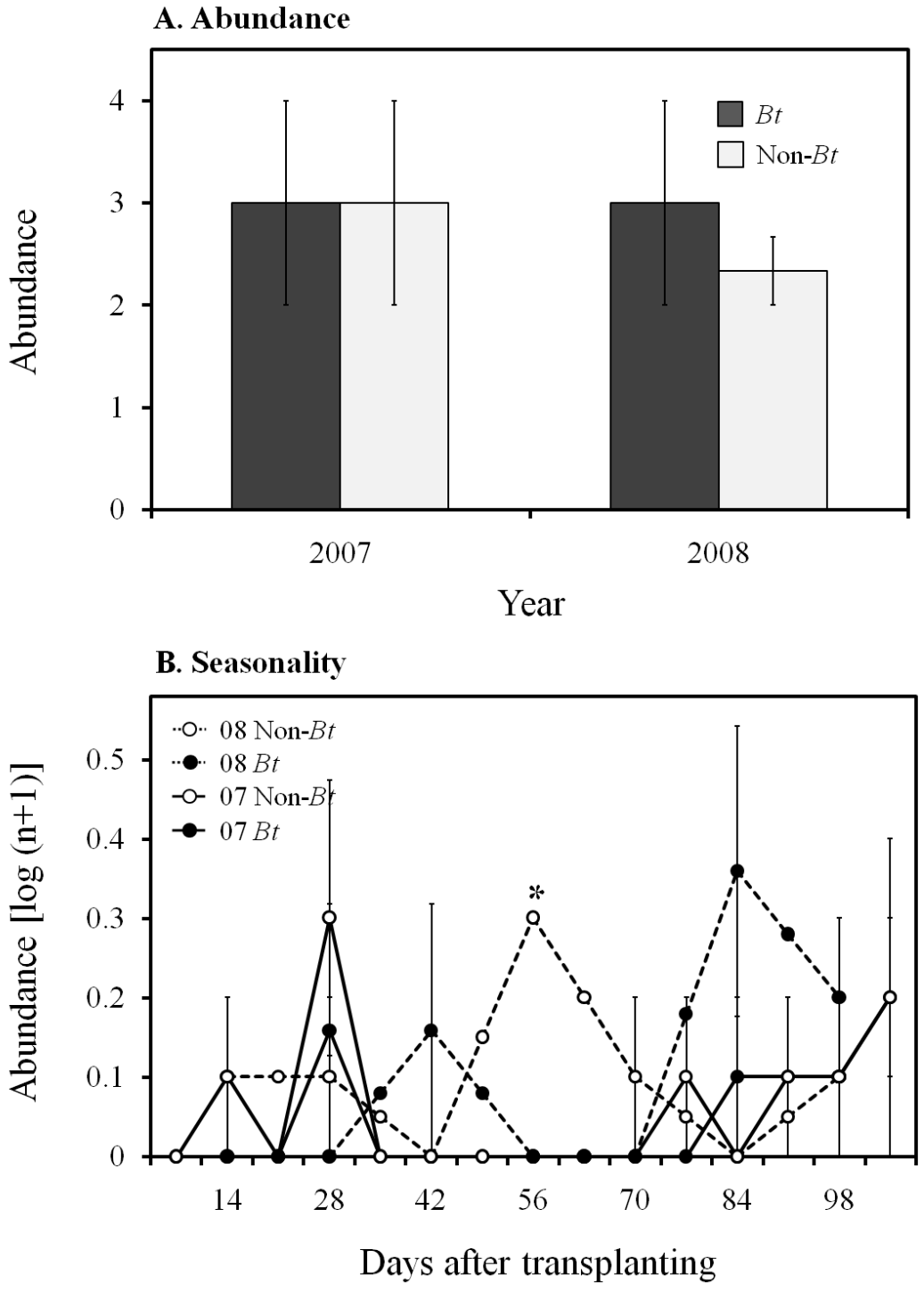

Figure 16-2 Abundance and seasonal dynamics of wander-ambush spiders (mean±SE, n = 3) per plot *Bt* and non-*Bt* rice throughout the rice growing season.

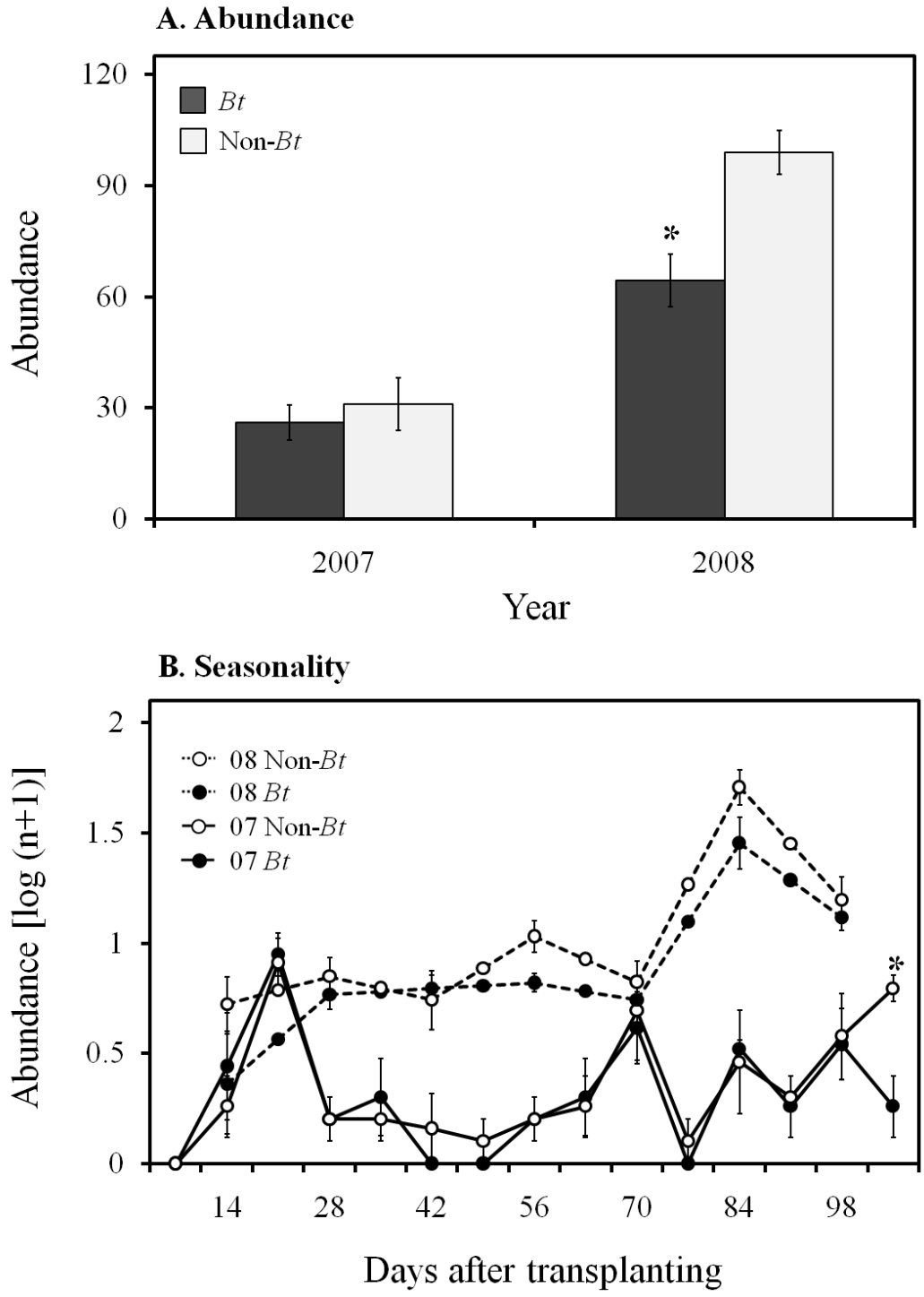

Figure 16-3. Abundance and seasonal dynamics of web-matrix spiders (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season.

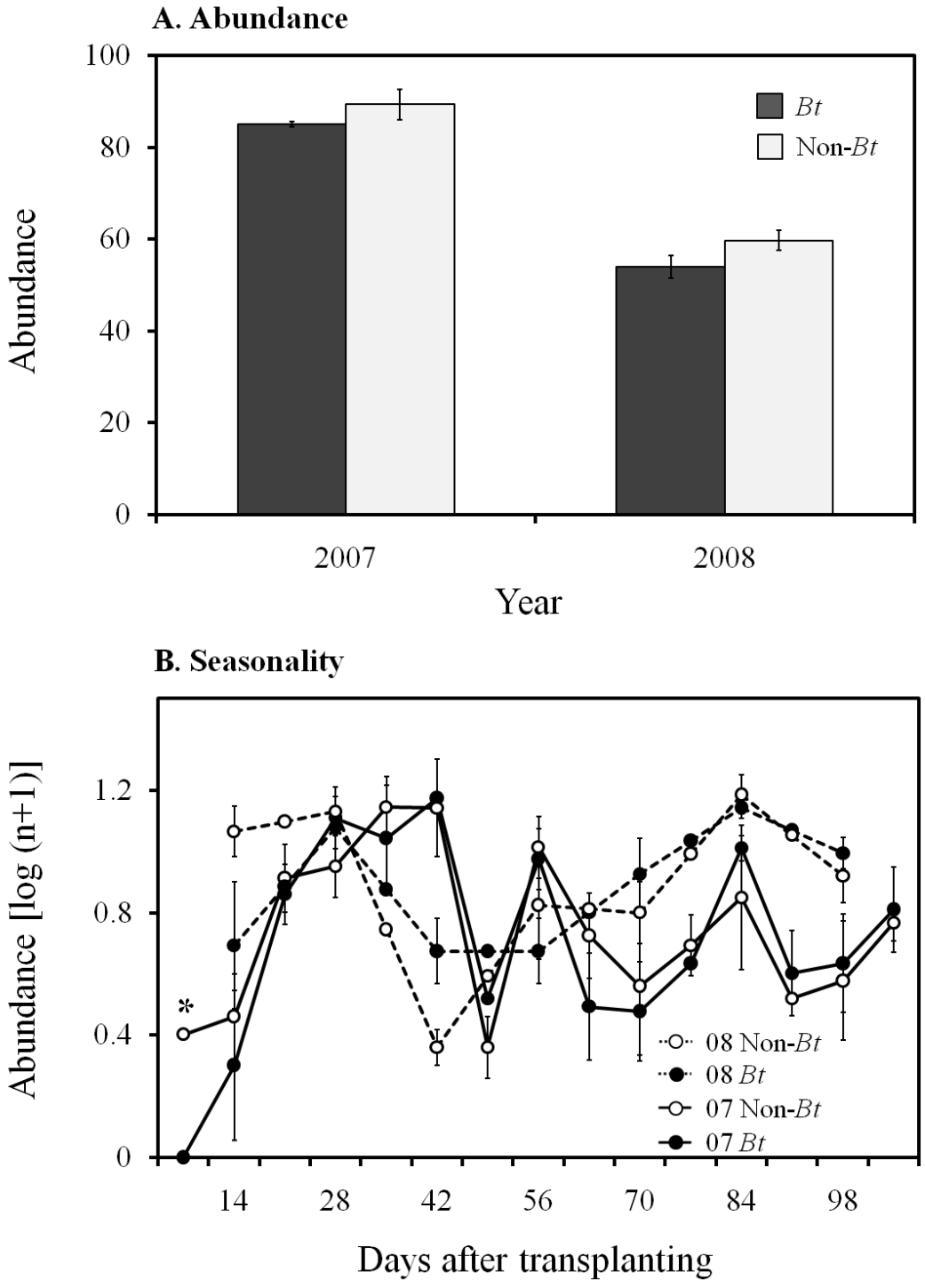

Figure 16-4. Abundance and seasonal dynamics of web-orb spiders (mean±SE, n = 3) per plot in *Bt* and non- *Bt* rice throughout the rice growing season.

