A THESIS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Ecology and insecticidal susceptibility of mycophagous *Illeis koebelei* Timberlake (Coleoptera: Coccinellidae: Halyziini)

식균성 노랑무당벌레의 생태 및 약제 감수성에 관한 연구

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UNDER THE DIRECTION OF ADVISER JOON-HO LEE
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF SEOUL NATIONAL UNIVERSITY

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FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
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Ecology and insecticidal susceptibility of mycophagous *Illeis koebelei* Timberlake (Coleoptera: Coccinellidae: Halyziini)

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ABSTRACT

This study was conducted to investigate ecology and insecticidal susceptibility of mycophagous ladybeetle, *Illeis koebelei.* Twelve species of plants infected with powdery mildew were surveyed as host of *I. koebelei* in Gyeonggi-do, Korea from 2010 to 2012. The pear tree, *Pyrus ussuriensis* var. *macrostipes* (Nakai), was the most preferred plant to *I. koebelei. I. koebelei* was found from early July to early November in pear orchards. There was no trace without fungal materials through finding the spores of powdery mildew in the gut of *I. koebelei*. All motile stages are obligate mycophagy, and the feeding potential is ranked as follows: fourth instar,

adults, third instar, second and first instar.

Development experiment was conducted at eight temperatures, ranging from 15.4 to 39.5 ℃. Development rates were well fitted with linear and nonlinear models. Lower developmental thresholds for egg, first instar, second instar, third instar, and fourth instar larva, pre-pupa, pupa, and total immature stage were estimated to be 3.6, 12.7, 12.1, 11.3, 11.3, 12.8, 14.7, and 14.2 ℃, respectively. Their respective thermal requirements in degree days (DD) were 86.6, 16.0, 22.5, 30.2, 49.3, 14.5, 43.8, and 217.4DD, respectively. Survivorship was highest at 25.1 ℃ for immature. Oviposition experiment was conducted at nine temperatures, ranging from 15.4 to 35.3 ℃. Mean fecundity ranged from 18.6 eggs at 29.3 ℃ to 205.3 eggs at 20.3 ℃. It was well described by the extreme value function. Adult survival and cumulative oviposition rates of *I. koebelei* were fitted to a sigmoid function and a two-parameter Weibull function, respectively. Findings of this study provide basic information for ecology of *I. koebelei*. They can be used to optimize environmental conditions for mass-rearing and shipping, comparing optimal occurrence conditions between *I. koebelei* and powdery mildew, and forecasting phenology and population dynamics of *I. koebelei* in the fields.

Bioassay was conducted to determine the relative toxicities of several pesticides used for cucumber production in Korea to *I. koebelei* and to provide a background for implementation of integrated powdery mildew management programs. Synthetic and environmental-friendly products used conventionally for the control of insect or

microbial pests on cucumber in Korea. Based on IOBC classification, three insecticides, bifenthrin + imidacloprid, acetamiprid + indoxacarb, acetamiprid + etopheprox are classified as having a Class 4 (harmful). Spiromesifen showed the low toxicity to the survival and the fecundity of *I. koebelei* when this chemical had been exposed to 3rd larva or newly emerged adult via feeding with cucumber powdery mildew. However, pyriproxyfen not only decreased the fecundity of female adult but also strongly prohibited from pupation. Many commercial biological or botanical pesticides can restrict the population of *I. koebelei*. However, Q pact (a.i. *Ampelomyces quisqualis* 94013), Top seed (a.i. *Paenibacillus polymyxa* AC-1), BT one (*Bacillus thuringiensis*) and Solbitchae (insecticidal microorganism) had no toxicity to *I. koebelei* when this chemical had been exposed to the third instar larva or newly emerged adult feeding with cucumber powdery mildew.

Key words: *Illeis koebelei*, powdery mildew, mycophagous, biological control, survival, development model, oviposition model, toxicity, pesticide

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INTRODUCTION

The family Coccinellidae is comprised of approximately 6,000 species worldwide (Hodek et al., 2012). Most coccinellid species are commonly entomophagous, and the major preys are hemipteran insects, although their preference is different according to the food species (Omkar and Bind, 1996; Giorgi et al., 2009). Therefore, they have been well known as natural enemies. Apart from this entomophagous feeding habit, some species are mycophagous, and they belong to the tribe Halyziini and Tytthaspidini of Coccinellinae (Giorgi et al., 2009; Sutherland and Parrella, 2009).

Of them, mycophagous ladybeetles in the tribe Halyziini are potentially attractive agents for the biological control of powdery mildew, but trophic ecology of these beetles is poorly understood. Mycophagous ladybeetles in tribe Halyziini are potential biological control agents for powdery mildew (Sutherland and Parrella, 2009), a common and economically important plant disease worldwide that infects more than 1,500 plant species (Amano, 1986; Braun, 1987; Ale-Agha et al., 2008; Glawe, 2008; Olena et al., 2014). The major diet of these ladybeetles is powdery mildew, and their alternative foods were known for several species, for instance sooty mold or pollen (Sasaji, 1998; Giorgi et al., 2009). Like the fungi that they feed on, the Halyziini exhibits a cosmopolitan distribution, and at least one species of mycophagous

ladybeetle is present wherever powdery mildews commonly occur (Sutherland and Parrella, 2009).

Oriental genus *Illeis* belonging to Halyziini has attracted by many entomologists or biologists for its unique mycophagous habit (Men et al., 2002; Giorgi et al., 2009; Sutherland and Parrella, 2009; Sharma and Joshi, 2010; Karuna et al., 2013; Thite et al., 2013). Among Halyziini species, the yellow ladybeetle, *Illeis koebelei* Timberlake, is one of most attractive species that can control powdery mildew due to its unique habitats and feeding behavior (Men et al., 2002; Giorgi et al., 2009; Sutherland and Parrella, 2009; Sharma and Joshi, 2010; Karuna et al., 2013; Thite et al., 2013). *I. koebelei* has been found in Asian countries, including Korea (Kim et al., 1994; Lee et al., 2015), Philippine (Recuenco-Adorada and Gapud, 1998), Japan (Takeuchi et al., 2000), China (Wu et al., 2011), and Taiwan (Lin et al., 2006). However, there is a little study about *I. koebelei* in Korea, in spite of arising interest as the solution of biological control of powdery mildew disease. The objective of this study was to investigate the natural occurrence and the biology of *I. koebelei* in various agricultural and horticultural systems in Gyeonggi-do, Korea. In addition, we observed the morphological characteristics of mycophagous *I. koebelei* in relation to the mycophagous habits.

Powdery mildew is the most common, widespread, and very economically important disease in many agricultural crops worldwide (Amano, 1986). They attack a wide range of plant species and infect many different plant structures (Glawe, 2008)

in all kinds of temperate, arid, subarctic and tropical habitats (Ale-Agha et al., 2008). Management of powdery mildew is mainly relying on regular fungicide application, but this chemical control have raised the high cost, causing fungicide resistance and residual effects on environment and human (Razdan and Sabitha, 2009). Therefore, as an alternative control method, biological control by arthropods or microbes has been considered; mycolytic microorganisms (Kiss 2003; Lee et al., 2007; Romero et al., 2007), mycophagous arthropods (Wu and Guo, 1987; Bhattacharjee et al., 1994; English-Loeb et al., 2007), and other potential non-fungal biological control agents (Segarra et al., 2009; Hegazi and El-Kot, 2010).

Despite its potential as a biological control agent for powdery mildew, *I. koebelei* has been rarely studied. In Korea, *I. koebelei* populations show two peaks per year (Lee et al., 2015). The first peak in early July might be related to the overwintering generation. The second peak from early October to early November could be important for *I. koebelei* as a good biological control agent against powdery mildew because this period is harvesting or post-harvesting period for many crops such as cucumber, pepper, tobacco, apple, and pear in Korea. Fungicide application is generally limited until the next crop season due to residual toxicity of harvested products and economic reason. Powdery mildew can become severe at this period due to decreased fungicide application and matured crop age (Lee et al., 2015). Thus, *I. koebelei* has high potential for controlling powdery mildew during this period in Korea. Management of powdery mildew would be more effective and feasible if

phenology and population dynamics of *I. koebelei* can be predicted reliably. However, no modeling study such as development and oviposition models has been conducted for *I. koebelei*. Such lack of models hampers understanding of population dynamics of *I. koebelei* and its use for powdery mildew management. Therefore, the objective of this study was to develop temperature-dependent development and oviposition models of *I. koebelei*. These models will be very useful for understanding population dynamics of *I. koebelei* and for developing biological control strategy to control powdery mildew using *I. koebelei*.

For the management of powdery mildew, grower heavily relies on chemical control using fungicide, but this conventional control method caused adverse effects: development of pesticide resistance in pests, environmental pollution, and disruption of natural enemies of target pests (Razdan & Sabitha, 2009). Nowadays, there is increasing worldwide public attention focused on minimizing the chemicals' side effect. For the biological control of powdery mildew, there are many trials have been made using microorganisms, mycophagous arthropods and other biological agents (Bhattacharjee et al., 1994; English-Loeb et al., 2007; Lee et al., 2007; Romero et al., 2007; Segarra et al., 2009; Hegazi and El-Kot, 2010).

As natural enemies can be exposed to pesticides, selective pesticides which have low toxic to natural enemy are good alternative strategy for conservation of natural enemy populations, and reducing the rate of pesticide application (Tanaka et al., 2000). Ultimately, all control tactics should be considered and mixed to total

solutions, what is called, Integrated Pest Management (IPM) that is a combination of biological, cultural, chemical, and other possible control skills that reduce pests with natural enemies to tolerable levels. For the implemental of an IPM, selection of chemicals that are high control effect to pest, but low toxic to natural enemies is necessary. So, effects of pesticides on natural enemy should be estimated for the success biological control using natural enemies. International Organization of Biological Control (IOBC) is active in identifying pesticides compatible with biological control. Based on IOBC classification, the effect on natural enemy is categorized as Class 1 toxicity level (harmless), Class 2 level (slightly harmful), Class 3 level (moderately harmful), and Class 4 toxicity level (harmful).

Many tests have assessed the effect of chemical insecticides, biopesticides, and genetically modified organisms (GMO) on natural enemies. Generally, the three types of tests used in most countries are the laboratory, semi-field, and field tests. For the effective investigation, the laboratory tests that can prevent various environmental factors are recommended (Tanaka et al., 2000). Most assessments have been examined under field and laboratory conditions and relied on mortality as an indicator of susceptibility. There are many wider and deeper studies of toxicity related to Asian ladybeetle *Harmonia axyridis* have been conducted with various insecticides, biopesticides, fungicides, herbicides and what is more insect-resistant transgenic crops (Koch, 2003). Cho et al. (1997) reported that synthetic pyrethroid insecticides were less toxic to *H. axyridis* than to aphids. Insect ecdysone agonists, halofenozide

and methoxyfenozide are known to cause the premature of larval molting, interruption of feeding and incomplete pupation (Carton et al., 2003). And several terpenoids derived from plants, carmphor, menthol, catnip, and grapefruit for instance are known to repel the ladybeetle (Riddick et al., 2008). In the case of ladybeetle *Hippodamia variegata* (Goeze), Almasi et al. (2013) selected the pirimicarb and pymetrozine as a low toxic in contrast to proteus that showed the high mortality rate, and. Rahmani et al. (2013) reported that a new neonicotinoid insecticide, thiamethoxam decreased the developmental period of pre-adult stage but did not effect on adult developmental period. In the case of ladybeetle *Coccinella septempunctata* L., imidacloprid and deltamethrin is known to relatively harmful (Bozsik, 2006), but two biopesticdes, Bioshower (a.i. 100% fatty acid) and insecticidal soap (a.i. 20% fatty acids) have no toxicity to the ladybeetle (Raudonis et al., 2010). Radha (2013) studied the comparative toxicity of biopesticide and synthetic pesticide against cowpea aphid, *Aphis craccivora* and its natural enemy ladybeetle, *Micrapis discolor*. They recommended the botanical insecticides (neem seed extracts) and microbial pesticide (spinosad) as alternatives to chemical insecticides for the IPM of *Aphis craccivora* in cowpea.

In spite of the broad distribution of mycophagous *I. koebelei* and its potential as a biological agent against powdery mildew disease, there is no trial to evaluate the side effect of pesticides on mycophagous *I. koebelei*. For planning successful IPM or organic strategy to control cucumber powdery mildew, information about the effect of

pesticides on natural enemy of a given ecosystem is very important. So, this study was conducted to determine the relative toxicities of several pesticides used in Korea cucumber production to mycophagous natural enemy, *I. koebelei* and to provide a background for implementation of integrated powdery mildew management programs.

Chapter I.

Occurrence and biological characteristics of *I. koebelei* Timberlake (Coleoptera: Coccinellidae: Halyziini) in Korea

Abstract

Mycophagous *Illeis koebelei* was collected from 12 species of plants infected with powdery mildew in Gyeonggi-do, Korea. The pear tree, *Pyrus ussuriensis* var. *macrostipes* (Nakai), was the most preferred plant to *I. koebelei.* This species was found from early July to early November in pear orchards. There was no trace without fungal materials through finding the spores of powdery mildew in the gut of *I. koebelei*. All stages except egg and pupa are obligate mycophagy, and the feeding potential is ranked as follows: fourth instar, adults, third instar, second and first instar. Developmental periods of four larval instars and adult feeding cucumber powdery mildew were 1.2, 2.3, 2.3, 4.6, 37.7 days respectively at 25℃. In this study, we could find the feeding potential of *I. koebelei* against the cucumber powdery mildew, and further more study will be needed to develop this species as a biological control agent e.g. mass rearing skill, selection of low toxic chemical agent for IPM and control technique against powdery mildew in agro-ecosystem.