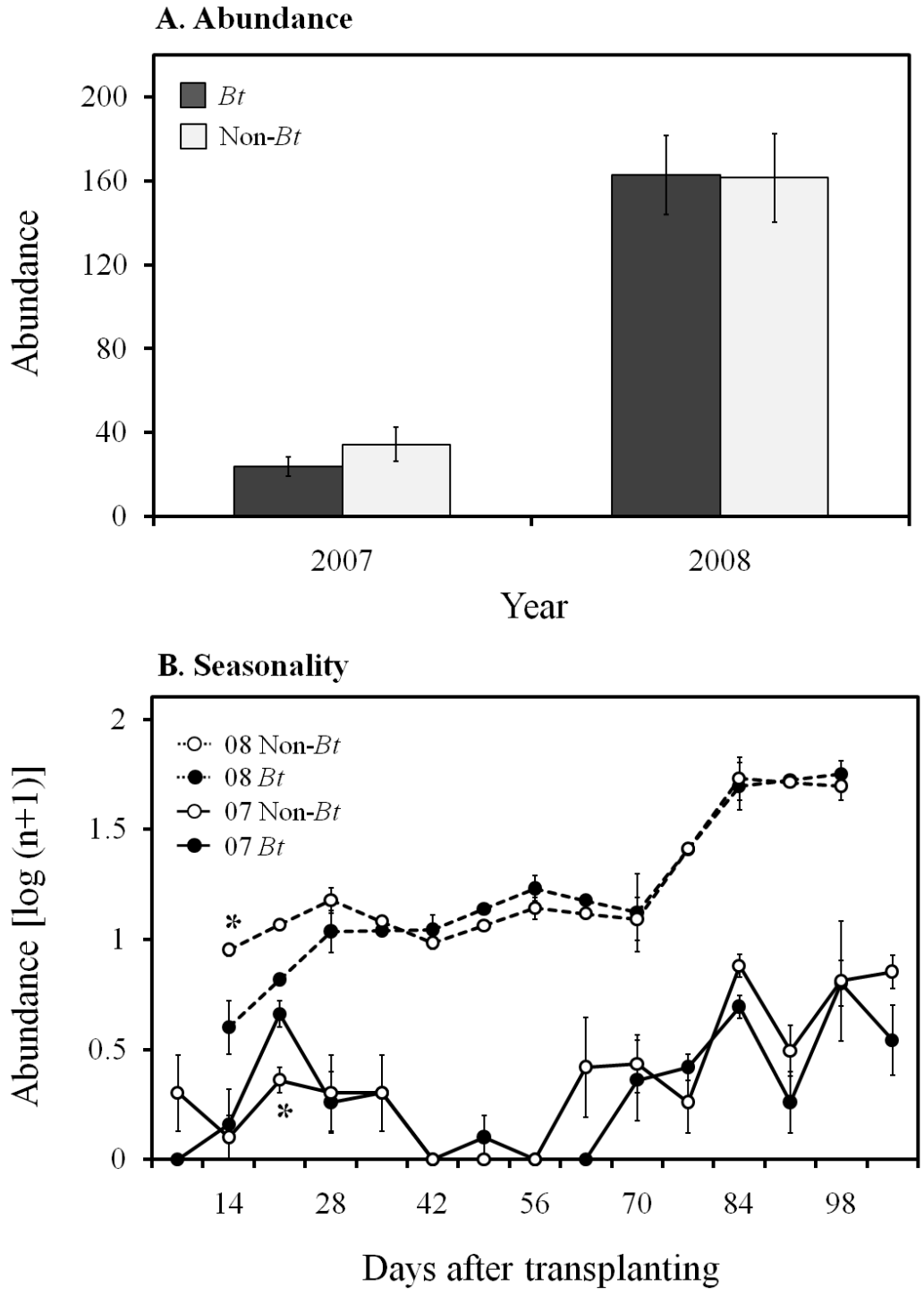

Figure 16-5. Abundance and seasonal dynamics of web-sheet spiders (mean±SE, n = 3) per plot in *Bt* and non-*Bt* rice throughout the rice growing season

Table 6. Similarity of spider communities between *Bt* and non-*Bt* rice throughout the rice growing season according to Sørenson quantitative coefficient

Spider Guild	Year	
	2007	2008
Total	0.952 (20/42)	0.818 (18/44)
Wander-active	1.000 (6/12)	0.923 (6/13)
Wander-ambush	1.000 (2/4)	0.667 (1/3)
Web-matrix	0.889 (4/9)	0.800 (4/10)
Web-orb	0.909 (5/11)	0.667 (4/12)
Web-sheet	0.857 (3/7)	0.857 (3/7)

Numbers in parentheses are shared species/total species

3.5. Discussion

Our 2-year field study was conducted in small plots (< 0.05 ha per plot) because of the regulation policy. Other previous studies were also conducted in small plots (0.03ha-0.07ha) for assessing *Bt* rice effects (Chen et al 2006, 2007, Li et al. 2007, Bai et al. 2010, Tian et al. 2010, Han et al 2011). The rarefaction curves indicate that our sample size was adequate because total spider and insect individual numbers collected in our study reach the asymptote in species richness (Fig. 4, 13).

Our data show that plots of *Bt* rice and non-*Bt* rice did not differ significantly in family richness, abundance and diversity of insect community although that of *Bt* rice were higher than non- *Bt* rice. Overall, no differences in family richness and abundance of insects by order level (Table 2) and by family level (Fig. 5) were found between *Bt* and non-*Bt* rice, resulting in high similarity in insect community structure between them (Table 3). The temporal patterns in insect family richness, insect abundance, and Shannon's index were very similar between *Bt* and non-*Bt* rice although some significant differences were observed in few occasions (Fig. 2). It seems that a few insect families of herbivores, Aphididae, Cicadellidae and Delphacidae, in 2007 and predators in 2008. The abundance of 3 families of herbivore was higher in non-*Bt* rice in 2007, but that was higher in *Bt* rice in 2008. The difference of herbivores occurred after the middle rice stage, and there was a low chance to exposure to *Bt* protein or prey containing *Bt* toxin in the fields due to low concentration of *Bt* protein in the rice plant. Moreover, most of the Aphidiidae was captured after 70 days of transplanting crop. The difference of Cicadellidae and Delphacidae was in a few occasions and overall, density of them was not significantly different in *Bt* rice comparing to non-*Bt* rice. Chen et al. (2012) reported that the population density of brown planthopper (BPH)

nymphs was significantly lower in cry1Ab *Bt* rice, but the temporal pattern of population dynamics of BPH adults was similar between the Bt and non-*Bt* rice and had no distinctive negative effects on the survival and developmental duration of BPH nymphs in laboratory study. In other field study, no marked effects on nontarget sucking insects were detected. Fu et al. 2003 reported that none of the development and reproduction parameters differed when measured in the BPH, *N. lugens,* and the white-backed planthopper, *S. furcifera* reared on *Bt* rice expressing a fusion protein of Cry1Ab/CpTI and non-*Bt* rice. Similarly, there was no difference in any of the five fittness parameters, survival to the adult stage, male and female weight, and male and female developmental time, of *N. lugens* reared on *Bt* rice and non-*Bt* rice (Bernal et al. 2002). Tan et al. (2006) also reported that *Bt* rice significantly affected neither oviposition behavior nor fecundity of the white-backed planthopper. The difference of predatos occurred at the early rice stage when predators immigrated into a rice field. Because rice fields are renewed every year, most of insects immigrate each season for colonization. Also, most of the predators were Coenagrionidae occupyng 45.5% of all predators in 2008, flight ability of this species was high enough to move over our plot boundary. Thus it may be difficult to confirm that difference of predators was caused by *Bt* rice. Community structure of spiders in our study was similar to previous reports conducted in Korean rice fields (Choi and Namkung 1976, Okuma et al. 1978, Paik and Namkung 1979, Kim 1995, Im and Kim 1996, 1999, Lee et al. 1997). Our study showed that there were no significant effects of *Bt* rice on the spider community. Overall, no differences in species richness and abundance of spiders by order level (Table 5) and by family level (Fig. 12) were found between *Bt* and non-*Bt* rice, resulting in high similarity in spider community structure between them (Table 6). The temporal patterns in spider species richness and spider abundance were very similar between *Bt* and non-*Bt* rice although some significant differences in species richness were

observed in a few occasions (Fig. 11). It seems that a few spider species such as *P. oculiprominens*, *T. maxillosa* and *P. clercki* caused this difference with that *P. oculiprominens* and *T. maxillosa* were more captured in non-*Bt* and vice versa in *P. clercki*. And this difference occurred at the early and late rice stages when these species immigrated into a rice field. Because rice fields are renewed every year, most of the spiders immigrate each season for colonization. *T. maxilosa* and *P. clercki* immigrate into rice fields at the early rice stage and *P. oculiprominens* is observed at the late rice stage. These species are web builders and are less likely to move to other habitats after colonization. Especially, *P. oculiprominens* and *P. clercki* share the same microhabitats in rice fields, which may result in interspecific competition for space and prey due to a substantial niche overlap. *P. clercki* occupied earlier than *P. oculiprominens* in rice fields, so *P. oculiprominens* might choose the habitat which has a relatively lower density of *P. clercki* to avoid the competition. Also, abundance of *G. dentatum*, which shared the same microhabitats with *P. oculiprominens* was approximately 34% higher in *Bt* rice than non-*Bt* rice but had no significant difference ($F_{1,4}$=2.72, P=0.1745). Interspecific competition in web building spiders has been demonstrated in relatively simple habitats, such as litter, estuaries, wetlands and agricultural ecosystems (Uetz 1979, Fasola 1999, Marshall and Rypstra 1999, Novak et al. 2010). Thus it seems that intraspecific competition and the difference of early immigration density affect the whole density of spiders during the rice growing season rather than by the *Bt* construct itself.

Spider guilds are another functional tool to translate community because those were organized reflecting spiders' biological and ecological characteristics. In terms of spider guilds, though proportion of web-matrix spiders was lower in *Bt* rice fields than non-*Bt* in 2008 due to *P. oculiprominens* with 69.4% occupation in web-matrix, there were no negative effects on the other guilds (Fig 5).

Previous studies were conducted to assess *Bt* rice effects on some spider species or families. On the other hand, our study examined the whole spider community at the species level and was the first trial of this kind conducted in rice fields. Previous studies also showed that *Bt* rice had no negative effects on spiders. Liu et al. (2003) reported that cry1Ab/cry1Ac-carrying transgenic *Bt* rice generally had no distinctive negative effects on the rice arthropod guilds and superior families. According to Chen et al. (2009), survivorship and fecundity of *P. subpiraticus* preying on *Bt* rice-fed *C. medinalis* were not significantly affected although its developmental time was significantly longer. Tian et al. (2010) also showed that *Bt* rice did not significantly affect the population density of *U. insecticeps*. Han et al. (2011) reported that three transgenic *Bt* rice strains expressing cry1Ab/cry1Ac, cry1C and cry2A had no significant adverse effects on three predator species, *Cyrtorhinus lividipennis*, *P. subpiraticus* and *Theridium octomaculatum*, in the rice fields.