Keywords: *Illeis koebelei*, Powdery mildew, Mycophagous, Biological control

1. Introduction

Powdery mildew is the most common, widespread, and very economically important disease in many agricultural crops worldwide (Amano, 1986). They attack a wide range of plant species and infect many different plant structures (Glawe, 2008) in all kinds of temperate, arid, subarctic, and tropical habitats (Ale-Agha et al., 2008). Management of powdery mildew is mainly relying on regular fungicide application, but this chemical control have raised the high cost, causing fungicide resistance and residual effects on environment and human (Razdan and Sabitha, 2009). Therefore, as an alternative control method, biological control by arthropods or microbes has been considered; mycolytic microorganisms (Kiss 2003; Lee et al., 2007; Romero et al., 2007), mycophagous arthropods (Wu and Guo, 1987; Bhattacharjee et al., 1994; English-Loeb et al., 2007) and other potential non-fungal biological control agents (Segarra et al., 2009; Hegazi and El-Kot, 2010).

Oriental genus *Illeis* belonging to Halyziini has been attracted by many entomologists or biologists for its unique mycophagous habit (Men et al., 2002; Giorgi et al., 2009; Sutherland and Parrella, 2009; Sharma and Joshi, 2010; Karuna et al., 2013; Thite et al., 2013).

Illeis koebelei is generally recorded in Asia, such as, Japan (Takeuchi et al., 2000), China (Wu et al., 2011), Philippine (Recuenco-Adorada and Gapud, 1998), Taiwan (Yu, 2011), and Korea (Kim et al., 1994; Lee et al., 2015). However, there is a little

study about *I. koebeleis* in Korea, in spite of arising interest as the solution of biological control of powdery mildew disease.

The objective of this study was to investigate the natural occurrence and the biology of *I. koebelei* in various agricultural and horticultural systems in Gyeonggi-do, Korea. In addition, we observed the morphological characteristics of mycophagous *I. koebeli* in relation to the mycophagous habits of them.

2. Materials and Methods

2.1. Natural occurrence

This study was carried out in the eight regions of Gyeonggi-do, Korea (Fig. 1), which is situated at 36 90' 51" to 38 16' 54" N latitude and 126 55' 03" to 127 82' 95" E longitude. *Illeis koebelei* was surveyed at five sites per region every 10 days during April-November from 2010 to 2012. Their presence or abundance was based on visual encounters of the plants with powdery mildew. Collection was made by aspiration or hand picking, depending on the types of habitats, and all stages were collected, if possible. They were kept in plastic bowls along with fungus infected leaves separately to avoid overcrowding and food limitation, and were brought to the laboratory.

Powdery mildew severity was assessed using the score chart of 0 to 5 scale (0 = No infection, 1 = 0.1 ~ 10 %, 2 = 10.1 ~ 15 %, 3 = 15.1 ~ 25 %, 4 = 25.1 ~ 50 %, and 5 = More than 50 % leaf area covered with mildew growth) as described by Anand et al.

(2008). *I. Koebelei* abundance were estimated visually to the five level of index (0 = No detection, 1 = Under 1, 2 = 1.1 ~ 2, 3 = 2.1 ~ 3, 4 = 3.1 ~ 4, and 5 = More than 4 individuals per five leaves of each plant).

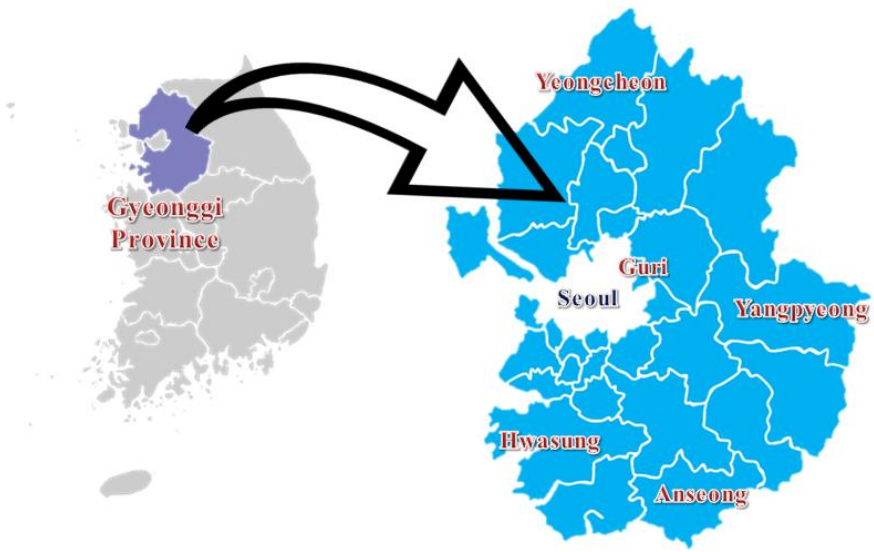


Fig. 1. Study area for investigating the natural occurrence of *Illeis. koebelei*.

2.2. Rearing of *I. koebelei*

Cucumis sativus L. (Beakdadagi) seeds were planted in plastic pots (100 cm diameter, 89 cm height) and grown in a thermostatic chamber at 26℃, 70±10% RH, and a photoperiod of 16:8 (L:D) h. Powdery mildew was collected from cucumber plants for inoculum. Powdery mildew collected was gently transferred to a single leaf of each two-week-old plant using a soft paintbrush. Four-week-old powdery mildew infected plants were used for the mass rearing of *I. koebelei* (Fig. 2).

The beetles were maintained in the rearing room at 23±1℃ and relative humidity at 60~80% under a photoperiod of 16:8 (L:D) h. Fifteen to twenty adult beetles were allowed to mate and lay eggs in plastic containers (30 × 30 × 30 cm) in which two powdery mildew infected cucumber. Eggs laid on plant were transferred to the translucent plastic cage (232 × 165 × 95 mm, with ventilation hole) with wet tissue paper laid on the bottom. Newly hatched larvae were kept on the powdery mildew infected cucumber plants. Before powdery mildew was depleted, larvae were gently transferred to a new powdery mildew infected cucumber plant using a soft brush until pupation. Pupae were placed in the other plastic cage until emergence.

Fig. 2. Mass rearing of *Illeis koebelei* larva using cucumber leaves infected by powdery mildew in laboratory.

2.3. Consumption and control of cucumber powdery mildew

The feeding capacity of *I. koebelei* against cucumber powdery mildew was investigated in laboratory. Cucumber leaf disc infected by powdery mildew was laid in 6-well flat-bottom plate (34.9 mm diameter, 20 mm height per cell; Dae-han Lab Tech, Suwon-si, Korea), and then put immature and female adult of *I. koebelei* in separate wells (Fig. 3). It was maintained in the insect rearing room at 26 ℃ with RH of 70% and a photoperiod of 16:8 (L:D) h, and powdery mildew infected leaf discs were supplied for feeding. Sum of fed area of immature and adult to the death was calculated.

The optimal density of *I. koebelei* against cucumber powdery mildew was investigated in greenhouse located in Hwaseong-si (Fig. 4). In order to determine the proper dosing density of *I. koebelei*, 1, 2, 3, 5, 7, and 9 3rd instar larvae were inoculated on cucumber leaves infected with powdery mildew in less than 2.5%, and then the disease severity was investigated for 22 days after inoculation. Disease incidence (%) was determined by calculating the number of infected leaves out of 30 leaves among the treated plants by modifying the method of Lamsal et al. (2011).

The control effect of cucumber powdery mildew was tested by the combination of *I. koebelei* and microorganism (*Paenibacilluspolymyxa*AC-1) for controlling powdery mildew. The test was conducted in Anseong and Hwaseong, and the specific treatment methods are shown in Table 1.

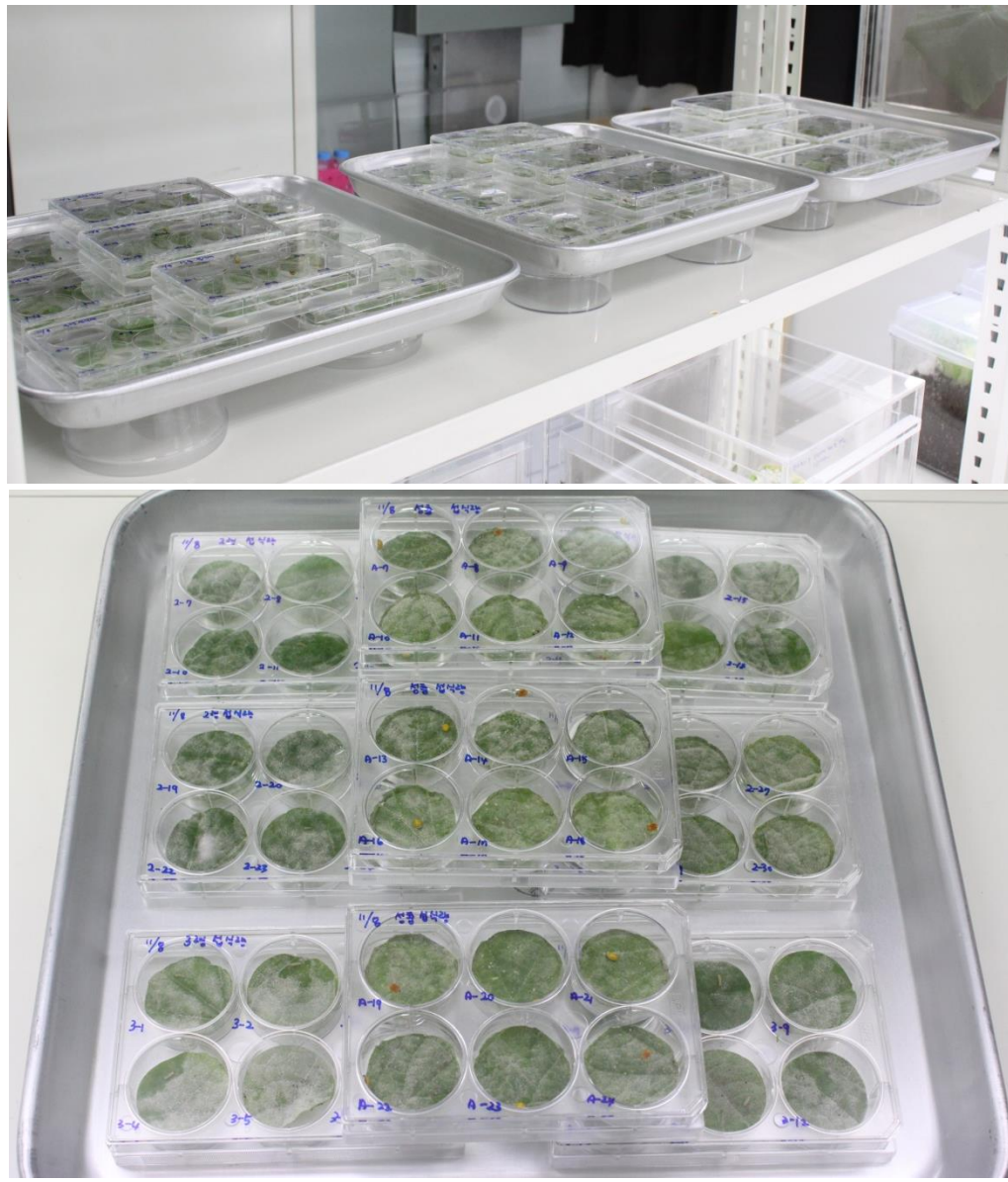


Fig. 3. Examination of the feeding amounts of *Illeis koebelei* to cucumber powdery mildew in laboratory.

Fig. 4. Examination of the control effect of *Illeis koebelei* on cucumber powdery mildew in the greenhouse.

Table 1. Study area and the input of *Illeis koebelei* and bio-pesticide for the control of cucumber powdery mildew

Area	Season (Month/day)	Biological agent	
		Illeis koebelei (3^{rd} larvae + adults)	Microorganism (*Paenibacilluspolymyxa*AC-1)
Anseong	Autumn (9/18～11/20)	·4 times (9/25, 10/2, 10/9, 10/16) ·1,600 larvae, 700 adults	·4 times (9/24 10/1, 10/8, 10/15)
Hwaseong	Autumn (9/25～11/15)	·3 times (10/2, 10/15, 10/30) ·800 larvae, 200 adults	·2 times (10/9, 10/22)

3. Results

3.1. Natural occurrence

Illeis koebelei was found on 12 species of plants infected with powdery mildew in Gyeonggi-do (Table 2). Of them, *I. koebelei* was most abundantly found on the pear tree, P*yrus ussuriensis* var. *macrostipes* (Nakai) of which powdery mildew belonging to Phyllactinia (Fig. 5). Phyllactinia powdery mildew appeared to be the most preferred food to *I. koebelei*. *I. koebelei* occurred from early July to early November in pear orchards (Table 3). All stages of *I. koebelei* were found from August to September, when the monthly mean temperature was 25.6℃ and 20.7℃, respectively.

Spores of cucumber powdery mildew were found in the gut of *I. koebelei* (Fig. 6). There was no trace of arthropod foods without fungal materials. There are several species of powdery mildews that *I. koebelei* visiting for feeding. But we could not find any relationships between the structures of powdery mildew spores and the occurrence of *I. koebelei* (Fig. 7, Table 2).

Table 2. Host plants infected with powdery mildew (PM) of *Illeis koebelei* in South Korea

PM (Genus)	Host Plant (Species)	PM Severity	*I. koebelei* Abundance	Area Collected
Leveillula	*Capsicum annuum* L.	3 [a]	2 [b]	A, H [c]
Sphaerotheca	*Lactuca indica* var. *laciniata* for. *indivisa* Hara	2	1	H, Y1
	Cucumis sativus L.	4	1	H
	Cucurbita moschata Duch	3	3	G, H
	Coreopsis lanceolata L.	3	2	H
	Impatiens balsamina L.	2	1	H
Phyllactinia	*Pyrus ussuriensis* var. *macrostipes* (Nakai) T. Lee	4	5	A, H
	Diospyros kaki Thunb	3	4	H
	Ailanthus altissima Swingle	2	3	H
Microsphaera	*Juglans sinensis* Dode	2	2	Y2
Golovinomyces	*Nicotiana tabacum* L.	1	1	Y2
	Plantago asiatica L.	2	1	H
5 Genera	12 Species			H

[a] 1 = 0.1 ~ 10 %, 2 = 10.1 ~ 15 %, 3 = 15.1 ~ 25 %, 4 = 25.1 ~ 50 % and 5 = More than 50 %

[b] 1 = Under 1, 2 = 1.1 ~ 2, 3 = 2.1 ~ 3, 4 = 3.1 ~ 4 and 5 = More than 4 individuals/five leaves

[c] A: An-seong, G: Gu-ri, H: Hwa-sung, Y1: Yeon-cheon, Y2: Yang-pyeong.

Fig. 5. The symptom of powdery mildew of pear (*Pyrus ussuriensis* var. *macrostipes*) leaves.

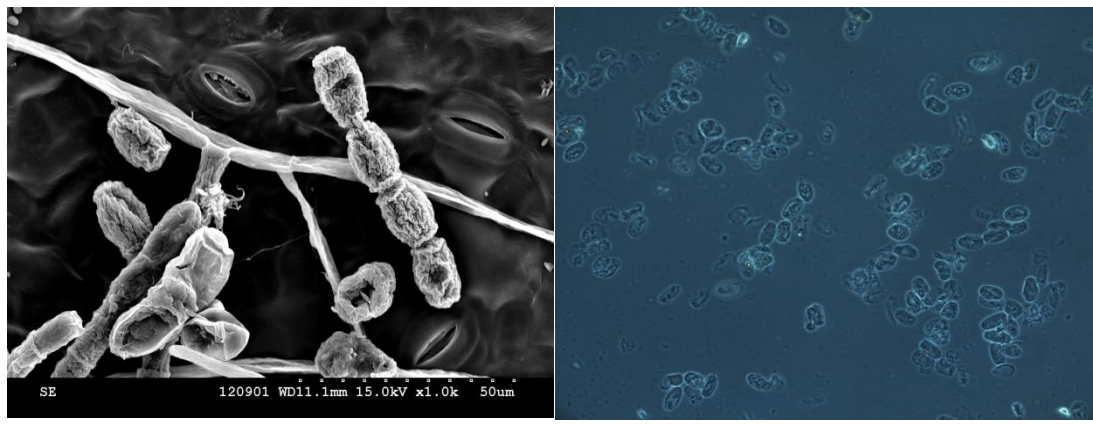


Fig. 6. Powdery mildew spores on the cucumber leaf (left) and that from the gut of *Illeis koebelei* adult (right) after feeding the cucumber powdery mildew.

Fig. 7. Spore structures of nine species of powdery mildew that *Illeis koebelei* visiting for feeding.

Table 3. Seasonal occurrence of *Illeis koebelei* on pear tree infected with powdery mildew in South Korea.

Stage	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.
Egg				○○	○○○	○○○		
Larva				●	●●●	●●●	●●	
Pupa					◆	◆◆◆	◆	
Adult				■■■	■■■	■■■	■■■	■

3.2. Biological and morphological characteristics

Table 4 shows the developmental characteristics and feeding capacity of *Illeis koebelei* feeding cucumber powdery mildew. The eggs (1.02 mm) were glued vertically and laid in cluster (Fig. 8). After embryonic development, the egg color changed to dark yellowish and finally dark greyish before hatching. There are 4 instars, all similar in appearance. Whole body length of gray first instar with black hairs was 1.38 mm, and yellowish second instar larvae with blackish dots and white hairs on the whole body was more elongated and flattened measured as 2.89 mm. From the third instar, their dots become much darker and body length was 3.96 mm. Fourth instar was almost similar to the third, but longer in its body length by 5.31 mm. Pupa was somewhat smaller than adult in body size and has yellow color with black spots on the whole body (Fig. 8). The female adult was 4.51 mm in length, and it is in elongated oval shape looking like shielded beetle and has two black spots on the pronotum (Fig. 8).

Developmental periods of four larval instars and adult feeding cucumber powdery mildew were 1.2, 2.3, 2.3, 4.6, and 37.7 days, respectively at 25℃.

We compared the morphologic characteristics of mycophgous *I. koebelei* with entomophagous *Propylea japonica* (Thunberg), especially mouth parts (Fig. 9). The mandible and maxillary palps of entomophagous *P. japonica* were fork-shaped for capturing aphids to feed, however, mycophagous *I. koebelei* showed comb-shaped mandible and maxillary palps that are convenient to rake together mycelium or spores

of powdery mildew. Actually it is easy to observe mycelium or spores adhering to mouth parts of *I. koebelei*. This morphologic difference showed the same appearance regardless of developmental stages.

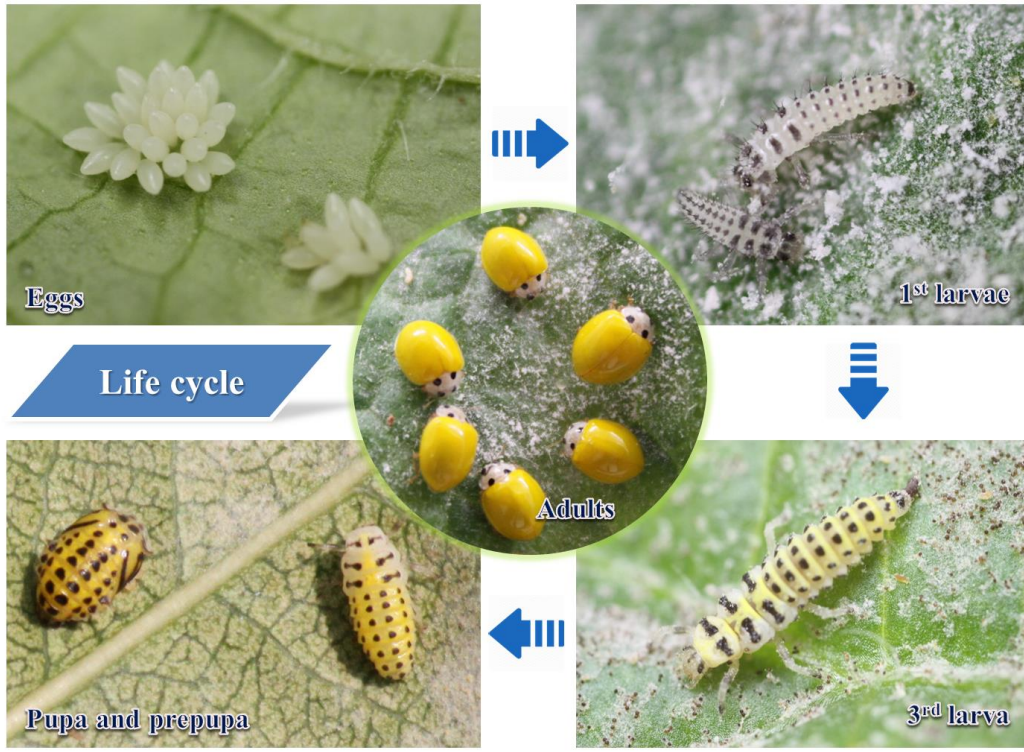
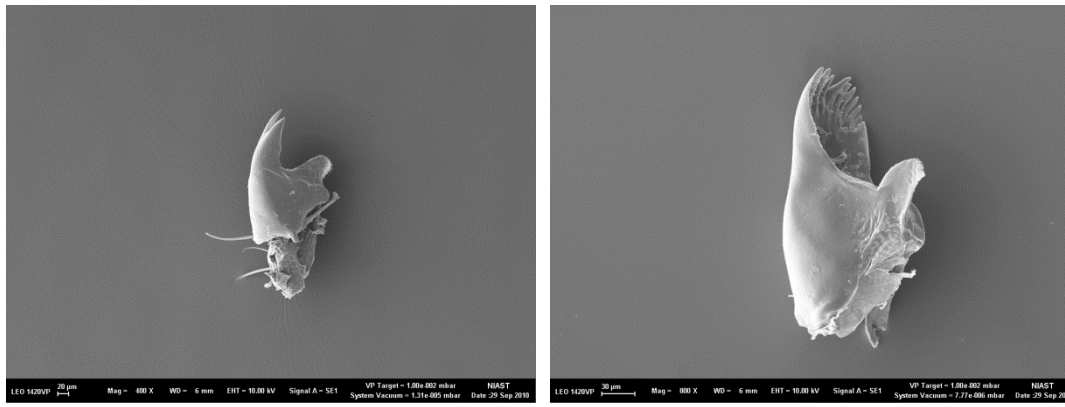
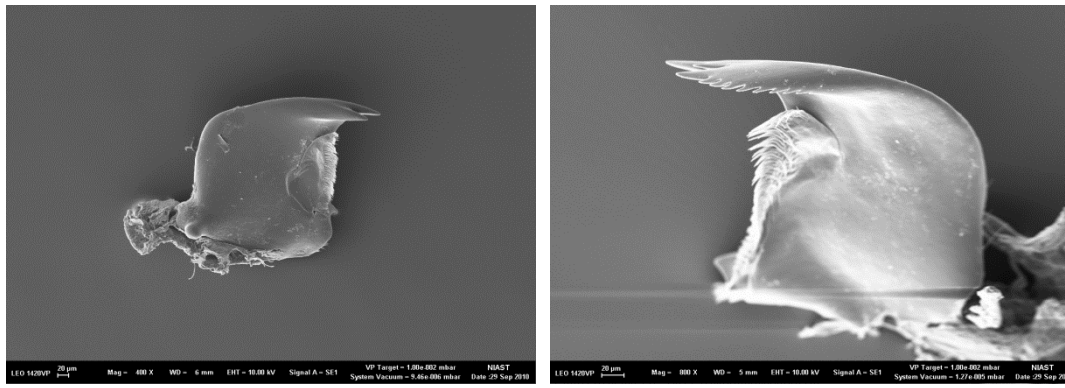


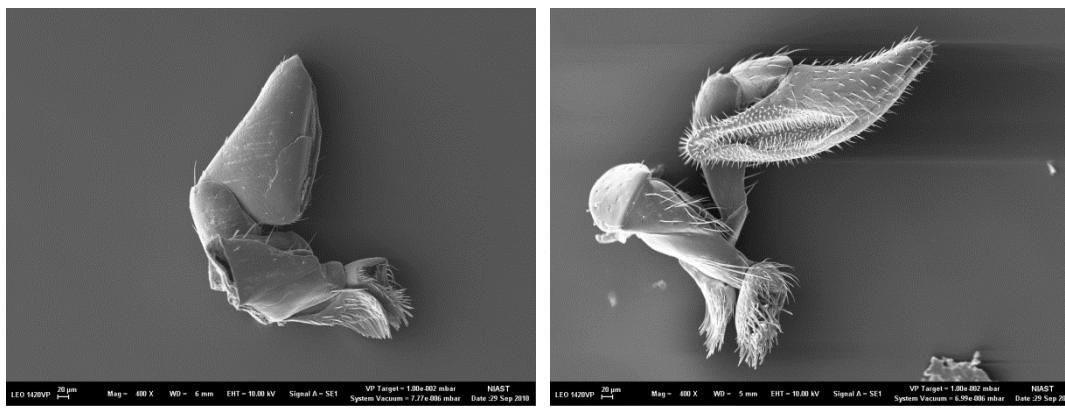
Fig. 8. Morphological characteristics of the developmental stages of *Illeis koebelei*.



<Dorsal views of mandible of *P. japonica*(left) and *I. koebelei*(right) larva>



<Dorsal views of mandible of *P. japonica*(left) and *I. koebelei*(right) adult>



< Dorsal views of maxillary palps of *P. japonica*(left) and *I. koebelei*(right) adult >

Fig. 10. Morphologic comparison of the mouth parts of mycophagous *Illeis koebelei* and entomophagous *Propylea japonica*.

Table 4. Developmental characteristics of each stages of *Illeis koebelei* feeding cucumber powdery mildew at 25℃ and 16:8 (L:D) h

Stages	Body length (mm±SD)	Developmental periods (day, Mean±SD)
Egg	10.2±0.04	3.9±0.6
1st instar	1.38±0.44	1.2±0.4
2nd instar	2.89±0.54	2.3±0.7
3rd instar	3.96±0.50	2.3±1.3
4th instar	5.31±0.57	4.6±1.4
Pupa (♀)	3.68±0.19	4.1±0.4
Egg-pupa	-	18.4±1.3
Adult (♀)	4.51±0.25	37.7±31.9

※ Sample size was 30 with three replications of 10 individual each.

3.3. Consumption and control of cucumber powdery mildew

Fed area by each stages of *I. koebelei* were 45.6, 144.4, 372.2, 628.1, 473.7 mm^2 of cucumber powdery mildew per day (Table 5). By age group, fourth instar larvae consumed cucumber powdery mildew the most followed by 4^{th} instar, adult, 3^{rd} instar, 2^{nd} instar, and 1^{st} instar. The fed area during larval period (1,190.3 mm^2) was about 2.5 times higher than that of adults.

When the incidence of powdery mildew was less than 2.5%, the greater the number of *I. koebelei* larvae inoculated, the higher the control effect against cucumber powdery mildew was found. However, inoculation of more than three larvae didn't show any significant difference (Fig. 9).

Table 6, 7 show the results of testing the effects of cucumber powdery mildew control by adding *I. koebelei* and microorganism simultaneously in Anseong and Hwaseong area. In the Hwaseong area, the control effect of cucumber powdery mildew disease caused by simultaneous introduction of *I. koebelei* and microorganism was very high at 31.5% at the beginning of harvest and 80.0% at the end of harvest. However, in the case of Anseong area, cucumber organic cultivation farm, the greenhouse area was too wide and the incidence of powdery mildew was more than 2.5%. Therefore, the control effect was relatively lower than that of Hwaseong area.

Table 5. Fed area of each stages of *Illeis koebelei* on cucumber powdery mildew

Fed area (mm^2/day±SD)(%)				
1^{st} instar	2^{nd} instar	3^{rd} instar	4^{th} instar	Adult (♀)
45.6±20.1 (10)	144.4±97.8 (21)	372.2±226.9 (79)	628.1±340.0 (133)	473.7±257.9 (100)

※ Sample size was 30 larvae and adults, three replications, at 25℃ and 16:8 (L:D)h.