The effect of transgenic plants on spiders at the community level has been examined in other crops. Peterson et al. (2011) conducted a meta-analysis of the effects of *Bt* cotton, corn, potato, rice and eggplant on spiders, and suggested that there are no consistent negative effects of *Bt* toxins against spiders. Several other studies also found no or no consistent effect of *Bt* crops on spiders (Sisterson et al. 2004, Naranjo 2005, Whitehouse et al. 2005, Meissle and Lang 2005, Ludy and Lang 2006a, 2006b, Řezáč et al. 2006, Rose and Dively 2007, Farinós et al. 2008, Meissle and Romeis 2009).

The *Bt*-rice line with a synthetic cry1Ac1 gene for control of *C. medinalis* generally has no negative effects on the insect and spider community in this study. However, uncertain negative effects on spiders may cause decline of naturally occurred biological control effects on non-target pests and it may demand for additional other control efforts. Thus future field studies should be conducted for longer periods to detect delayed risk and additional

laboratory experiments which is relatively easy to control extraneous variables that are needed to clarify direct and indirect field effects of the cry1Ac toxin on arthropods.

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Chapter 4.

Suggestion for assessment methodology on test of *Bt* rice effects on non-target arthropods

4.1. Abstract

One of the primary concerns related to the adoption of GM crops in the environment is its negative effect on non-target organisms. However, standardized protocols for assessing potential risks of GM crops are not established although several approaches are implemented. A tiered risk assessment is suggested as the most appropriate approach to assess non-target affects of GM crops because it can save time and resources by organizing the studies in a cohesive and coherent manner and avoiding unnecessary lines of investigation. For risk assessment of *Bt* rice on non-target arthropods in Korea, this study suggest a tiered system and five test species, *N. lugens*, *O. japonica*, *P. japonica*, *P. subpiraticus* and *P. clercki*, considering standards of selection test species. To suggest appropriate sampling methods, sampling plot size, sampling timing and sampling occasion for field assessment, the efficacy of two sampling methods, sweep net and suction, was compared to survey insect and spider community, species richness and diversity of spiders in rice fields of three different plot sizes, 270 m^2, 1000 m^2 and 3300 m^2, throughout the rice growing season. Suction sampling captured more spider species and individuals than sweep net sampling, but insects were captured more by sweep net sampling. In this study, biodiversity of spiders did not increase with increase of plot size. The similarity of the community was higher between 1000 m^2 and 3300 m^2 than others. Thus it seems that 1000 m^2 plot size test is resonable to detect *Bt* crop effects on arthropods community in rice fields. This study used cluster analysis with similarity of community to find appropriate sampling time and occasion, and sampling time were divided into five clusters with 65.6% similarity and these clusters generally corresponded to five rice growing stages in the fields.

4.2. Introduction

The cultivating area of GM crops expressing *Cry* proteins derived from the soil bacterium *Bacillus thuringiensis* Berliner (*Bt*) has been risen steadily since the first *Bt* crop was released commercially in 1996 (James 2011). Several countries developed regulatory systems for investigating the risk analysis of GM crops in order to assess possible long-term or unexpected effects (Conner 2003). However, standardized protocols for assessing potential risks of GM crops do not exist and several approaches are proposed (Jepson et al. 1994, Dutton et al. 2003, Wolt et al. 2003, Howard and Donnelly 2004, Romeis et al. 2008, Hillbeck et al. 2011). A tiered risk assessment which is generally used for assessing the effects of pesticides is considered the most appropriate approach to assess non-target affects of GM crops because it can save valuable time and resources by organizing the studies in a cohesive and coherent manner and avoiding unnecessary lines of investigation (Romeis et al. 2006, 2008, Rose 2007, Hillbeck et al. 2011). The procedure starts with laboratory tests followed by semi-field and field tests according to decision on the risk hypothesis (Hassan 1998, Candolfi et al. 2000, 2001, Dutton et al. 2003). Lower-tier laboratory tests are conducted under worst-case exposure conditions. Species representative of non- target arthropods (NTAs) in the target environment are exposed to the arthropod-active protein in excess of the field exposure level. Lower tiers allow tighter control over experimental variables and exposure conditions, resulting in a greater ability to produce statistically reliable results at relatively low cost though realism in terms of exposure pathway or level is usually relatively low (Roemis et al. 2011). Higher-tier tests are conducted on a larger temporal and/or spatial scale like greenhouses, semi-fields or fields, and can more realistically assess potential exposure to the insecticidal protein than lower-tier tests (Rose 2007, Romeis et al. 2008, 2009). Movement to the next tier occurs either when the available information is insufficient to accept the risk hypothesis of ‘no effect’ or when this hypothesis is rejected. If sufficient data and experience from toxicological tests and

exposure analyses are available to characterize the potential risk as being acceptable, then there is no need to undertake additional tests (Romeis et al. 2006, 2008, Rose 2007, Hillbeck et al. 2011).

For a risk assessment of GM crops, it is impossible to test all species that may potentially be exposed to the arthropod-active protein, e.g. *B. thuringiensis*. Therefore, test organisms should be selected to represent different habitat types (e.g., soil- or plant-dwelling arthropods) or different ecosystem services such as ecological functions (e.g., predator, parasitoid or decomposer), taxonomic groups. Also, the availability of the test organisms and their likelihood of exposure to GM crops as well as a possible sensitivity to products of transgenic plants should be also considered for selection of test species (Jepson et al. 1994, Hilbeck and Andow 2002, Dutton et al. 2003, Rose 2007, Romeis et al. 2008, 2011, Hilbeck et al. 2011). Ranking of species according to its geographic distribution, habitat specialization, abundance, phenology, linkage and association with the crop can reduce the number of potential test species existing in a given crop system and its surrounding habitats, but we should acknowledge the limitations of the available knowledge about species and their function and identifying important gaps of information (Hilbeck et al. 2011).

On assessing the effects of *Bt* crops on non-target arthropods in the fields (e.g., biodiversity), it is important to choose appropriate sampling methods, plot size, sampling timing and frequecy, because differences in behavior and ecology of non-target arthropods may affect sampling efficacy (Delabie et al. 2000, King and Porter 2005, Valverde and Lobo 2005).

Present study suggested a tiered assessment system of *Bt* rice on non-target arthropods in Korea based on my laboratory and field study. For selection of test non-target species for the lower tier test, it should be considered expression sites of insecticidal protein in plant tissue, exposure possibility to insecticidal expression sites, inhabiting sites on rice plant, ecologically functional role, relatively importance on rice, easiness and control for tests and

feeding mode of test species. For the field trial, the result of comparing the efficiency of two sampling methods, the influence of plot size in three different plot sizes and various sampling time and sampling occasions with arthropods diversity.

4.3. Material and Methods

Study area

This study was conducted in 3 rice fields in Ye-san, Su-won and In-cheon, Korea and the field plot size was 270 m^2, 1000 m^2 and 3300 m^2. The field trail was conducted in Ye-san in 2007, and in Su-won and In-cheon in 2009. The field trial of different size was replicated three timesThe mean monthly air temperature in study sites ranged between 21^oC and 26^oC from June to September in both years. The mean monthly rainfall ranged from 106.7mm to 470.6mm, 56.3mm to 766.0mm and 51.0mm to 470.6mm in Ye-san, Su-won and In-cheon, respectively.

All rice seedlings were transplanted in a 15 cm × 30 cm spacing on 25 May in Ye-san, on 1 June in Su-won, and on 23 May in In-cheon. The field was managed according to the standard rice cultivating practices, but insecticides and herbicides were not treated.

Sampling

Arthropod sampling was conducted from 28 June to 4 October in 2007, from 27 June to 5 October in 2009. Sampling was conducted at one week intervals with 15 sampling occasions in both years. A battery-powered suction device (DC 12V, Bioquip Co., Rancho Dominguez, CA, USA) was used to collect spiders and insects inhabiting the lower and middle parts of the rice plant. Four rice hills were sucked for one suction sample. A sweep net (39cm in diameter) was used to collect spiders and insects inhabiting the upper and top parts of the rice plant. Sweep net sample was made at an angle of 180^o and repeated three times per a sweep net sample. Suction and sweep net samples were taken 5 times each on every sampling date. Also, sampling was made by keeping a certain distance (3m) cross-diagonally while avoiding

interference of each sampling. The direction of the sampling was changed on each sampling date. Sampling was not made on the board (1 m) of each plot.

Spider and insect identification

Collected spiders were fixed in 85% ethanol and identified to species level under a dissecting microscope according to Namkung (2003), Chikuni (2008) and Ono (2009). Species names followed Namkung et al. (2010) and order of families followed taxonomic orders of Platnick's catalogue ver. 12.5 (2012), and adopted the latest taxonomical transformation. All collected insects were fixed in 85% ethanol and identified to family level except Psocoptera. Specimens were deposited in Laboratory of Insect Ecology, College of Agriculture and Life Sciences, Seoul National University.

Data analysis

Data of spiders and insects were used for comparison of efficacy of sampling methods and for the others comparisons only spider data were used. A repeated measurement ANOVA (Proc RM ANOVA) in SAS 9.2 (SAS Institute 2004) was used to analyze the effect of "plot size" on number of species and Shannon's diversity index. To evaluate sample size adequacy and compare species richness among the three different plot sizes, rarefaction curves (Gotelli and Colwell 2001) were constructed using Species Diversity and Richness v3.0 computer program (Henderson and Seaby 2002). Samples were randomly reordered 50 times and standardized to the number of individuals caught to avoid the problem that the samples are listed can have a large impact on the result when calculating species richness or fitting a species accumulation curve. Also, diversity analysis was conducted for comparison of the spider community structures (species richness and species diversity) in three plot sizes using

PRIMER-5 (Clacrke and Warwick 2001). Shannon's -diversity, H′, (Shannon and Weaver 1949) is:

$$H' = -\sum p_i \ln p_i$$

where p_i is the proportion of the *i*th species in the total sample.

Cluster analysis, using the paired group method and Bray-Curtis similarity measures, was used to check the similarity of the predefined groups, to depict similarity of spider assemblages and to find appropriate sampling time and occasion from all plot sizes.

4.4. Results

4.4.1. Selection of test species for *Bt* rice effects under laboratory conditions

For a lower tier of risk assessment of *Bt* rice, six non-target species were selected as test organizms: 1) *S. lurida*, 2) *O. japonica*, 3) *N. lugens*, 4) *P. japonica*, 5) *P. subpiraticus*, 6) *P. clercki*. Following were considered for species selction.