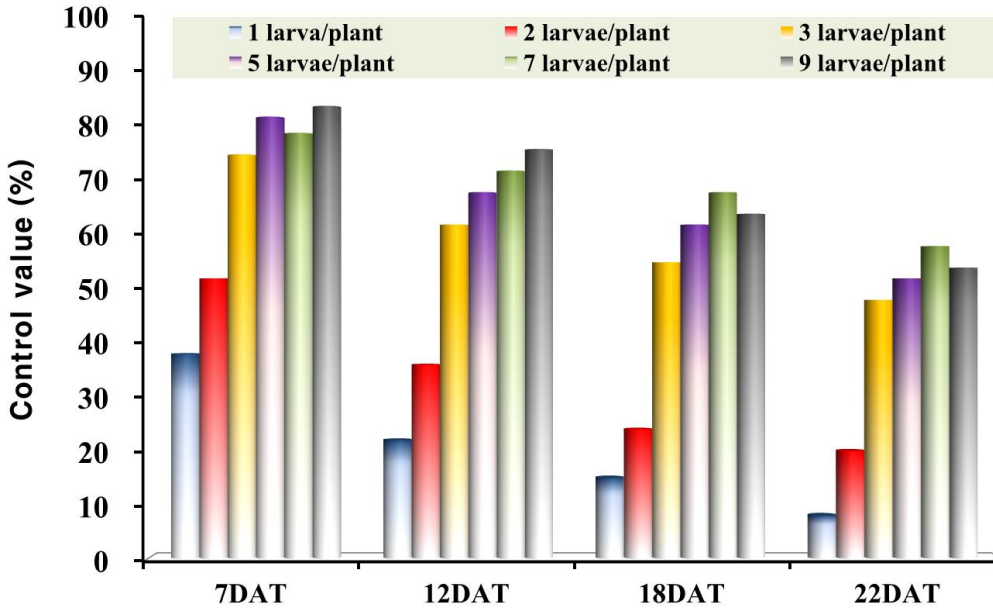


Fig. 10. Comparison of the effect of *Illeis koebelei* larva (3rd instar) inoculation density on the control of cucumber powdery mildew infected less than 2.5% per plant.

Table 6. Control effect of cucumber powdery mildew by *I. koebelei* in greenhouse of Hwaseong area

Date	10/5	10/16	10/23	10/30	11/6
Control value (%)	15.2	32.7	33.1	38.0	37.2

Table 7. Control effect of cucumber powdery mildew by *I. koebelei* in greenhouse of Anseong area

Date	9/18	9/30	10/9	10/23	11/6
Control value (%)	15.2	32.7	33.1	38.0	37.2

4. Discussion

Takeuchi et al. (2000) reported that *I. koebelei* feeds on 11 species of powdery mildews (e.g., *Sphaerotheca fusca*, *S. cucurbitae*, and *Phyllactinia moricola*) and the feed (powdery mildew) effects on their developmental time and survival rate. It seems to be positively correlated between powdery mildew severity and *I. koebelei* abundance (Table 1). However, in spite of the abundant density of cucumber powdery mildew, the *I. koebelei* density was relatively low in some locations. It might be caused by the chemical or physical control measure used in the commercial greenhouses.

The characteristics of host plant can influence the interactions between insect and their prey (Morath et al., 2012; Weber et al., 2012). The secondary metabolites emitted from plants infected with powdery mildew could help the mycophagous insect detecting the food sources easily. Morath et al. (2012) reported that volatile organic compounds (VOCs) produced by fungi play important signaling roles between fungi and plants, arthropods, bacteria, and other fungi.

Illies koebelei was detected from early July to early November in pear orchard. In this season, all stage of *I. koebelei* were observed on the under surface of pear leaves infected with powdery mildew severely (Table 1). Although most of ladybeetles could be found easily before July in Gyeonggi-do, Korea, we could not find *I. koebelei* in these seasons. Various circumstances (e.g., prey species, prey density, temperature) affect the oviposition selection of adult ladybeetle (Hodek and Evans,

2012). It seems that *I. koebelei* is alive in these seasons by feeding alternative diet, e.g., pollen. As pollen can be the good alternative food source for many ladybeetles (Lundgren, 2009).

We reconfirmed the mycophagy by measuring fed area of cucumber powdery mildew in each stages of *I. koebelei.* For the mass rearing of *I. koebelei* using cucumber powdery mildew for food, the adequate temperature was known as 24℃ in the range from 16℃ to 26℃ (Yarwood et al., 1954).

Chapter II.

Temperature-dependent development and oviposition models of *I. koebelei* Timberlake (Coleoptera: Coccinellidae: Halyziini)

Abstract

The yellow ladybeetle, *Illeis koebelei* Timberlake, is a potential biological agent for powdery mildew. The objective of this study was to construct development and oviposition models of *I. koebelei*. Development experiment was conducted at eight temperatures ranging from 15.4 to 39.5 ℃. Development rates were well fitted with linear and nonlinear models. Lower developmental thresholds for egg, first instar, second instar, third instar, and fourth instar larva, pre-pupa, pupa, and total immature stage were estimated to be 3.6, 12.7, 12.1, 11.3, 11.3, 12.8, 14.7, and 14.2 ℃, respectively. Their respective thermal requirements in degree days (DD) were 86.6, 16.0, 22.5, 30.2, 49.3, 14.5, 43.8, and 217.4, respectively. Survivorship was the highest at 25.1 ℃ for immatures. Oviposition experiment was conducted at nine temperatures, ranging from 15.4 to 35.3 ℃. Mean fecundity ranged from 18.6 eggs at 29.3 ℃ to 205.3 eggs at 20.3 ℃. It was well described by extreme value function. Adult survival and cumulative oviposition rates of *I. koebelei* were fitted to a sigmoid function and a two-parameter Weibull function, respectively. Findings of this study can be used to optimize environmental conditions for mass-rearing and shipping, comparing optimal occurrence conditions between *I. koebelei* and powdery mildew, and forecasting phenology and population dynamics of *I. koebelei* in the fields.

Keywords: *Illeis koebelei*; yellow ladybeetle; powdery mildew; survival; development model; oviposition model

1. Introduction

Beetles belonging to Coccinellidae are mostly entomophagous, but some species are mycophagous. These mycophagous beetles belong to tribes Halyziini and Tytthaspidini (Giorgi et al., 2009; Sutherland and Parrella, 2009). Mycophagous ladybeetles in tribe Halyziini are potential biological control agents for powdery mildew (Sutherland and Parrella, 2009), a common and economically important plant disease worldwide that infects more than 1,500 plant species (Amano, 1986; Braun, 1987; Ale-Agha et al., 2008; Glawe, 2008; Olena et al., 2014). Although these ladybeetles mainly feed on powdery mildew, they also feed on alternative foods such as sooty molds and pollens depending on species (Sasaji, 1998; Giorgi et al., 2009). Among Halyziini species, the yellow lady beetle, *Illeis koebelei* Timberlake, is one of most attractive species that can control powdery mildew due to its unique habitats and feeding behavior (Men et al., 2002; Giorgi et al., 2009; Sutherland and Parrella, 2009; Sharma and Joshi, 2010; Karuna et al., 2013; Thite et al., 2013). They occur only on plants contaminated by powdery mildew. *I. koebelei* has been found in Asian countries, including Korea (Kim et al., 1994; Lee et al., 2015), Philippine (Recuenco-Adorada and Gapud, 1998), Japan (Takeuchi et al., 2000), China (Wu et al., 2011), and Taiwan (Lin et al., 2006).

Despite its potential as a biological control agent for powdery mildew, *I. koebelei* has been rarely studied. In Korea, *I. koebelei* populations show two peaks per year (Lee et al., 2015). The first peak in early July might be related to the overwintering

generation. The second peak from early October to early November could be important for *I. koebelei* as a good biological control agent against powdery mildew because this period is harvesting or post-harvesting period, during which fungicide application is generally prohibited, for many crops such as cucumber, pepper, tobacco, apple, and pear in Korea. Powdery mildew can become severe at this period due to decreased fungicide application and matured crop age (Lee et al., 2015). Thus, *I. koebelei* has high potential for controlling powdery mildew during this period in Korea. Management of powdery mildew would be more effective and feasible if phenology and population dynamics of *I. koebelei* can be predicted reliably. However, no modeling study such as development and oviposition models has been conducted for *I. koebelei*. Such lack of models hampers understanding of population dynamics of *I. koebelei* and its use for powdery mildew management. Therefore, the objective of this study was to develop temperature-dependent development and oviposition models of *I. koebelei*. These models will be very useful for understanding population dynamics of *I. koebelei* and for developing biological control strategy to control powdery mildew using *I. koebelei*.

2. Materials and methods

2.1. Insect rearing

Cucumber (*Cucumis sativuss*) seeds were sown in plastic pots (12 cm diameter ×10.5 cm height; Gonongtech garden; Uijeongbu-si, Korea) and grown in an insect

rearing room at 26 ℃ with relative humidity (RH) of 70% and a photoperiod of 16:8 (L:D) h in Gyeonggi-do Agricultural Research & Extension Services (GARES) in Hwaseong-si, Korea. Cucumber leaves contaminated with spores of powdery mildew were collected from cucumber plants at greenhouses in GARES. These collected spores were transferred to a single leaf of two-week-old plants using a soft paintbrush (SL14045; Shinil Science; Paju-si, Korea). At two weeks after inoculation, two powdery mildew infected plants were supplied in each rearing cage (30×30×30 cm; Gyeonggi art; Suwon-si, Korea) for *I. koebelei*.

Laboratory colony of *I. koebelei* was initiated from approximately 200 individuals collected from a pear orchard in Hwaseong-si in 2011. These insects were reared for more than three generations before being used for experiments. Separate colonies of immature stages and adults were maintained in the insect rearing room at 26 ℃ with RH of 70% and a photoperiod of 16:8 (L:D) h. Powdery mildew infected plants were supplied for feeding. Fifteen to 20 adult *I. koebelei* were placed together for mating and oviposition in rearing cages (30 × 30 × 30 cm) with powdery mildew infected cucumber plants. Plants with eggs were then transferred to translucent plastic containers (232 × 165 × 95 cm, HPL825; Lock & Lock; Seoul, Korea) lined by wet tissue paper laid on the bottom. The center part (75% of the area) of the lid of the container was cut off and a mesh screen was attached for ventilation. Newly hatched

larvae were transferred to rearing cages (30 × 30 × 30 cm) to complete their development. Again, adults were moved to adult rearing cages as described above.

2.2. Experimental procedures

All experiments were conducted under a photoperiod of 16:8 (L:D) h with RH of 60-80 % in environmental chambers (MIR-153; Sanyo Electronic Co.; Osaka, Japan). Temperature and relative humidity inside these chambers were monitored using HOBO data loggers (H08-007-02; OnSet Computer Corp.; Pocasset, MA, USA) and mean temperatures were used for data analyses. Temperatures for egg and larval development experiments were 15.4, 20.3, 24.9, 28.2, 29.6, 33.4, 35.3, and 39.5 ℃. Temperatures for adult oviposition experiment were 15.4, 18.2, 20.3, 22.9, 24.9, 27.9, 29.3, 33.4, and 35.3 ℃.

For egg development experiment, eggs (< 1 day old) were collected from the colony. Cucumber leaves with eggs were placed inside insect breeding dishes (50 mm diameter×15 mm height; SPL Sciences; Pocheon-si, Korea) with a wetted tissue paper placed at the bottom of the dish. Dishes with 20 to 48 eggs were randomly allocated in environmental chambers resulting in total 53 to 204 eggs per each temperature. Eggs were checked daily for hatching.

For larval development experiment, five dishes with eggs were placed for each temperature by the same method described above. Thirty newly hatched larvae (< 1 day old) were transferred individually into the insect breeding dish (50 mm diameter,

15 mm height) for each temperature. One cucumber leaf contaminated with powdery mildew was supplied for each larva. Larval development was checked daily and powdery mildew contaminated cucumber leaf was replenished before depletion. Because no 1st instar larvae survived at 33.4 ℃ and no eggs hatched at 35.3 or 39.5 ℃, 20 individuals (< 1 day old) of each stage reared at 24.1 ℃ were tested at these temperatures for further examination of larval development for specific stages.

For oviposition experiment, pre-pupae or pupae were collected from the colony and placed in breeding cages (50 mm diameter, 15 mm height). Newly-emerged adults (< 1 day old) were then collected. One pair of newly-emerged adults was placed in an insect breeding dish (50 mm diameter, 15 mm height). These dishes were then placed in environmental chambers at each temperature. Adults were provided with one cucumber leaf contaminated with powdery mildew. Female adult survival (longevity) and the number of eggs laid were examined daily.

2.3. Model development and data analysis

Normality and variance homogeneity of data was tested using Shapiro-Wilk test and Bartlett test in SAS, respectively (SAS Institute, 2011). The data, which do not show normality and variance homogeneity, were transformed with $\log_{10}$ before analysis of variance (ANOVA) test. ANOVA was conducted to examine the effect of temperatures on development time for each stage of *I. koebelei* using PROC GLM in

SAS (SAS Institute, 2011). Means were separated by Tukey HSD test ($P < 0.05$; SAS Institute, 2011).

Development rates were expressed as the reciprocal of developmental times (in days) of each stage and applied to a linear model (Davidson, 1994) and a non-linear model (Lactin et al., 1995). For linear regression analysis, development rates of linear portion for each stage were regressed against temperature using PROC REG in SAS (SAS Institute, 2011). The linear model was:

$$r(T) = aT + b \quad (1)$$

where *r(T)* is the development rate at temperature *T* (℃), *a* is the slope, and *b* is the *y*-intercept. For each stage, lower developmental threshold was calculated as *–a/b* (Arnold, 1959) while thermal constant in degree-day (DD) required to complete development was calculated as 1/*a* (Campbell et al., 1974).

A non-linear model was used to fit development rates over the entire temperature range. The non-linear model (Lactin et al., 1995) used in this study was:

$$r(T) = exp^{(pT)} - exp^{[pT_m - (T_m - T)/\Delta T]} \quad (2)$$

where $r(T)$ was the mean development rate at temperature T (℃), p was the shape of the curve, T_m was the upper threshold temperature (°C), and ΔT was the temperature range over which physiological breakdown became the overriding influence.

Operative thermal range (B_{80}) which indicated ≥80 % performance of maximal rate was also determined using the protocol of Lutterschmidt and Hutchison (1997). The operative thermal range (B_{80}) is a temperature range based on an optimal temperature (T_{opt}) that might be more applicable to various environment changing conditions (i.e., insect rearing, pesticide application, natural enemy release, optimal sampling timing, and so on) and conflicting issues (i.e., selection of rearing temperature based on different optimal developmental, ovipositional, and survivorship temperatures) (Baek et al., 2017).

Variation in development time for each stage was described using the standardized cumulative distribution of frequency of development time. Development times were standardized by physiological time (px) of each stage using Eq. (8). Two-parameter Weibull function was then applied:

$$p(px) = 1 - exp^{-(px/\alpha)^{\beta}} \quad (3)$$

where $p(px)$ was the cumulative proportion of individuals that completed development at physiological time (px), and α and β were fitted constants.

Stage-specific survival rate (percentage) was calculated by dividing the number of individuals survived to the next stage by the initial number of individuals at each temperature. The following model (Eq. 4) suggested by Sanchez-Ramos et al. (2015) was used:

$$S(T) = 100 - exp^{a+bT+cT^2} \quad (4)$$

where $S(T)$ was the survival rate (%) at temperature T (℃), and a, b, and c were fitted parameters. The operative thermal range (B_{80}) with ≥ 80 % performance of maximal rate was determined according to recommendations of Lutterschmidt and Hutchison (1997).

Data transformation, if necessary, was also conducted for adult data as described previously. ANOVA was conducted to examine the effect of temperature on longevity, fecundity, and pre-oviposition period using PROC GLM in SAS (SAS Institute, 2011). Means were separated by Tukey HSD test ($P < 0.05$; SAS Institute, 2011).

Adult development rate, the reciprocal of longevity (1/day) as a function of temperature (℃), was fitted to a function from the library of TableCurve 2D (Jandal Scientific, 1996). The following equation (Eq. 5) was used:

$$r(T) = exp^{[a+bT^{2.5}+cT^3]} \quad (5)$$

where $r(T)$ was the development rate at temperature T (℃), and a, b, and c were fitted constants.

Total number of eggs per female (fecundity) was obtained by summing the number of eggs laid daily by an adult female during its whole life span. The mean fecundity was calculated based on females examined in this study. The relationship between the mean total fecundity and temperature T (℃) was fitted to the following extreme value function (Eq. 6) of Kim and Lee (2003):

$$f(T) = a \cdot exp^{[1+(b-T)/k-exp^{(b-T)/k}]} \qquad (6)$$

where $f(T)$ was the mean number of total eggs produced by a female at temperature T (℃), a was the maximum reproductive capacity, b was the temperature (℃) at which the maximum reproduction occurred, and k was a fitted constant.

Cumulative oviposition rate according to physiological time was calculated at each temperature examined. Each cumulative oviposition rate was scaled to the maximum. Physiological time (px) of females was then calculated using Eq. (8). The two-parameter Weibull function (Eq. 3) was used to model scaled cumulative oviposition rates. This model indicates age-specific cumulative oviposition rate. It can define the cumulative proportion of eggs laid by a female adult until physiological time (px).

Age-specific survival (proportion) of females at physiological time (*px*) was fitted to the following sigmoid function (Eq. 7):

$$s(px) = exp^{(\gamma - px)/\delta} \quad (7)$$

where *s*(*px*) was the percentage of live females at physiological time (*px*), γ was the physiological time at 50% survival, and δ was a fitted constant.

Physiological time (*px*) for models above was calculated using Eq. (8) which used non-linear development rate models of immatures or adults:

$$px = \sum_{i=1}^{n} r(T_i) \quad (8)$$

where $r(T_i)$ was the development rate at temperature *T* (℃) at day *i*. Parameters of the adult development rate model were estimated using TableCurve 2D (Jandal Scientific, 1996). Other non-linear models for fecundity and survivorship of adults were estimated using PROC NLIN in SAS (SAS Institute, 2011).

3. Results

3.1. Development of immature stages

I. koebelei could complete its development at 20.3-29.3 ℃. However, it suffered significant mortality (Table 1), and at 15.4 ℃, less than half of eggs hatched.

Although some larvae could develop to pre-pupal stage, they all failed further development. At 33.4 ℃, individuals of a few stages (i.e., egg, third instar larva, pre-pupa, pupa) successfully completed its development, although their survivorship was very low except for pre-pupa. At 35.3 and 39.5 ℃, all stages of *I. koebelei* could not survive except for pre-pupa at 35.3℃. Overall, pre-pupal stage appeared to have high durability for these temperature conditions. Development of *I. koebelei* was significantly affected by temperature for all stages: egg (F = 75.4; df = 5, 646; P < 0.001), first instar larva (F = 72.5; df = 4, 132; P < 0.001), second instar larva (F = 77.9; df = 4, 100; P < 0.001), third instar larva (F = 11.5; df = 5, 81; P < 0.001), fourth instar larva (F = 24.3; df = 4, 63; P < 0.001), pre-pupa (F = 55.7; df = 6, 87; P < 0.001), pupa (F = 7.3; df = 4, 46; P < 0.001), and total immature (from egg to adult emergence) stage (F = 60.4; df = 3, 34; P < 0.001) (Table 1).

Linear portions of developmental rate data were well fitted to the linear model (Fig. 1). Lower developmental thresholds for egg, first, second, third, and fourth instar larva, pre-pupa, pupa, and total immature stages were estimated to be 3.6, 12.7, 12.1, 11.3, 11.3, 12.8, 14.7, and 14.2 ℃, respectively. Their respective thermal requirements (in degree days) were 86.6, 16.0, 22.5, 30.2, 49.3, 14.5, 43.8 and 217.4, respectively (Table 2).

Table 1. Developmental time (day, mean ± SD) of *Illeis koebelei* at constant temperature

Temperature (℃)	Egg	1st instar	2nd instar	3rd instar	4th instar	Pre-pupa	Pupa	Egg to adult
15.4	7.5±3.80a[1)] (66/161)[2)]	4.4±1.47a (26/30)	6.7±1.79a (11/26)	6.2±3.31a (6/11)	10.0±0.00a (1/6)	5.0±0.00a (1/1)	-	-
20.3	5.1±1.40b (156/204)	2.8±0.80b (28/30)	2.4±1.04b (23/28)	4.0±1.84b (21/23)	5.9±1.18b (16/21)	2.1±0.60c (9/16)	7.3±1.53a (4/9)	28.7±3.06a (156/204)x(4/30)
24.1	4.0±0.49c (145/181)	1.2±0.42d (28/30)	2.3±0.61b (28/28)	2.2±1.34c (27/28)	3.9±1.06c (22/27)	1.2±0.38d (18/22)	4.0±0.00b (15/18)	20.3±1.88b (145/181)x(15/30)
27.9	3.6±0.93c (134/187)	2.0±0.00c (28/30)	1.3±0.46c (22/28)	1.8±0.44c (13/22)	2.7±1.11d (13/13)	1.9±0.70c (11/13)	3.3±0.50b (9/11)	16.6±1.01c (134/187)x(9/30)
29.3	3.3±1.15c (126/187)	1.3±0.45d (23/30)	2.0±0.35bc (17/23)	1.8±0.70c (14/17)	3.3±1.15cd (12/14)	1.1±0.33d (10/12)	3.2±0.41b (7/10)	15.2±0.41c (126/187)x(7/30)
33.4	3.8±0.41bc (20/53)	-	-	4.0abc (1/20)	-	2.8±0.41b (20/20)	3.9±2.19b (12/40)	-
35.3	-[3)]	-	-	-	-	3.0±0.00b (19/20)	-	-
39.5	-	-	-	-	-	-	-	-

[1)] Means within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD test at 95% confidence intervals)

[2)] Numbers in parentheses indicate the numbers of individuals that survived / total number of individuals tested

[3)] No individuals survived or tested

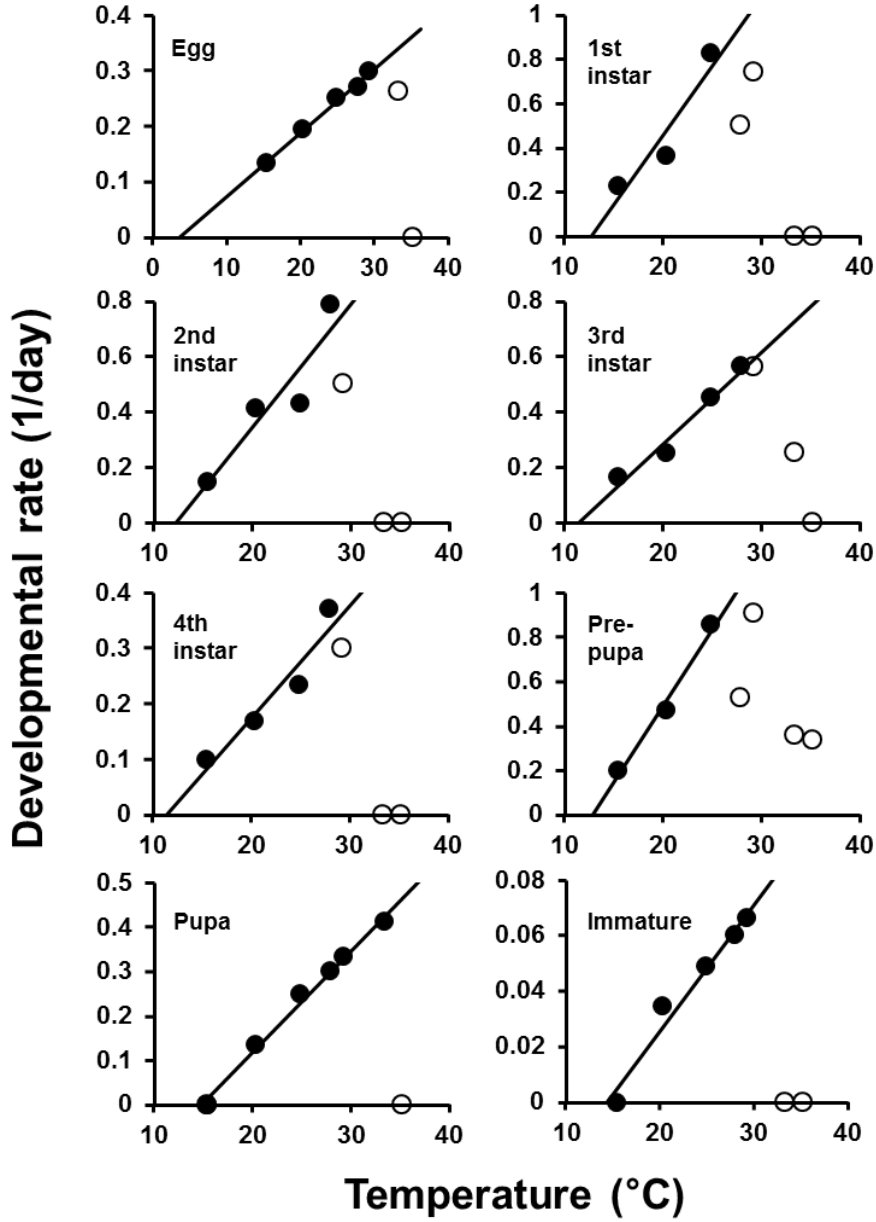


Fig. 1. Linear model of developmental rates (1/day) for each stage of *Illeis koebelei* at constant temperature (●, Observed; ○, Excluded in the linear model; —, Estimated).

Table 2. Parameter estimates (± SEM) of the linear development model, lower temperature threshold, and thermal requirement for each stage of *Illeis koebelei*

Stage	Parameters		r^2	Lower temperature threshold (℃)	Thermal requirement (Degree-days)
	a	*b*			
Egg	0.0115 ± 0.0006	-0.0413 ± 0.0144	0.99	3.6	86.6
1st instar	0.0625 ± 0.0207	-0.7916 ± 0.4264	0.90	12.7	16.0
2nd instar	0.0445 ± 0.0125	-0.5397 ± 0.2837	0.86	12.1	22.5
3rd instar	0.0331 ± 0.0043	-0.3755 ± 0.0961	0.97	11.3	30.2
4th instar	0.0203 ± 0.0044	-0.2296 ± 0.0990	0.91	11.3	49.3
Pre-pupa	0.0690 ± 0.0079	-0.8841 ± 0.1631	0.99	12.8	14.5
Pupa	0.0228 ± 0.0010	-0.3362 ± 0.0251	0.99	14.7	43.8
Egg to adult	0.0046 ± 0.0005	-0.0653 ± 0.0114	0.97	14.2	217.4

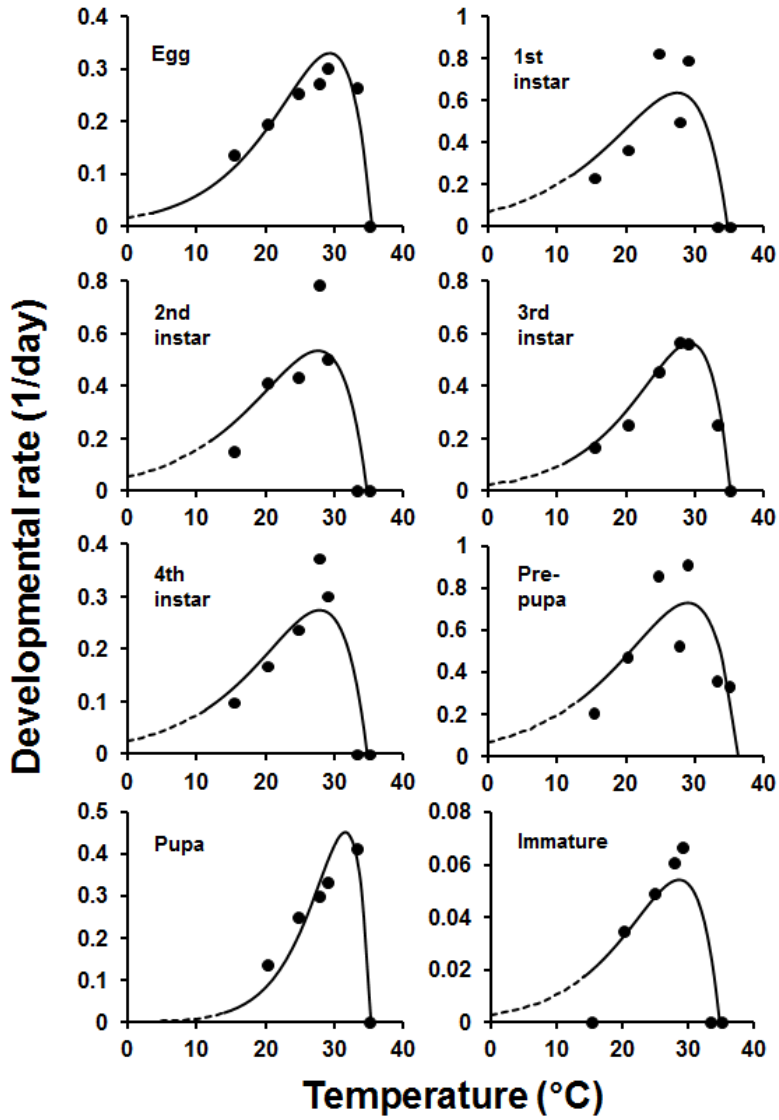


Fig. 2. Nonlinear model of developmental rates (1/day) for each stage of *Illeis koebelei* with low temperature inhibition below the lower developmental threshold estimated from the linear developmental rate model (●, Observed; —, Estimated; ----, Excluded).