1) Expression sites of insecticidal protein in plant tissue, leaf, stem and pollen,
2) Exposure possibility to insecticidal expression sites, direct exposure by feeding *Bt* rice part and indirect feeding through prey fed *Bt* rice,
3) Inhabiting sites on rice plant such as upper part of rice plant and lower part of rice plant,
4) Ecological functional role such as herbivore and predator
5) Relative importance on rice such as prey of natural enemies and natural enemy
6) Easiness and control for tests such as visual check or feeding supply
7) Acquirement of test species, generation, field collecting and lab colonization
8) Feeding mode of test species such as tissue feeder, phloem-sap feeder, pollen feeder and predator. All of six species showed high likelihood of exposure on *Bt* toxin in the rice fields representing different habitats, different mode of feeding and feeding part of test species. These species are easy to acquire, considering generation number per year, host range, field collecting and laboratory rearing. Also, these species are appropriate to test due to the easiness of visual check, feeding supply and control. Standard of mortality under test condition was classified with low (1-30 %), medium (31-70 %) and high (71-100 %) according to mortality of organisms raring on non-*Bt* rice (negative control) (Table 1).

Although *P. subpiraticus, P. clerki and P. japonica* distribute nationwide, *O. japonica* usually distributes in the middle part of Korea, and *S. lurida* usually distribute in west-coast of Korea. *N. lugens* is a major migratory rice pest. With these 6 species, we can suggest the tiered system for assessment of *Bt* rice effects on non-target arthropods in Korea (Fig. 1). Risk hypothesis is that *Bt* rice (Cry1Ab) has no negative effects on non-target arthropods. Tier I is a laboratory test of selected non-target species using exposure levels representing at least 10x the highest Expected Environmental Concentration (EEC). Insecticidal protein mixed with artificial diet is the preferred test compound. Endpoint is the mortality of test species and exposure duration is from few days to for specific ages (3-5 ages) and a little shorter than that of higher tier. Negative control is test species fed on artificial diet and positive control is ELISA. When there are some effects on test species or it is difficult to progress Tier I for some reason (e.g., no suitable artificial diet with high *Bt* concentration), we can move on Tier II. Tier II is a laboratory test using *Bt* rice alone or mixed with artificial diet like in pollen or leaf discs from the *Bt* rice. Endpoints are mortality, fecundity, development and fitness of test species and exposure duration is full life-cycle or 1 generation of test species. Negative control is test species fed on non-*Bt* rice and positive control is ELISA. If there are some effects on test species, we can move on Tier III. Tier III is a semi-field test and is conducted under greenhouse conditions with *Bt* rice. Endpoint is the population size of test species and exposure duration is 2-3 generation of test species (Fig 1).

Table 1. Selection of test species for *Bt* rice effect under laboratory condition

		O. japonica	***N. lugens***	***S. lurida***	***P. subpiraticus***	***P. clercki***	***P. japonica***
Likelihood of exposure	Feeding parts	Leaf	Leaf /Stem	Leaf /Stem/Grain	Herbivore	Herbivore	Pollen/Herbivore
	Mode of feeding	Chewing	Piercing-sucking	Piercing-sucking	Piercing-sucking	Piercing-sucking	Chewing
	Habitats	Plant-welling/ upper part	Plant-dwelling/ lower part	Plant-dwelling/ lower part	Plant/ Soil-dwelling/ lower part	Plant-dwelling/ lower part	Plant-dwelling/ upper part
Acquirement of test species	Generation	1/year	Multi-/year	1/year	2/year	2/year	Multi-/year
	Host range	Multi	Rice	Rice	Multi	Multi	Multi
	Field collecting	Easy	Easy	Easy	Easy	Easy	Easy
	Lab colonization	Medium	Easy	Medium	Easy	Medium	Easy
Easiness for tests	Visual check	Easy	Medium	Easy	Easy	Easy	Easy
	Feeding supply	Easy	Easy	Easy	Medium	Medium	Medium
	Control	Easy	Easy	Medium	Easy	Easy	Easy
Mortality under test condition		Low	Low	High	Medium	Medium	Medium
Abundance		Medium	High	Medium	High	High	High
Geographic distribution		Medium	Medium	Medium	Wide	Wide	Wide
Taxonomic group		Orthoptera	Hemiptera	Hemiptera	Araneae	Araneae	Coleoptera
Ecological function		Herbivore (Tissue feeder)	Herbivore (Phloem-sap feeder)	Herbivore (Phloem-sap feeder)	Predator (Hunter)	Predator (Web-builder)	Herbivore/Predator (Pollen-feeder)

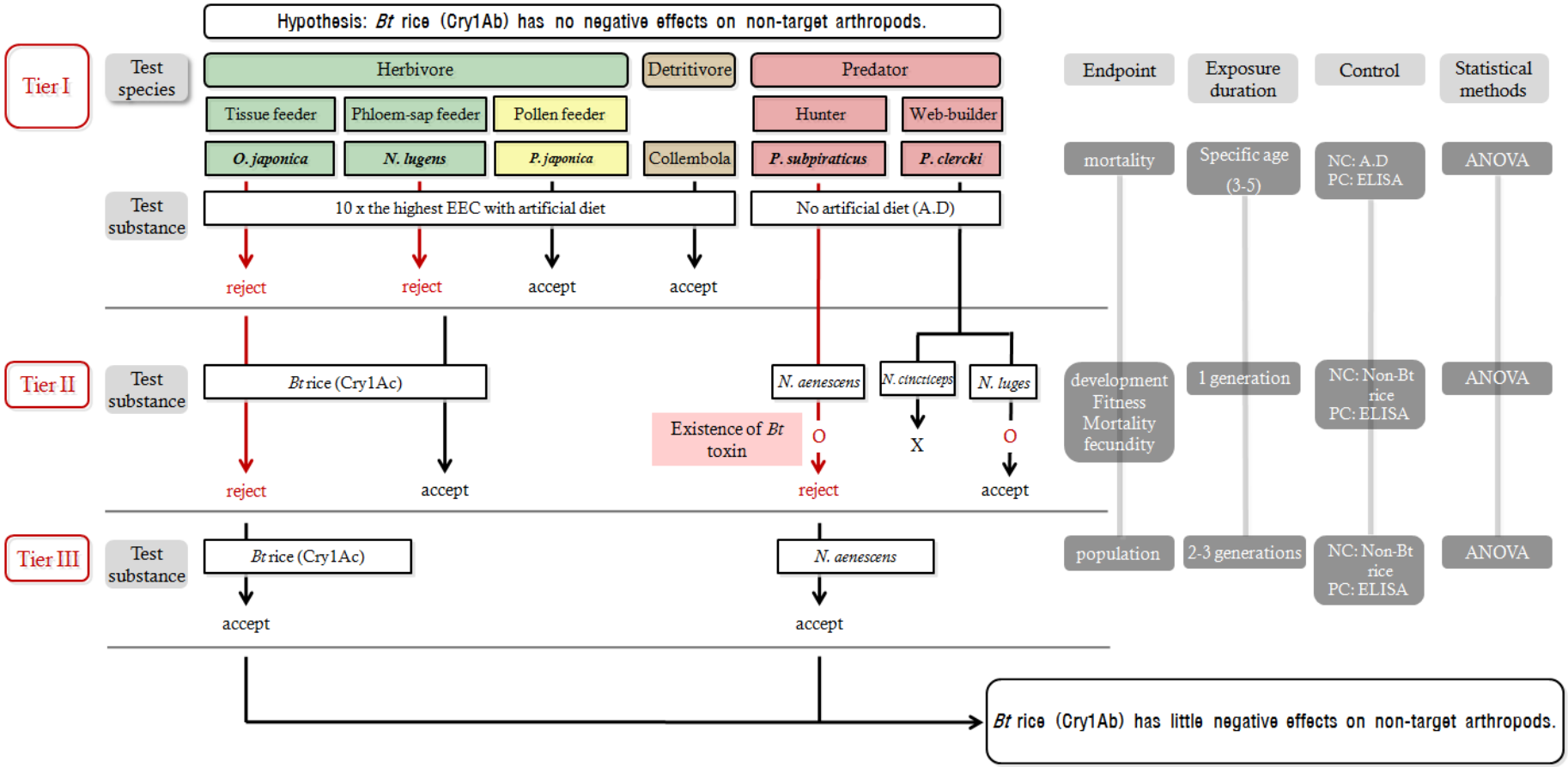

Figure 1. Suggestion of the tiered system for assessment of *Bt* rice effects

4.4.2. Sampling protocol for *Bt* rice effects under field conditions

4.4.2.1 Sampling methods

Among a total 64,099 individuals of insects captured for 2 years in this study, 53,810 individuals were captured by the sweep net sampling method. Among ecological guilds, detritivore containing Psocoptera and Collembola were mostly captured by the suction sampling method and the majority of herbivores, predators and parasitoids were captured by the sweep net sampling method. Also in the family level, 2 families of Coleoptera (Elateridae and Carabidae) and a family of Hemiptera (Hydrometridae) and Hymenoptera (Formicidae) were captured only by the suction sampling method and 4 families of Hemiptera (Alydidae, Beritidae, Coreidae and Rhopalidae), 2 families of Hymenoptera (Diapriidae and Scelionidae) and Odonata (Aeshnidae and Libelluidae) and a family of Diptera (Syrphidae) and Othoptera (Pyrgomorphidae) were captured only by the sweep net sampling method (Fig. 2).

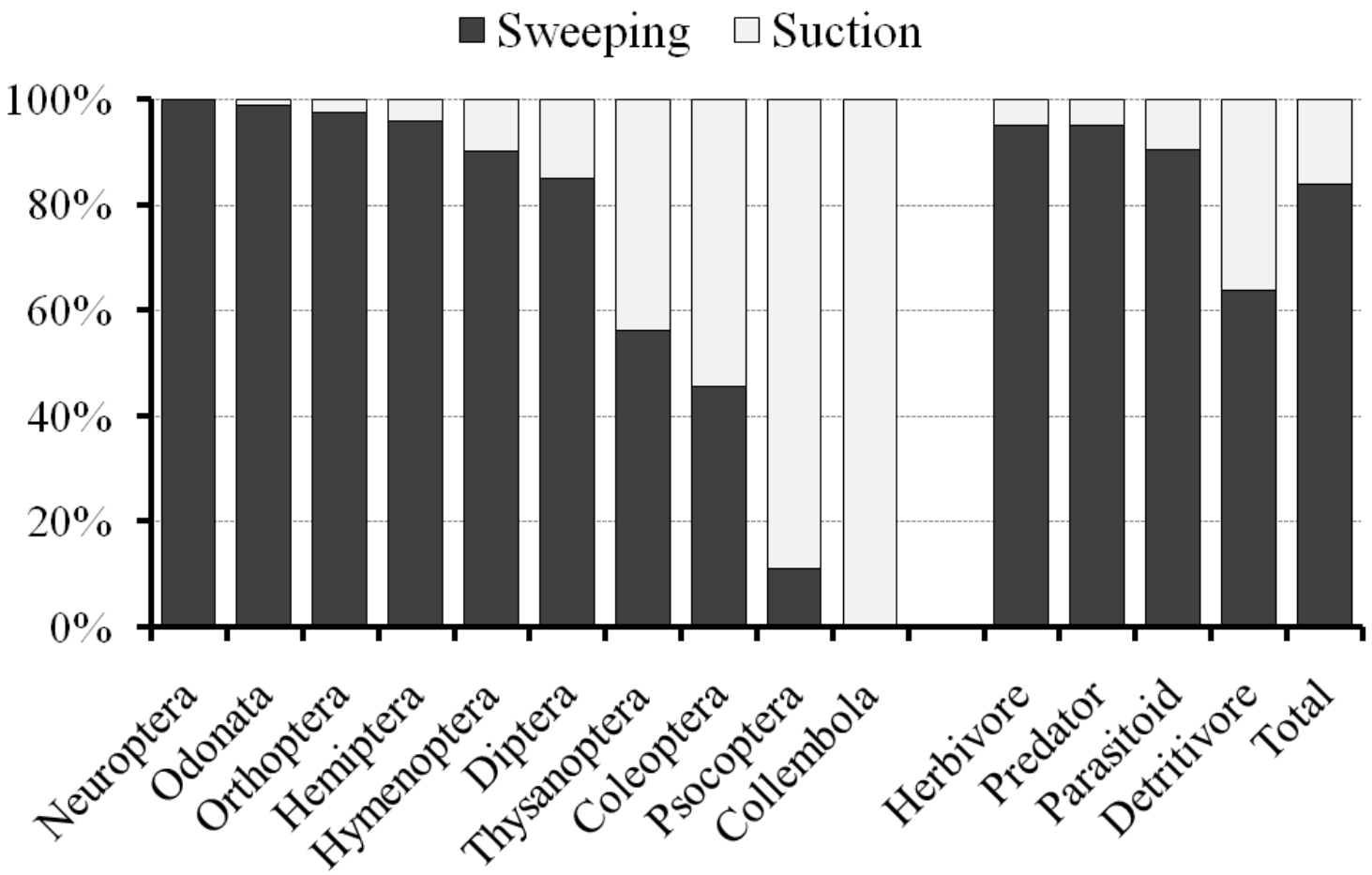

Figure 2. Proportion of insect orders according to sampling methods in rice fields. The data are pooled for two years