Table 3. Parameter estimates (± SEM) of the nonlinear development model for each stage of *Illeis koebelei*

Stage	Parameters			r^2
	p	T_m	ΔT	
Egg	0.1622 ± 0.0190	35.5032 ± 0.3038	6.1478 ± 0.7106	0.98
1st instar	0.1353 ± 0.0434	34.7679 ± 0.7312	7.2760 ± 2.1724	0.88
2nd instar	0.1397 ± 0.0434	34.7678 ± 0.7009	7.0779 ± 2.0827	0.88
3rd instar	0.1686 ± 0.0144	35.1686 ± 0.1879	5.9071 ± 0.4921	0.99
4th instar	0.1446 ± 0.0385	34.7899 ± 0.5992	6.8821 ± 1.7884	0.90
Pre-pupa	0.1353 ± 0.0414	36.3355 ± 1.1691	7.2840 ± 2.0883	0.93
Pupa	0.2681 ± 0.0315	35.3452 ± 0.1830	3.7296 ± 0.4374	0.91
Egg to adult	0.1620 ± 0.0474	34.8092 ± 0.6136	6.1696 ± 1.7991	0.87

Table 4. Parameter estimates (± SEM) of the distribution model of developmental time for each stage of *Illeis koebelei*

Stage	Parameters		r^2
	α	β	
Egg	0.9172 ± 0.0268	3.4594 ± 0.4424	0.98
1st instar	0.9381 ± 0.0472	3.0274 ± 0.6950	0.97
2nd instar	0.9019 ± 0.0309	3.4545 ± 0.6002	0.98
3rd instar	0.9019 ± 0.0440	2.5410 ± 0.4501	0.97
4th instar	0.9846 ± 0.0326	4.4399 ± 0.9847	0.95
Pre-pupa	0.8180 ± 0.0265	4.8571 ± 1.0174	0.99
Pupa	0.9061 ± 0.0120	8.9136 ± 1.2922	0.99
Egg to adult	0.9877 ± 0.0065	17.3218 ± 2.5233	0.98

The non-linear Lactin model provided a good fit for development rate of different stages: egg (F = 72.0; df = 2, 6; P < 0.001; r^2 = 0.98), first instar larva (F = 9.7; df = 2, 6; P = 0.026; r^2 = 0.88), second instar larva (F = 9.8; df = 2, 6; P = 0.026; r^2 = 0.88), third instar larva (F = 118.5; df = 2, 6; P < 0.001; r^2 = 0.99), fourth instar larva (F = 12.6; df = 2, 6; P = 0.017; r^2 = 0.90), pre-pupa (F = 17.1; df = 2, 6; P = 0.010; r^2 = 0.93), pupa (F = 15.3; df = 2, 5; P = 0.027; r^2 = 0.92), and total immature (F = 9.2; df = 2, 6; P = 0.029; r^2 = 0.87) (Fig. 2, Table 3). Upper developmental thresholds (T_m) for egg, first, second, third and fourth instar larva, pre-pupa, pupa, and total immature stages were 35.5, 34.8, 34.8, 35.1, 34.8, 36.3, 35.3, and 34.8 ℃, respectively (Table 3). Their respective optimal temperatures (T_{opt}) indicated by the maximal rate were 29.3, 27.4, 27.6, 29.2, 27.9, 29.0, 31.6, and 28.6 ℃, respectively. Thermal ranges (B_{80}) with ≥ 80 % of the maximum value for egg, first, second, third and fourth instar larva, pre-pupa, pupa, and total immature stages were 24.3-32.5, 21.4-31.3, 21.8-31.4, 24.4-32.3, 22.3-31.5, 23.0-32.8, 28.6-33.3, and 23.6-31.8 ℃, respectively.

Developmental variations (i.e., distribution of development time) of all stages were well described by the two-parameter Weibull function: egg (F = 690.7; df = 1, 33; P < 0.001; r^2 = 0.98), first instar (F = 266.9; df = 1, 18; P < 0.001; r^2 = 0.97), second instar (F = 420.7; df = 1, 21; P < 0.001; r^2 = 0.98), third instar (F = 332.0; df = 1, 25; P < 0.001; r^2 = 0.97), fourth instar (F = 217.8; df = 1, 24; P < 0.001; r^2 = 0.95), pre-pupa (F = 128.3; df = 1, 15; P < 0.001; r^2 = 0.99), pupa (F = 489.5; df = 1, 16; P

< 0.001; r^2 = 0.99), and immature stage (F = 426.3; df = 1, 21; P < 0.001; r^2 = 0.98) (Fig. 3, Table 4).

The survival model also well described the relationship between survival of different stages and temperatures: egg (F = 1,224.0; df = 2, 6; P < 0.001; r^2 = 0.99), first instar (F = 712.3; df = 2, 6; P < 0.001; r^2 = 0.99), second instar (F = 173.8; df = 2, 6; P < 0.001; r^2 = 0.99), third instar (F = 89.0; df = 2, 6; P = 0.006; r^2 =0.94), fourth instar (F = 78.3; df = 2, 6; P = 0.002; r^2 = 0.99), pre-pupa (F = 115.1; df = 2, 6; P < 0.001; r^2 = 0.99), pupa (F = 47.0; df = 2, 6; P = 0.001; r^2 = 0.97), and immature stage (F = 6.7; df = 2, 6; P = 0.049; r^2 = 0.83) (Fig. 4, Table 5). Temperatures (T_m) with the maximal survival (%) for egg, first instar, second instar, third instar, fourth instar, pre-pupa, pupa, and immature stages were estimated to be 24.2, 21.8, 23.7, 22.9, 24.2, 29.1, 25.3, and 25.1 ℃, respectively. Thermal ranges (B_{80}) with ≥ 80 % of the maximum value for respective stages above were 17.7-30.8, 13.5-30.0, 17.5-30.0, 15.9-29.9, 18.1-30.4, 21.8-35.9, 15.7-30.9, and 20.7-29.6 ℃, respectively.

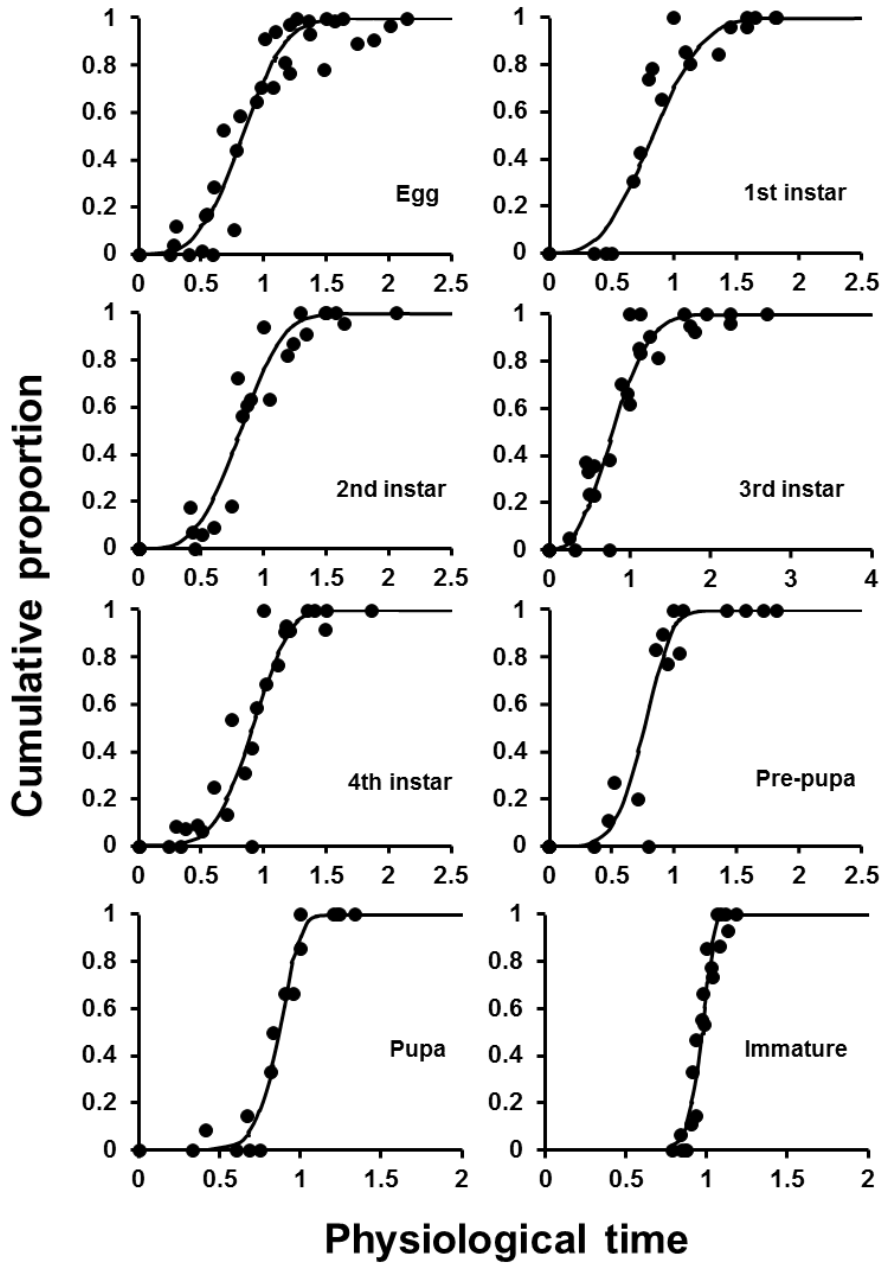


Fig. 3. Cumulative distribution of development for each stage of *Illeis koebelei* against physiological time (●, Observed; —, Estimated).

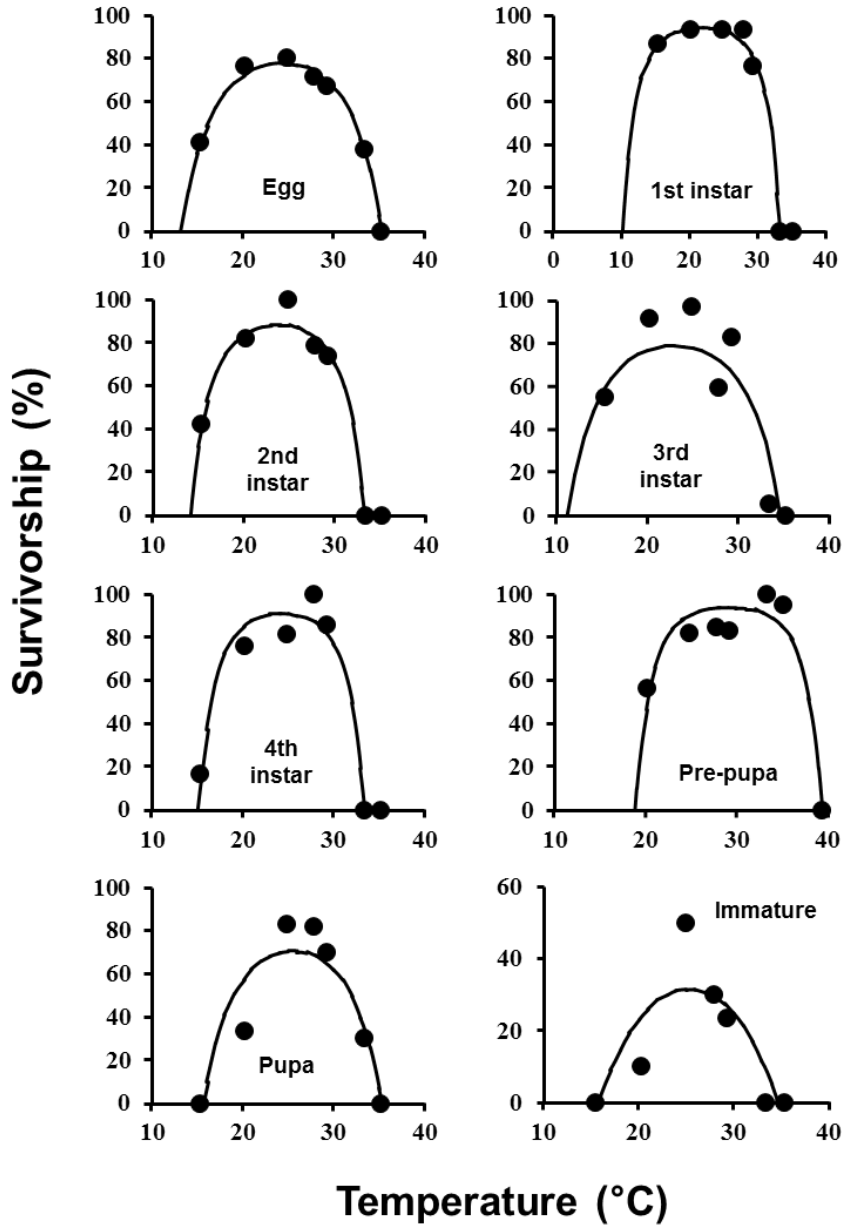


Fig. 4. Survival rate model for each stage of *Illeis koebelei* at constant temperature (●, Observed; —, Estimated).

Table 5. Parameter estimates (± SEM) of the survival rate model for each stage of *Illeis koebelei*

Stage	Parameters			r^2
	a	*b*	*c*	
Egg	10.3826 ± 0.3623	-0.6013 ± 0.0315	0.0124 ± 0.0006	0.99
1st instar	11.8140 ± 2.0052	-0.9270 ± 0.1666	0.0213 ± 0.0032	0.99
2nd instar	15.5513 ± 1.8792	-1.1048 ± 0.1739	0.0233 ± 0.0036	0.99
3rd instar	8.9797 ± 3.2902	-0.5182 ± 0.2714	0.0113 ± 0.0052	0.94
4th instar	19.1596 ± 3.3386	-1.4013 ± 0.3143	0.0289 ± 0.0064	0.99
Pre-pupa	24.6827 ± 6.1392	-1.5773 ± 0.4525	0.0271 ± 0.0075	0.99
Pupa	12.0179 ± 1.3399	-0.6887 ± 0.1207	0.0136 ± 0.0024	0.97
Egg to adult	6.3207 ± 0.6514	-0.1594 ± 0.0545	0.0032 ± 0.0011	0.83

3.2. Adult longevity and fecundity

Temperature had a significant effect on the longevity of female adults of *I. koebelei* (F = 26.2; df = 8, 179; $P < 0.001$). The longevity of female adults was decreased with increasing temperature (Table 6). Adult development rate over thermal ranges tested were well described (Fig. 5a, F = 213.9; df = 2, 8; $P < 0.001$; r^2 = 0.98, Table 7). Expected adult survival rate over adult physiological time was well described by the sigmoid function (Fig. 5d, F = 2,840.7; df = 1, 141; $P < 0.001$; r^2 = 0.98, Table 7).

The pre-oviposition period of adult females was the highest at 15.4 ℃ (F = 14.3; df = 3, 46; $P < 0.001$, Table 6). The relationship between fecundity and temperature was well described by the extreme value function (Fig. 5b, F = 56.5; df = 2, 8; $P < 0.001$; r^2 = 0.97, Table 5). Fecundity was the highest at 20.3 ℃. It was the lowest at 29.3 ℃ (F = 2.5; df = 6, 139; P = 0.025, Table 6). Temperature (T_f) with the maximal fecundity was estimated to be at 20.9 ℃. Thermal range (B_{80}) with ≥ 80 % of the maximum value was determined to be from 18.8 to 23.7 ℃. Age-specific cumulative oviposition rate over physiological time of female adults was well fitted by the two parameter Weibull function (Fig. 5c, F = 7,055.9; df = 1, 571; $P < 0.001$; r^2 = 0.96, Table 7).

Table 6. Mean longevity, fecundity, and pre-oviposition period (mean ± SE) of female *Illeis koebelei* at constant temperature

Temperature (℃)	*n*	Longevity (day) (min – max)	Fecundity (min – max)	Pre-oviposition period (day)
15.4	20	323.7 ± 43.44a* (24 – 547)	50.8 ± 25.79ab (0 – 471)	138.7 ± 36.39a
18.2	20	165.2 ± 32.01b (1 – 524)	128.3 ± 49.07ab (0 – 802)	30.0 ± 12.62b
20.3	20	57.9 ± 10.70bc (4 – 142)	205.3 ± 59.74a (0 – 956)	8.8 ± 2.60b
22.9	20	90.5 ± 20.46bc (5 – 371)	161.1 ± 43.44ab (0 – 645)	12.7 ± 4.02b
24.9	20	50.5 ± 9.80c (2 – 138)	173.4 ± 58.28ab (0 – 971)	9.7 ± 2.79b
27.9	20	38.3 ± 6.42c (10 – 104)	78.2 ± 36.03ab (0 – 696)	13.1 ± 4.51b
29.3	20	23.7 ± 6.84c (2 – 130)	18.6 ± 7.24b (0 – 112)	11.1 ± 5.18b
33.4	20	5.1 ± 0.43c (2 – 10)	0	-**
35.3	20	3.4 ± 0.29c (2 – 6)	0	-

* Means within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD test at 95% confidence intervals)

** No individuals survived

Table 7. Parameter estimates (± SEM) of the oviposition models of *Illeis koebelei*

Models	Parameters	Estimated values	r^2
Developmental rate model	*a*	-7.0431 ± 1.5289	0.98
	b	$2.332 \times 10^{-3} \pm 1.5802 \times 10^{-3}$	
	c	$-2.5991 \times 10^{-4} \pm 2.3264 \times 10^{-4}$	
Fecundity model	*a*	201.8000 ± 19.4716	0.97
	b	20.9490 ± 0.4287	
	k	3.7309 ± 0.4584	
Cumulative oviposition rate model	α	0.7234 ± 0.0110	0.96
	β	1.4733 ± 0.0501	
Survival rate model	γ	0.9833 ± 0.0234	0.98
	δ	1.0542 ± 0.0434	

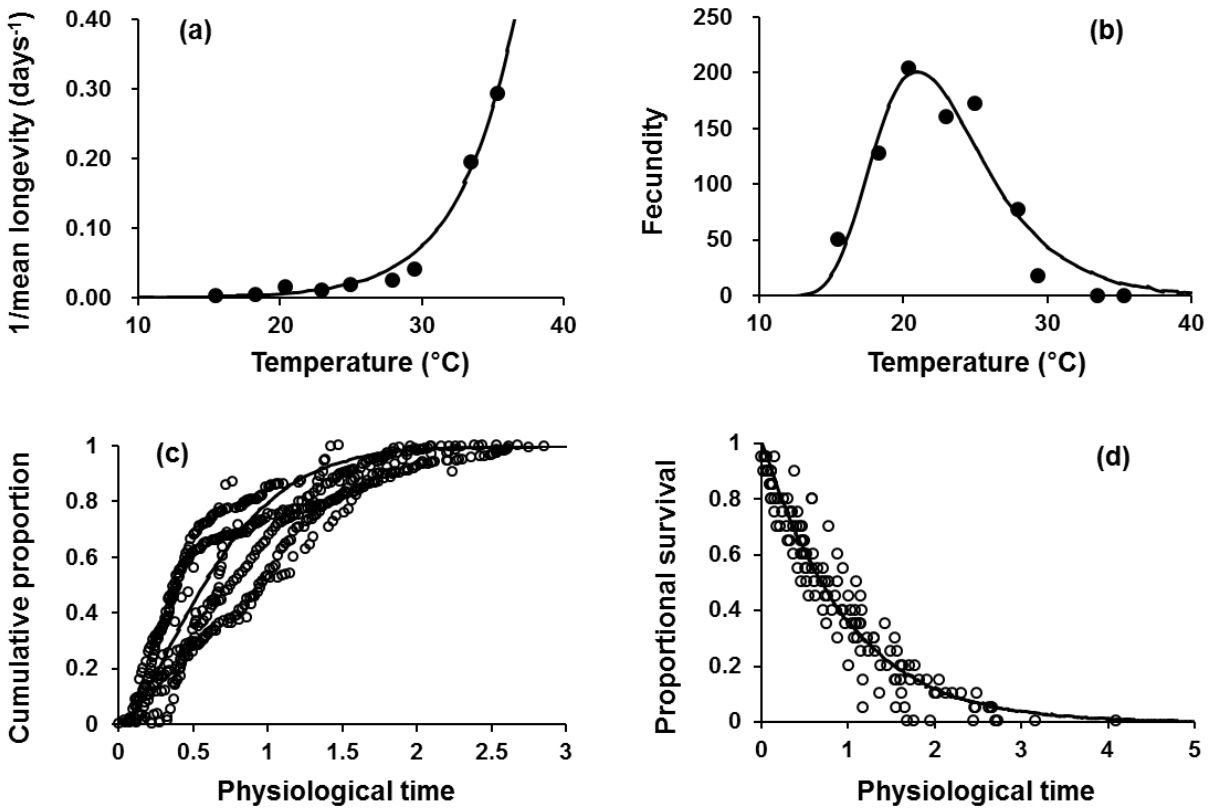


Fig. 5. Temperature dependent oviposition models of *Illeis koebelei*. (a) Adult development rate curve (1/mean longevity) of *Illeis koebelei* (●, Observed; —, Estimated). (b) Temperature-dependent fecundity curve of *Illeis koebelei* (●, Observed; —, Estimated). (c) Age-specific cumulative oviposition rate curve of *Illeis koebelei* (○, Observed; —, Estimated). (d) Age-specific adult survival rate curve of *Illeis koebelei* (○, Observed; —, Estimated).

4. Discussion

Temperature played a critical role in determining development, survival, and oviposition of *I. koebelei*. Eggs developed from much lower temperatures (i.e., lower threshold temperature) compared to other developmental stages. However, suitable thermal range of egg development (i.e., B_{80}) was not wider than that of any other developmental stage. Pre-pupae showed the widest thermal range in survivorship (i.e., B_{80}) among developmental stages of *I. koebelei*. Fecundity of *I. koebelei* was the highest at the relatively low temperature (see parameter, T_f) compared to optimal development (i.e., T_{opt}) and survival temperatures (i.e., T_m).

The estimated 4th instar and pre-pupa stage-specific development rate models of *I. koebelei* in this study have some weakness because their development rate at 15.4 ℃ was based on very small sample size due to death of tested individuals. However, the r^2 values of these fitted models were high (Table 2). We speculate that *I. koebelei* might rarely survive when they are exposed to low temperatures throughout immature stages, and the development time at 15.4 ℃ obtained in this study would be reliable for fitting the model.

Effects of temperature on the development of powdery mildew have been tested on multiple host plants. Rose powdery mildew (*Sphaerotheca pannosa* var. *rosae*) showed the highest development at 23 ℃ (Xu, 1999). In begonia, optimal temperatures of germination and colony development for the causal agent (*Oidium begonia*) of powdery mildew were 23-25 ℃ and 20-21 ℃, respectively (Quinn and

Powell, 1982). Oh (1997) has reported that the germination rate of grape powdery mildew (*Uncinula necator*) was the highest at 26 ℃. The range of optimal germination temperature for strawberry powdery mildew (*Sphaerotheca macularis* f. sp. *fragariae*) was from 15 to 25 ℃ (Amsalem et al., 2006). Although there were differences in germination rate and development of powdery mildew according to causal agents and host plants from previous studies (Quinn and Powell, 1982; Oh, 1997; Xu, 1999; Amsalem et al., 2006), optimal temperature ranges for germination and development of powdery mildew were 15-26 ℃ and 21-23 ℃, respectively. These optimal temperature ranges of powdery mildew were overlapped with optimal thermal ranges (B_{80}) with ≥ 80% of the maximum value of developmental and survival rates for immatures of *I. koebelei* and B_{80} of female adult fecundity for *I. koebelei*. Much broader optimal temperatures of *I. koebelei* compared to casual agents of powdery mildew might increase the chance of survival and establishment of *I. koebelei* in the field and greenhouse conditions.

Findings of this study are applicable to rearing system for *I. koebelei*. Separated rearing of adults from other developmental stages is highly recommended based on B_{80} of developmental and survival rates for their immatures and B_{80} of female adult fecundity. Rearing temperatures for *I. koebelei* adults are highly recommended to range from 18.8 to 23.7 ℃. Collected eggs from the adult rearing system might be reared at temperature range of 23.6 to 29.6 ℃ until emergence of adults to ensure high survivorship and rapid development. In the case of non-separated rearing,

rearing temperature for *I. koebelei* are highly recommended to be 23.6 or 23.7 ℃. In these temperatures, *I. koebelei* might show rapid development and high survival rates of immatures with high reproduction of adults.

The lower threshold temperature obtained by the linear model in this study can be used to determine proper storage of *I. koebelei*. Specifically, eggs would be the best for storage among all developmental stages of *I. koebelei* because eggs have more tolerance against low temperature. Optimal thermal ranges (B_{80}) of survivorship rate can be applied to determine proper shipping of *I. koebelei.* Since eggs and pre-pupae have broader thermal ranges (B_{80}) of survivorship rate compared to pupa stage, as well as no food requirement, these stages are highly recommended for shipping of *I. koebelei*.

Lower threshold temperature and thermal requirement (degree-days) obtained by linear models in this study coupled with weather data and monitoring data of field populations can be used to predict the phenology of *I. koebelei* in the field (Pruess, 1983; Higley et al., 1986). In the prediction of insect population dynamics under field conditions, precision level of prediction may increase by using upper threshold because upper threshold of insect development has been commonly used to more accurately calculate degree-days from weather data among high temperature cut-off techniques (Roltsch et al., 1999).

In summary, this study reports the effect of temperature on the development and oviposition of *I. koebelei* and synchronization of optimal temperatures between

powdery mildew and *I. koebelei*. This study also suggested optimal temperature for mass-rearing and proper developmental stage for storage and shipping of *I. koebelei.*

These models developed in this study might be useful for predicting phenology and population dynamics of *I. koebelei* in the field (e.g., Baek et al., 2008), estimating the establishment possibility in new areas (e.g., Hart et al., 2002; Hatherly et al., 2005), and simulating the impact of global warming on its distribution, abundance, and occurrence pattern in the future (e.g., Hance et al. 2007; Dixon et al. 2009) with environmental data.