Among a total 4,937 individuals of spiders captured for 2 years, 4,220 individuals were captured by the suction sampling method. Among 9 families, Nesticidae, Liny phiidae, Lycosidae and Theridiidae were mostly captured by the suction sampling method and Araneidae, Thomisidae and Pisauridae were captured more by the sweep net sampling method. *N. mogera*, *Paidiscura subpallens*, *E. koshiensis*, *U. feminea* and *P. tenera* were captured only by the suction sampling method and *P. angulithorax*, *T. extensa*, *A. bruennichi*, and *E. tricuspidata* were captured only by the sweep net sampling method (Fig. 3).

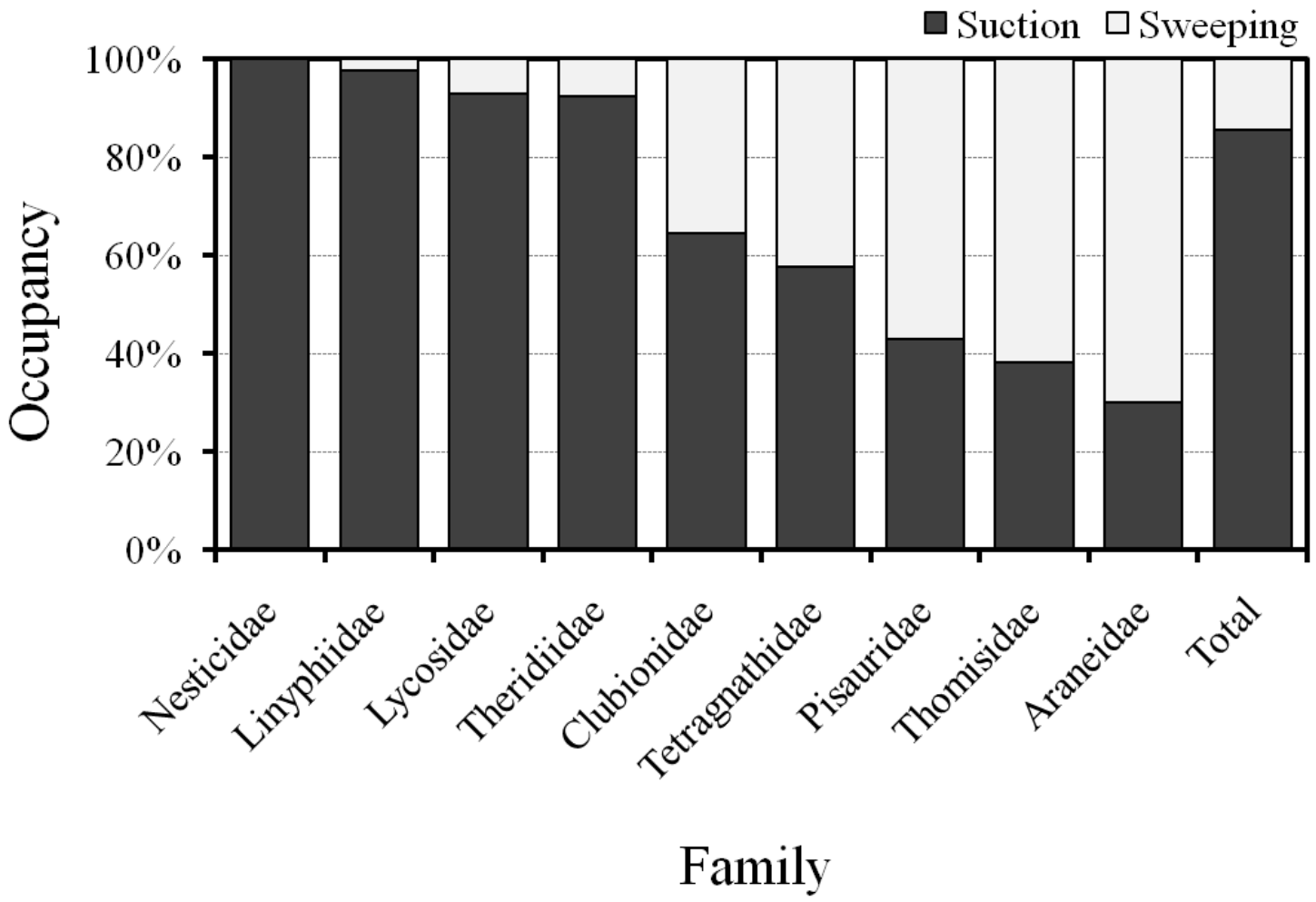

Figure 3. Proportion of spider families according to sampling methods in rice fields. The data are pooled for two years

4.4.2.2 Sampling plot size

For spiders, twenty two species in 22 genera and 9 families with 1,191 individuals, 30 species in 23 genera and 9 families with 1,168 individuals and 27 species in 22 genera and 10 families with 1,792 individuals were collected in 270 m^2, 1000 m^2 and 3300 m^2 plots, respectively (Table 2). Among them, 16 species in 16 genera and 8 families commonly occurred in 3 different plot size fields. Species richness and Shannon's index of spiders during the season were the highest in the 1000 m^2 plot and the lowest in 270 m^2 plots and in 3300 m^2 plots, respectively (Table 3). There was no significantly different in species richness among the plot size, however, Shannon's index was significantly different among 3 different sized plots (Table 3). Although significant differences were found in species richness at 7 ($F_{2,6}$=7.59, P=0.0283), 35 ($F_{2,6}$=12.26, P=0.0076) and 91 ($F_{2,6}$=8.55, P=0.0175) DAT, overall, the seasonal pattern of spiders was very similar among each plot size (Fig. 4).

Table 2. Spiders recorded in the rice fields throughout the rice growing season.

Family	Scientific name	270 m^2	1,000 m^2	3,300 m^2
Nesticidae	*Nesticella mogera* (Yaginuma 1972)	1	-	1
Theridiidae	*Chrysso octomaculata* (Bösenberg et Strand 1906)	9	12	-
	Enoplognathaabrupta (Karsch 1879)	55	47	42
	Paidiscura subpallens (Bösenberg et Strand 1906)	15	9	11
	Parasteatoda oculiprominens (Saitō 1939)	13	3	6
Linyphiidae	*Bathyphantes gracilis* (Blackwall 1841)	-	1	3
	Erigone koshiensis Oi 1960	-	4	5
	Gnathonarium dentatum (Wider 1834)	76	16	53
	Nippononeta ungulate (Oi 1906)	-	1	-
	Ummeliata feminea (Bösenberg et Strand 1906)	7	1	-
	Ummeliata insecticeps (Bösenberg et Strand 1906)	20	109	133
Tetragnathidae	*Pachygnatha clercki* Sundevall 1823	65	156	138
	Pachygnatha quadrimaculata	-	18	62

	Pachygnatha tenera Karsch 1879	-	-	7
	Tetragnatha caudicula	-	-	1
	Tetragnatha maxillosa Thorell 1895	163	106	30
	Tetragnatha pinicola L. Koch 1870	14	-	-
	Tetragnatha squamata Karsch 1879	-	1	-
	Tetragnatha vermiformis Emerton 1884	-	7	20
Araneidae	*Argiope bruennichi* (Scopoli 1772)	1	1	1
	Hypsosinga sanguine (C. L. Koch 1944)	-	11	3
	Larinioides cornutus (Clerck 1757)	1	4	3
	Neoscona adianta (Walckenaer 1802)	24	4	1
Lycosidae	*Arctosa ebicha* Yaginuma 1960	-	2	-
	Arctosa stigmosa (Thorell 1879)	50	7	35
	Pardosa laura Karsch 1879	1	8	11
	Pirata subpiraticus (Bösenberg et Strand 1906)	596	529	1011
	Trochosa ruricola (De Geer 1778)	7	-	-

Pisauridae	*Dolomedes sulfureus* L. Koch 1877	5	5	37
Clubionidae	*Clubiona japonicola* Bösenberg et Strand 1906	-	2	-
	Clubiona kurilensis Bösenberg et Strand 1906	59	64	129
Thomisidae	*Ebrechtella tricuspidata* (Fabricius 1775)	5	17	22
	Xysticus concretus Utochkin 1968	-	16	23
	Xysticus hedini Schenkel 1936	4	1	-
Salticidae	*Mendoza canestrinii* (Ninni 1868)	-	6	3
	Mendoza elongate (Karsch 1879)	-	-	1

Table 3. Biodiversity (mean±SE, n = 3) of the spider community per plot in different plot size throughout the rice growing season

	Treatment			RM-ANOVA		
	270 m^2	1000 m^2	3300 m^2	*df*	*F*	*p*
Species richness	16.87±0.88	23.00±1.15	21.33±0.67	2. 6	4.71	0.0588
Shannon's index	1.83±0.04ab	1.97±0.02a	1.75±0.06b	2. 6	6.03	0.0366

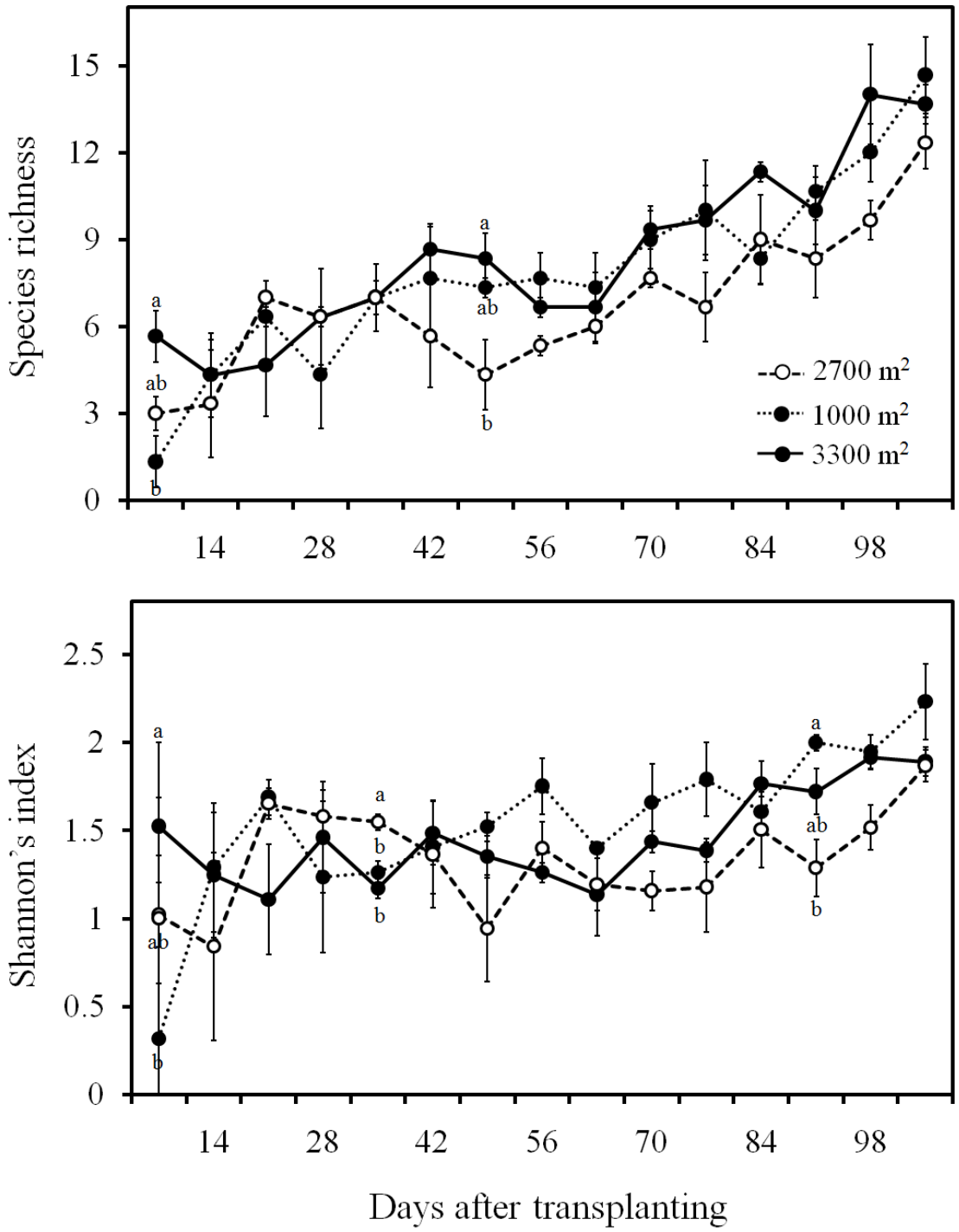

Figure 4. Seasonal dynamics of spiders (mean±SE, n = 3) per plot in different plot sizes throughout the rice growing season. Abundance was individual numbers of 5 samples and a sample consisted of one suction sampling and five sweep net samplings

Cluster analysis showed higher similarity among different plot size (Fig 4). β-diversity across plots was clearly represented in the dendrogram. Similarity in the spider community divided plots firstly into two clusters with 67.6% similarity: 270 m^2 and, 1000 m^2 and 3300 m^2. Plots of 1000 m^2 and 3300 m^2 showed 81.4% similarity (Fig. 5).

Sample-based and individual-based rarefaction curves for plot sizes (Fig. 6) reached asymptote as sample size and spider individual numbers increased revealing that samples of three plot sizes were nearly identical in the number of species each captured and plots of 1000 m^2 captured more species than 270 m^2 and 3300 m^2 (Fig. 6a).

The composition of dominant families and species were very similar among 3 plot sizes. The spider community was dominated by five families. Of them, Lycosidae and Tetragnathidae were the most abundant families in 3 different plot sizes, occupying 54.9% and 20.3% in 270 m^2 plots, 46.7% and 24.7% in 1000 m^2 plots and 58.9% and 14.4% in 3000 m^2 plots, respectively (Fig. 7a). Six dominant spider species, ranging from 4.2% to 56.4%, were *G. dentatum* and *U. insecticeps* (Linyphiidae), *P. clercki* and *T. maxillosa* (Tetragnathidae), *P. subpiraticus* (Lycosidae), and *C. kurilensis* (Clubionidae) (Fig. 7b). The most dominant species was *P. subpiraticus* in 3 plot sizes, occupying 50.4% in the 270 m^2 plot, 45.3% in the 1000 m^2 plot and 56.4% in the 3000 m^2 plot.

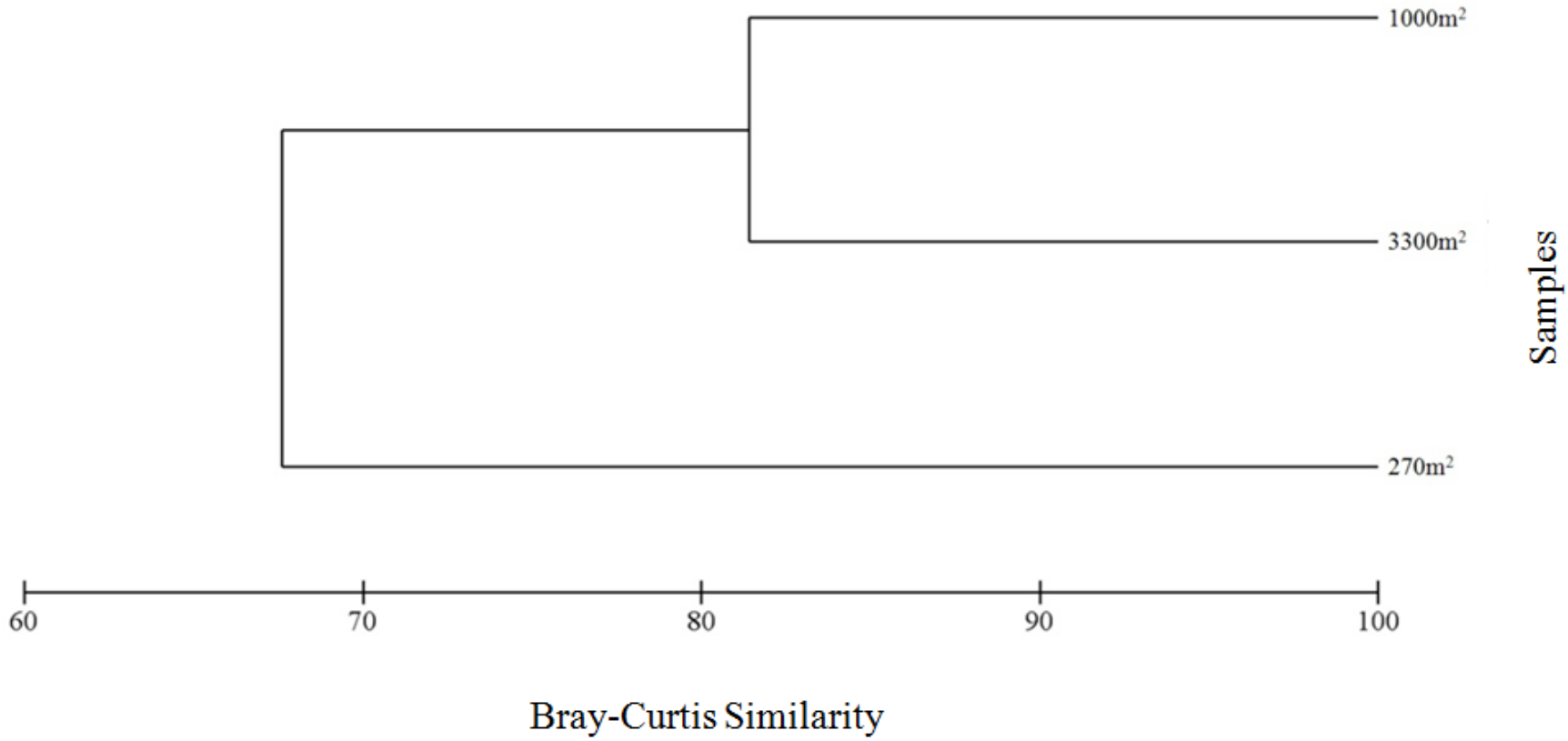

Figure 5. Cluster diagram formed utilizing the Bray-Curtis method showing the relationship among the different plot sizes throughout the rice growing season.

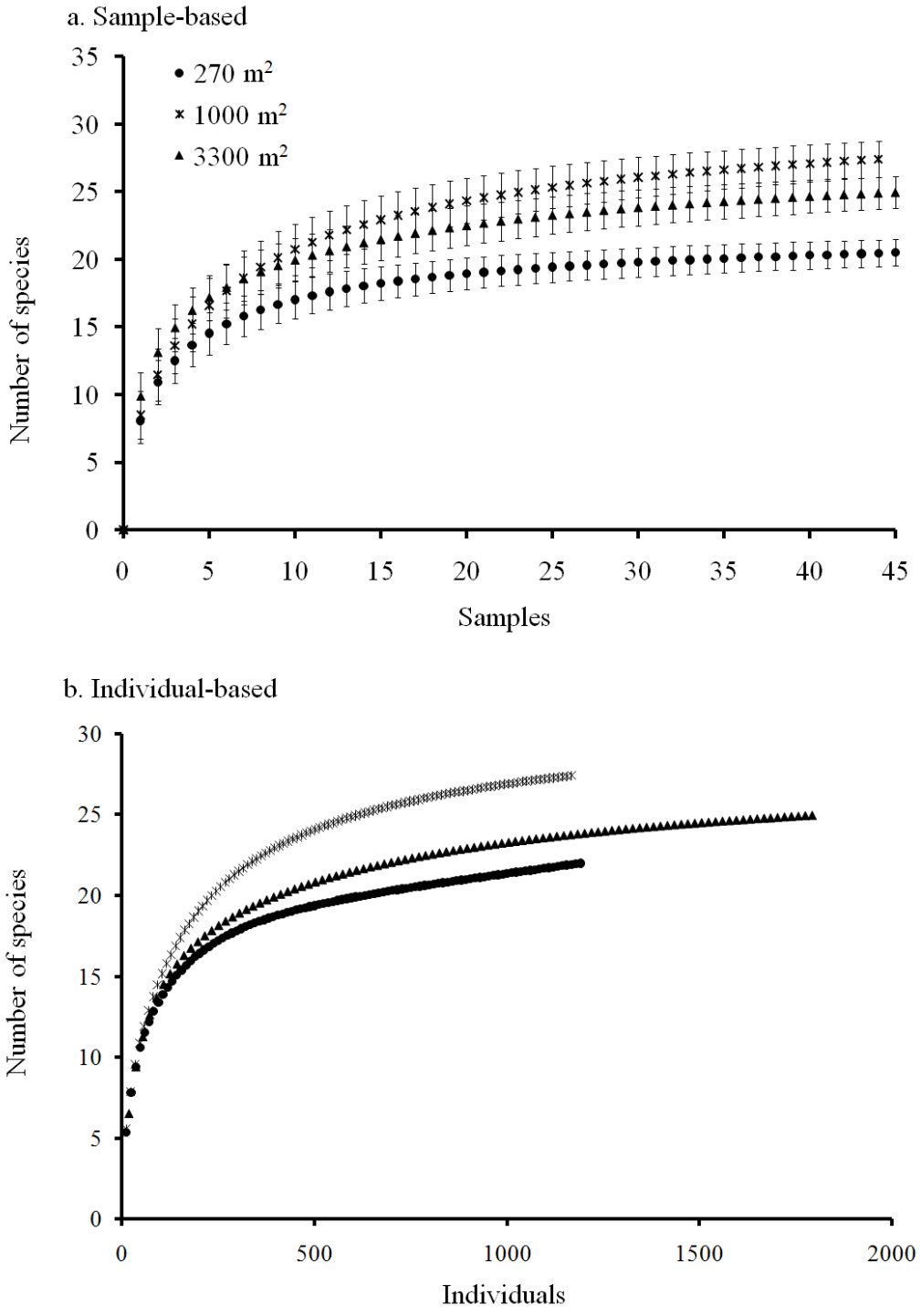

Figure 6. Comparison of species rarefaction curves of spiders in three plot size of rice fields throughout the rice growing season.

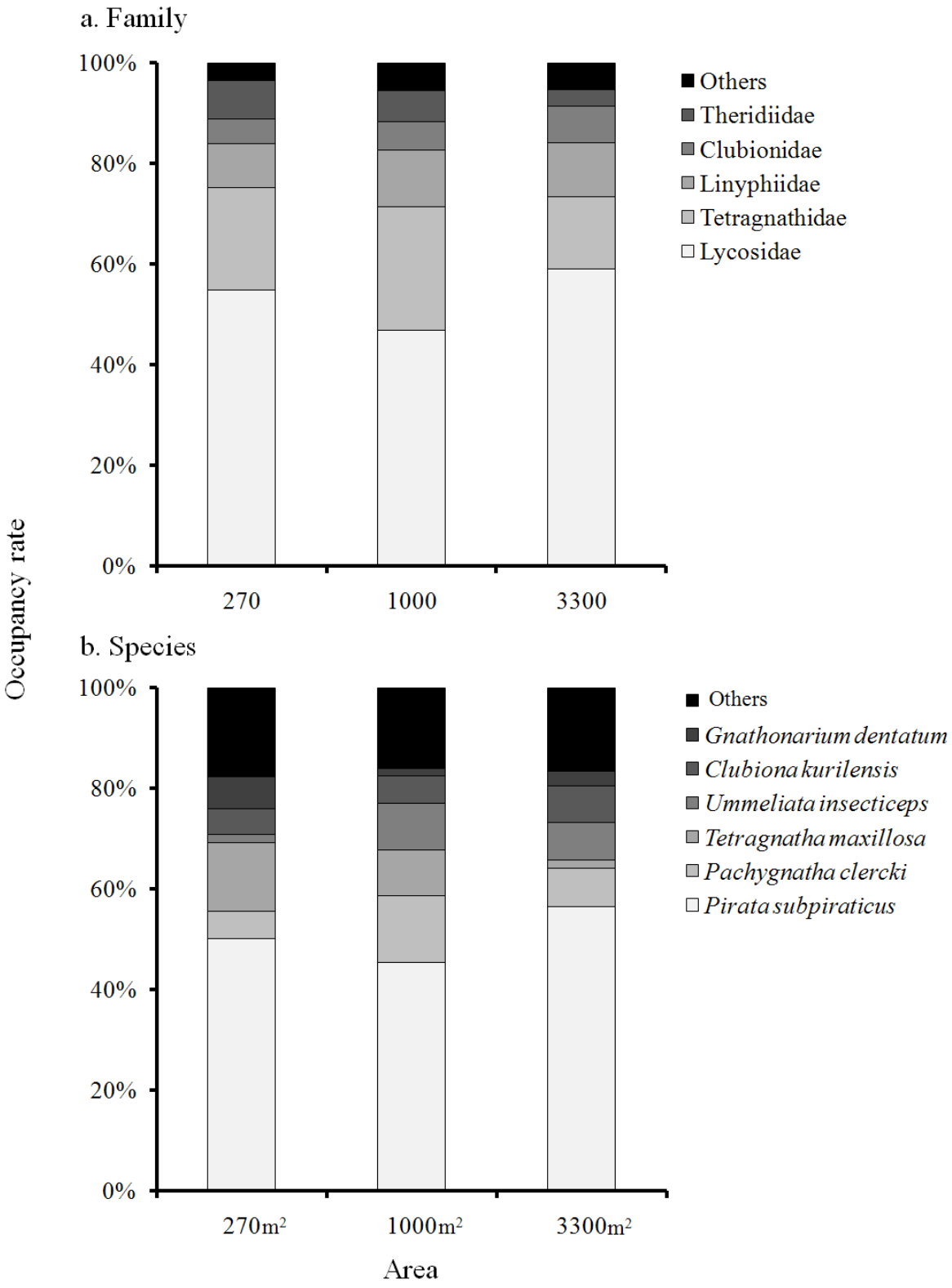

Figure 7. Comparison of dominant spiders in different plot size throughout the rice growing season

4.4.2.3 Sampling time and sampling occasion

Our survey was conducted at weekly intervals throughout the rice growing season and a total of 15 sampling windows were made. Cluster analysis showed similarity of spiders among different sampling times (Fig 8). β-diversity across plots was clearly represented in the dendrogram. Spiders divided times firstly into two clusters with 44.3% similarity, and five clusters with 65.6% similarity: time 1 (1-20 DAT), time 2 (21-34 DAT), time 3 (35-55 DAT), time 4 (56-83 DAT) and time 5 (84-105 DAT) (Fig. 8). Species richness was sharply increased until 56 DAT and we applied this group to accumulation curve of species richness (Fig. 9).

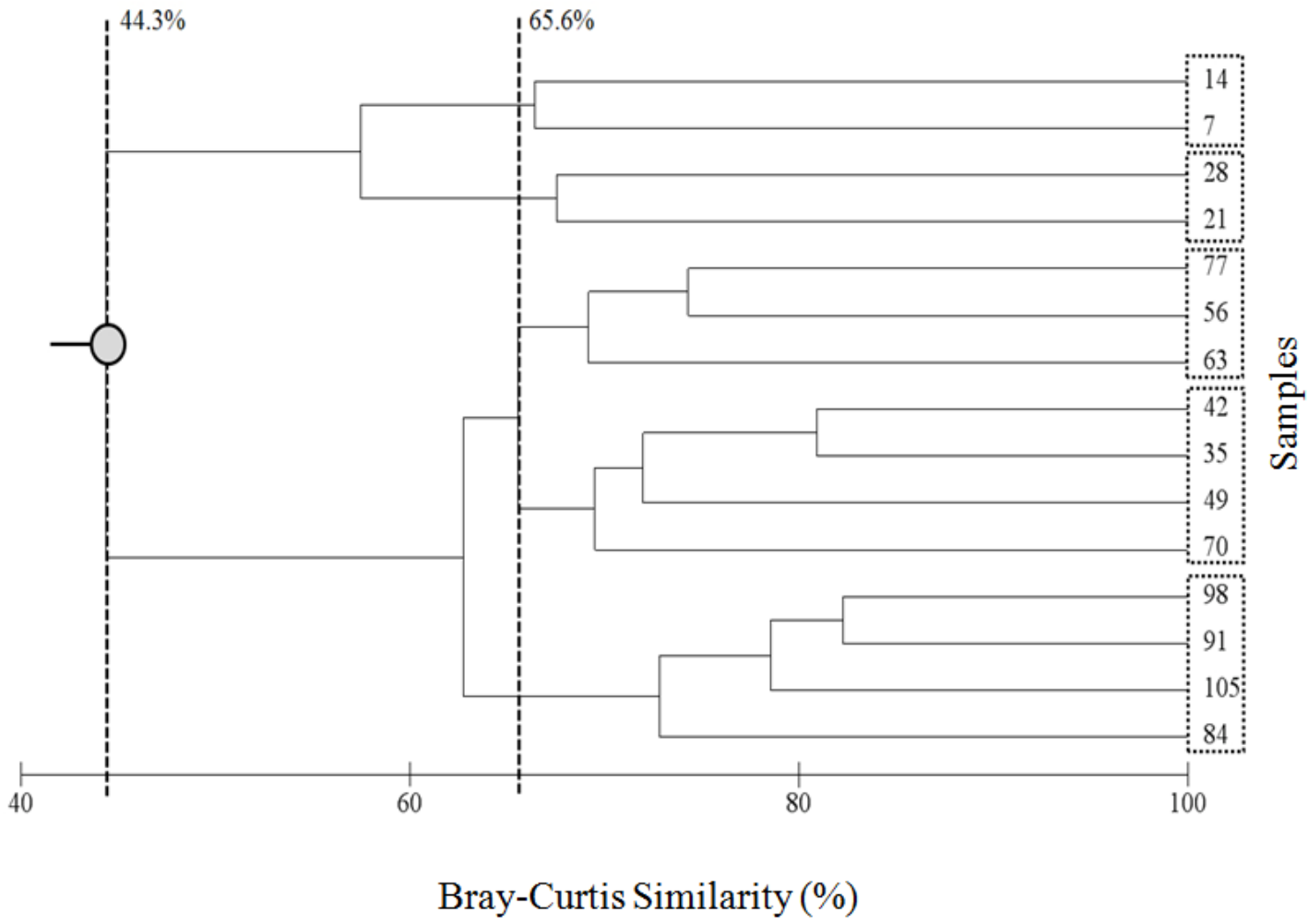

Figure 8. Cluster diagram formed utilizing the Bray-Curtis method showing the relationship among the sampling time and window throughout the rice growing season.

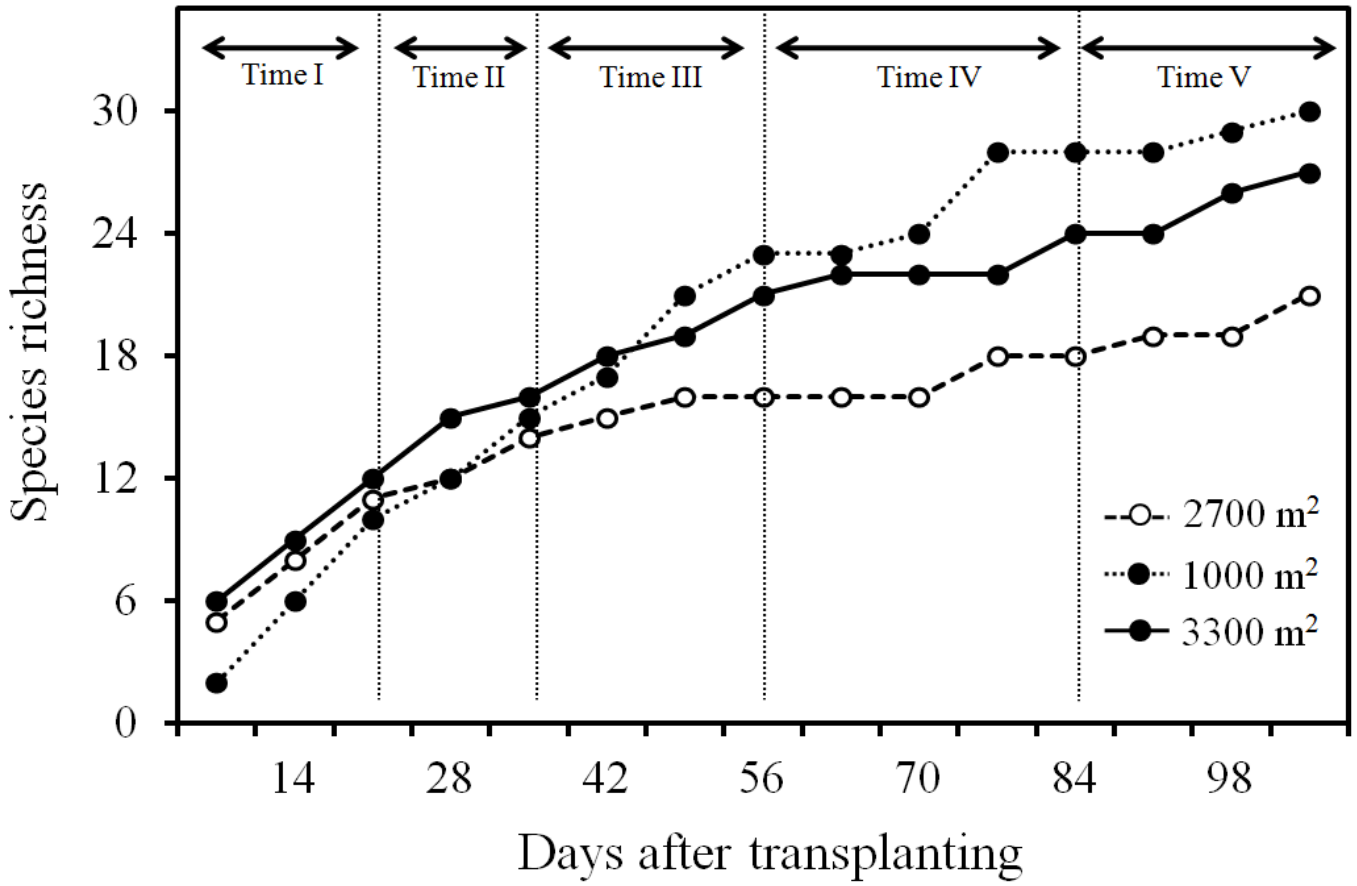

Figure 9. Accumulation curve of species richness according to sampling time throughout the rice growing season.

4.5. Discussion

A test base tiered system has been adapted to assess the effect of GM crop on non-target arthropods and includes a selection of suitable test organism at first tier conducted under laboratory conditions (Romeis et al. 2008, 2011). For a risk assessment of GM crops, using selective test organisms are indispensable. On selecting non-target herbivore, it is considered the test organism's mode of feeding and the site and the time of protein expression in the *Bt* crop (Dutton et al. 2002, Romeis et al. 2008). The six test species in this study were selected by considering these factors, and to ensure the reliability of the obtained results, test organisms should not show unacceptably high mortalities in the negative controls. Principles of basic toxicity testing dictate that test organisms should be healthy and of high quality and not otherwise stressed by factors other than the "*Bt*" (Rose 2007). All of these species represented different ecosystem services, various taxonomic groups and broad geographic distribution with high density in the fields (Table 1). Consequently, five of these species were appropriate to evaluate of *Bt* rice effects on non-target organisms, although *S. lurida* was not suitable for assessment under laboratory condition due to high mortality (Table 1).

The implication of *Bt* toxin exposure on the performance of non-target arthropods is still not clear in the case of long-term exposure occurring in the field. Therefore, field studies can be useful in identifying the overall effect on non-target arthropods. Thus as previously stated it is important to choose appropriate sampling methods, plot size, timing and occasion. Use of more than one sampling method is often recommended in a biodiversity survey depending on the taxa targeted (Resources Inventory Committee 1998). In this study, two sampling methods, suction and sweep net method, were used by considering spider and insect habitat and their activity. These two sampling methods are frequently employed in the rice arthropod

survey (Schoenly et al. 1996, Bambaradeniya and Edirisinghe 2008, Barrion et al. 2011). In this study, suction sampling captured more species and individuals of spiders than sweep net sampling while insects were more captured by sweep net sampling. Sweep net method is generally used for capturing arthropods having flight ability or living in the upper part of the plant. Chen et al. (2006) reported that vacuum-suction sampling should be the preferred method for detecting the effects of *Bt* rice on arthropods (e.g., epigeic Arthropods) without flight ability. However, in the current study Araneidae, Thomisidae were captured more by sweep net method and some species such as *P. angulithorax, T. extensa, A. bruennichi, and E. tricuspidata* were captured only by the sweep net method. In addition, the sweep net method showed higher efficacy in insect sampling except a few epigeic Coleoptera and Collembola. Thus, both suction and sweep net sampling methods should be employed because these methods are more likely complementary.

The potential influence of the plot size on the evaluation of non-target effects is another issue for study of non-target effects of GM crops. Small plots may give misleading results (Cantelo 1986, Witmer et al. 2003), in part because various non-target species establish in or re-colonize disturbed areas at different rates. Also, concern has been raised that small plot sizes for field studies may not differentiate the potential differences in arthropod diversity and abundance between *Bt* and non- *Bt* crops because of relatively high dispersal ability of arthropods (Sisterson et al. 2004, Farinós et al. 2008). However, large plot field studies may result in significant within-plot variation leading to the need for additional sampling for precise estimation (Rose 2007). In this study, biodiversity of spiders was not increased with increasing of plot size. But similarity of the community was higher between 1000 m^2 and 3300 m^2 than that of among three different plot sizes (Fig. 5). Thus, it seems that 1000 m^2 plot size test is resonable to detect *Bt* crop effects on arthropods community in rice fields. To

find appropriate sampling time, our study used cluster analysis with similarity of community, and sampling time were divided into five clusters with 65.6% similarity: Time 1 (1-20 DAT), Time 2 (21-34 DAT), Time 3 (35-55 DAT), Time 4 (56-83 DAT) and time 5 (84-105 DAT) (Fig. 9). Thus, field survey should be conducted at least five times according to time clusters that we suggested for detect *Bt* effect on arthropod community and these clusters generally corresponded to five rice growing stages in the fields (seedling, tilling, booting, flowering and heading, maturity).

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유전자변형벼(Cry1Ac)가 비표적절지동물에게 미치는 영향평가방법 개발

서울대학교 대학원

농생명공학부 곤충학전공

이 수 연

초록

혹명나방저항성유전자변형벼(Cry1Ac)가 표적절지동물 및 비표적절지동물에 미치는 영향을 평가하고 평가방법을 개발하기 위해 본 연구를 수행하였다. 표적절지동물인 혹명나방(*C. medinalis*)의 산란선호도와 벼애나방(*N. aenescens*)의 영기별 사망률에 미치는 영향을 평가한 결과, 혹명나방은 혹명나방저항성벼와 비유전자변형벼 사이에 산란선호도가 없었고 벼애나방의 영기별 사망률은 혹명나방저항성벼에서 높은 사망률을 보이면서 통계적으로 유의한 차이를 보였다. 비표적절지동물에서는 초식곤충군인 벼메뚜기(*O. japonica*), 먹노린재(*S. lurida*)와 벼멸구(*N. lugens*), 화분섭식군인 꼬마남생이무당벌레(*P. japonica*)와 천적군인 황산적거미(*P. subpiraticus*)와 턱거미(*P. clercki*)에서 혹명나방저항성벼의 영향을 평가하였다. 벼메뚜기, 먹노린재, 벼멸구의 발육기간, 생존율, 우화율 및 발육형질은 혹명나방저항성벼와 비유전자변형벼간에 차이가 없었고 꼬마남생이무당벌레는 진딧물만 공급한 경우

혹명나방저항성벼와 비유전자변형벼의 화분을 같이 공급한 경우보다 통계적으로 짧은 발육기간과 낮은 발육형질의 수치를 보였으나 혹명나방저항성벼와 비유전자변형벼의 화분을 공급한 처리간에는 차이를 보이지 않았다. 벼멸구의 벼의 묘령에 따른 발육실험에서는 60 일묘를 공급한 것보다 20 일묘를 공급한 경우 짧은 발육기간을 보이면서 통계적으로 차이가 있었다. 황산적거미와 턱거미는 혹명나방저항성벼를 먹인 벼멸구를 섭식한 경우 종아리마디 길이(Tibia length)가 비유전자변형벼를 섭식한 경우보다 통계적으로 길었으나 발육기간, 생존율, 우화율 및 다른 성충의 발육형질에서는 차이를 보이지 않았다.

포장조건에서 진행된 2 년간의 조사에서는 곤충군집은 전체 10 목, 43 과, 64,099 개체의 곤충이 채집되었고 초식곤충군, 포식자군, 기생포식자 및 분해자군 등 4 개의 생태적 기능군으로 분류되었다. 혹명나방저항성벼와 비유전자변형벼포장의 곤충군집의 발생양상은 유사하였고 2007 년의 초식곤충군의 발생밀도가 비유전자변형벼포장에서 높았으나 곤충군집의 과수, 발생밀도, 과다양성지수 및 우점과와 우점종의 비교에서는 차이가 없었다. 거미군집은 9 과 23 속 29 종 4,937 개체가 채집되었고 2008 년 색동꼬마거미, 턱거미와 민갈거미의 발생밀도가 혹명나방저항성벼와 비유전자변형벼 포장간에 차이를 보였다. 전반적으로 혹명나방저항성벼와 비유전자변형벼 포장에서의 거미군집의 발생밀도는 비슷한 양상을 보였고 종수, 발생밀도, 종다양성 지수 및 우점종에서 차이가 없었다.

해충저항성유전자변형벼의 (Cry1Ac) 비표적절지동물에 대한 위해성평가시 평가종의 선정기준과 위해성평가시스템인 Tier-system 을 논의 절지동물에 적용하였고 포장에서의 적절한 조사방법, 조사면적, 조사시기 및 횟수를 제시하기

위해 논에서 대표적으로 쓰이는 흡충기를 이용한 조사방법과 포충망을 이용한 조사방법의 효율성을 비교한 결과 조사종의 서식환경내에서의 생태학적 특성을 반영하고 다양한 종을 채집하기 위해서는 두 가지 방법을 모두 사용하는 것이 효율적인 것으로 나타났다. 논 포장에서 적절한 조사면적을 제시하기 위하여 면적이 다른 세 개의 논 포장(270m^2, 1000m^2, 3300m^2)의 거미 군집을 비교한 결과, 거미의 종다양성은 면적증가에 비례하여 증가하지 않았으며 1000m^2 에서의 생물다양성이 가장 높았고 다른 두 면적의 포장들과 군집유사도를 비교한 결과 높은 것으로 나타났다. 조사시기별 거미군집의 유사도 및 누적다양성을 분석한 결과, 5 회 이상의 조사가 필요한 것으로 나타났고 이는 벼의 생육단계(이앙기, 유묘기, 분얼기, 출수 · 개화기, 등숙기)와 일치하였다.

검색어: 유전자변형벼(Cry1Ac), 위해성평가, 비표적절지동물, 혹명나방, 벼애나방, 벼메뚜기, 먹노린재, 벼멸구, 꼬마남생이무당벌레, 황산적거미, 턱거미, 곤충군집, 거미군집

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