Chapter III.

Toxicity of conventional pesticides used in cucumber cultivation to *I. koebelei* Timberlake (Coleoptera: Coccinellidae: Halyziini)

Abstract

This is the first study to report the toxicity of pesticides to mycophagous ladybeetle, *Illeis koebelei*. We investigated the selective toxicities of synthetic or environment-friendly biopesticides to *I. koebelei* for the development of integrated powdery mildew management programs. Three synthetic insecticides, bifenthrin + imidacloprid WP, acetamiprid + indoxacarb WP, and acetamiprid + etofenprox WP, were very toxic (IOBC classification, Class 4) to *I. koebelei*. Spiromesifen SC was less toxic to the survivorship and fecundity of *I. koebelei* when the third instar larvae or newly emerged adults were exposed to this pesticide via feeding with spiromesifen SC-treated cucumber powdery mildew. Pyriproxyfen EC showed very high residual toxicity, and the pupation rate and fecundity decreased significantly. Many environment-friendly biopesticides restricted the population of *I. koebelei*. However, Q pact (a.i. *Ampelomyces quisqualis* 94013) and Top seed (a.i. *Paenibacillus polymyxa* AC-1) showed toxicity to *I. koebelei* larvae. BT one (a.i. *Bacillus thuringiensis*) showed no residual toxicity on the fecundity of *I. koebelei* adults.

Keywords: Ladybeetle, *Illeis koebelei*, mycophagous, pesticide, toxicity

1. Introduction

Pesticide use results in the unavoidable exposure to natural enemies of pests. Therefore, use of selective pesticides that have low toxicity to natural enemies is essential for the conservation of natural enemy populations (Tanaka et al., 2000). Thus, the use of selective pesticides is important in integrated pest management (IPM). The International Organization for Biological Control (IOBC) is active in identifying pesticides compatible with biological control. Based on IOBC classification, the effect of pesticides on natural enemy is categorized as Class 1 (harmless), Class 2 (slightly harmful), Class 3 (moderately harmful) and Class 4 (harmful) toxicity levels.

Powdery mildew disease is the most common and economically important plant disease in agricultural ecosystems worldwide (Amano, 1986). This disease damages a wide range of agricultural plant species (Glawe, 2008). The management of powdery mildew heavily relies on fungicides. However, owing to various adverse effects, such as development of pesticide resistance, environmental pollution, and killing of natural enemies (Razdan and Sabitha, 2009), the application of biological agents including microorganisms, and mycophagous arthropods has been increasingly studied (Bhattacharjee et al., 1994; English-Loeb et al., 2007; Lee et al., 2007; Romero et al., 2007; Segarra et al., 2009; Hegazi and El-Kot, 2010).

Of the coccinellid group, mycophagous ladybeetles in the tribe Halyziini are considered biological control agents against powdery mildew. Halyziini ladybeetles

feed primarily on powdery mildews and are distributed worldwide in regions where powdery mildews commonly occur (Sasaji, 1998; Giorgi et al., 2009; Sutherland and Parrella, 2009; Tabata et al., 2011). Mycophagous ladybeetle, *Illeis koebelei* is generally distributed in Asian countries, including China, Japan, Korea, Philippines, and Taiwan (Kim et al., 1994; Recuenco-Adorada and Gapud, 1998; Takeuchi et al., 2000; Lin et al., 2006; Wu et al., 2011). A recent study by Lee et al (2015) reported that *I. koebelei* was found from early July to early November in Korea, and has the potential for controlling cucumber powdery mildew.

Despite the broad distribution of mycophagous *I. koebelei* and its potential as a biological agent against powdery mildew disease in Korea, very few information are available on the toxicity of pesticides to *I. koebelei*. Information on pesticide toxicity to *I. koebelei* would help farmers to select the appropriate pesticides for the chemical control of other pest and to develop a successful IPM program to control cucumber powdery mildew using this mycophagous ladybeetle. Therefore, in this study, we aimed to determine the toxicity of pesticides, which are, commonly used in cucumber production in Korea, to *I. koebelei*. The results of this study would provide a guideline for selecting appropriate pesticides for controlling powdery mildew using *I. koebelei*.

2. Materials and Methods

2.1. Rearing of *I. koebelei*

I. koebelei and cucumber powdery mildews were collected from pear orchards and cucumber plants in commercial greenhouses, respectively, in Gyeonggi-do, Korea. *I. koebelei* was cultured on cucumber seedlings infected with powdery mildew in laboratory at 24±1℃, 60-80% (RH) and a photoperiod of 16:8 (L:D) h. Approximately 30 adult *I. koebelei* were placed in acrylic containers (30 × 30 × 30 cm) with two pots of cucumber seedlings infected with powdery mildew. The eggs laid on cucumber leaves were transferred to a translucent plastic cage (L 232 × W 165 × H 95 mm) with ventilation holes on the sides. A wet paper-towel was placed at the bottom of cage to provide moisture. Neonates were moved to cucumber leaf infected with powdery mildew by using a soft brush. The larvae were always were provided new cucumber seedlings before the exhaustion of food. The pupae were placed in acrylic containers until eclosion.

2.2. Pesticides

Sixteen synthetic insecticides (Table 1) for controlling *Trialeurodes vaporariorum* or *Bemisia tabaci*, and 22 environment-friendly commercial pesticides (Table 2), which are generally used in cucumber cultivation in Korea, were tested for toxicity to *I. koebelei*. All pesticides were diluted in water to the respective field-recommended application rate. The concentration of each pesticide was selected based on the 2012 Agrochemicals Use Guide Book (KCPA, 2012).

Table 1. List of commercial synthetic insecticides tested for toxicity against mycophagous ladybeetle *Illeis koebelei*

No.	Trade name	Active gradient (%)	Formulation	Recommended Concentration
1	Actara	Thiamethoxam (10)	WG [a)]	2,000 X
2	Affrim	Emamectin benzoate (2.15)	EC	2,000 X
3	Acellt	Spinetoram (5)	SC	2,000 X
4	Bigcard	Clothianidin (8)	SC	2,000 X
5	Cheonhamujuck	Bifenthrin+imidacloprid (2+8)	WP	2,000 X
6	Decis	Deltamethrin (1)	EC	1,000 X
7	Hanaro	Bistrifluron (10)	EC	2,000 X
8	Limone	Novaluron (10)	SC	2,000 X
9	Limousine	Gamma-cyhalothrin (1.4)	CS	2,000 X
10	Maengta	Acetamiprid+indoxacarb (4+5)	WP	1,000 X
11	Manjangilchi	Acetamiprid+etofenprox (2.5+8)	WP	1,000 X
12	Moseupiran	Acetamiprid (8)	WP	2,000 X
13	Oshin	Dinotefuran (10)	WP	1,000 X
14	Sanmaroo	Pyridaben (20)	WP	1,000 X
15	Shingiroo	Pyriproxyfen (10)	EC	2,000 X
16	Zizone	Spiromesifen (20)	SC	2,000 X

[a)]WG=water dispersible granule, EC=emulsifiable concentration, SC=suspension concentration, WP=wettable powder, CS=capsule suspension.

Table 2. List of environment-friendly commercial pesticides tested for toxicity against mycophagous ladybeetle *Illeis koebelei*

No.	Trade name	Active gradient (%)	Formulation	Recommended Concentration
1	Barogaru alpha	Plant Extracts+ *Bacillus subtilis*	EC [a)]	1,000 X
2	Barojin alpha	Plant Extracts + Microorganism	EC	1,000 X
3	Barotok alpha	Plant Extracts+ *Bacillus subtilis*	EC	1,000 X
4	Bijin alpha	Plant Extracts	EC	1,000 X
5	BT one	*Bacillus thuringiensis*	WP	1,000 X
6	Daeyou ecocide	*Bacillus thuringiensis* serovar	EC	2,000 X
7	Daeyou eungjinssak	Natural seed extracts	EC	1,000 X
8	Daeyou plazmaneem	Neem extracts	EC	500 X
9	Ddook plus	Plant Extracts + Microorganism	EC	1,000 X
10	Dyna	Plant Extracts	EC	1,000 X
11	Eungaetan alpha	Plant Extracts+ *Bacillus subtilis*	EC	1,000 X
12	Eungsami	Plant Extracts + Microorganism	EC	1,000 X
13	Jinap	Plant Extracts	EC	1,000 X
14	Jinsami	Plant Extracts	EC	1,000 X
15	Neem seed oil	Neem extracts	EC	1,000 X
16	Nobug	Plant Extracts	EC	1,000 X
17	Onsami	Plant Extracts	EC	1,000 X
18	Q pact	*Ampelomyces quisqualis* 94013	WP	1,000 X
19	Solbitchae	Microorganism	EC	400 X
20	Suncho	Plant Extracts	EC	1,000 X
21	Toggagi power	Plant Extracts	EC	1,000 X
22	Top seed	*Paenibacillus polymyxa* AC-1	SC	200 X

[a)]EC=emulsifiable concentration, WP=wettable powder, SC=suspension concentration.

2.3. Toxicity bioassay

To test the toxicity of the 38 pesticides listed above, the third instar larvae and adults of *I. koebelei* (<48 h old) were exposed to pesticides at the recommended field application rate of each commercial formulation. The larvae were sprayed with pesticide or water control until the body was soaked, and then placed in the insect breeding dish (Ø 150 × H 73 mm, ventilation allowed). The adults were dipped in pesticide or water control for 5 s and placed in the insect breeding dish (Ø 150 × H 73 mm, ventilation allowed). Cucumber leaf disc (Ø 100 mm) infected with powdery mildew was provided in the dish as a food source. The experiment was repeated three times with 15 individuals in each replicate for each treatment. Kimwipe (Kimberly-Clarke® , Kimwipes® EX-L) was placed at the bottom of the dish and moistened with water every day. Mortality was assessed 48 h after treatment. An insect was recorded as dead if it did not move or could not turn the body by itself when touched.

Pesticides that showed low toxicity to *I. koebelei* were further tested to evaluate the residual toxicity on survival and fecundity of *I. koebelei* at the same concentration. For the evaluation of residual toxicity to larvae, 30 third instar larvae were provided with cucumber leaves infected with powdery mildew, which had been exposed to pesticides. The amount of leaves provided was sufficient to feed the larva to pupation. After adult emergence, 10 adult couples were provided with pesticide-free cucumber seedlings, and the number of eggs laid by female adults was investigated for 20 days after the pre-oviposition period. For the evaluation of residual toxicity to adults, 15

adult couples (<24h) were treated, and the survival rate, pre-oviposition period and fecundity were investigated by the same method. All experiments were conducted in the laboratory at 24±1℃, 60-80% RH and a photoperiod of 16:8 (L:D) h.

2.4. Statistical analysis

The mortalities (%) caused by synthetic insecticides and commercial environment-friendly pesticides were corrected for control mortality using the Abbott's correction formula. Then, the mortalities (%) were transformed using the arcsine square root function prior to analysis of variance (ANOVA) test. The survival rate (%), pre-oviposition period, and fecundity of *I. koebelei* adults by the residual toxicity of pesticides, and pupation rate (%), emergence rate (%), pre-oviposition period, and fecundity of *I. koebelei* by the residual toxicity of pesticides were analyzed by one-way ANOVA. The means were separated with the Tukey's Studentized Range (HSD) Test (SAS Institute, 2008).

3. Results

3.1. Toxicity of synthetic insecticides

The effects of 16 synthetic insecticides on the mortality of *I. koebelei* under laboratory conditions are shown in Table 3. The susceptibility of *I. koebelei* to insecticides varied according to the insecticide and developmental stages. In general, the larvae were more susceptible to pesticides than the adults were. Neonicotinoid insecticides and a mixture chemicals, such as acetamiprid WP, acetamiprid +

indoxacarb WP, acetamiprid + etofenprox WP, bifenthrin + imidacloprid WP, clothianidin SC, and dinotefuran WP showed strong toxicity to *I. koebelei*. However, thiamethoxam WG was relatively less toxic to *I. koebelei* than other neonicotinoid insecticides. In particular, thiametoxam WG showed very less toxic effect on adult *I. koebelei*. Pyrethroid insecticides and a mixture with pyrethroids, such as bifenthrin + imidacloprid WP, deltamethrin EC and gamma-cyhalothrin CS were also highly toxic to *I. koebelei*, regardless of its developmental stages. The insect growth regulator, pyriproxyfen EC and the new class of spirocyclic tetronic acids, spiromesifen SC, were less toxic to *I. koebelei* with < 20% mortality. Other insect growth regulators, bistrifluron EC and novaluron SC, were also less toxic to adult *I. koebelei*, but were highly toxic to its larvae.

Pyrethroid insecticides and the mixture with that, such as bifenthrin + imidacloprid, deltamethrin and gamma-cyhalothrin were also very toxic to the larvae and adults of *I. koebelei*. The field recommended concentrations of the insect growth regulators, pyriproxyfen and the new class of spirocyclic tetronic acids, spiromesifen were so safe to the larvae and adults of *I. koebelei* that caused less than 20% mortality. Another the insect growth regulators, bistrifluron and novaluron had not the least effect on the adults of *I. koebelei*.

Table 3. Effects of commercial synthetic insecticides on mortality of *Illeis koebelei* under laboratory conditions

No.	Pesticide	Mortality (%, mean ± SE)	
		Larvae	Adults
1	Thiamethoxam	78.0 ± 7.35 bc [a)]	6.8 ± 3.90 efg
2	Emamectin benzoate	92.7 ± 7.30 ab	72.7 ± 13.65 b
3	Spinetoram	46.4 ± 7.68 d	25.0 ± 6.80 cd
4	Clothianidin	100 ± 0.00 a	68.2 ± 10.41 b
5	Bifenthrin + imidacloprid	100 ± 0.00 a	100 ± 0.00 a
6	Deltamethrin	100 ± 0.00 a	90.9 ± 3.90 a
7	Bistrifluron	60.1 ± 8.43 cd	0 ± 0.00 g
8	Novaluron	43.9 ± 8.43 d	6.8 ± 3.90 efg
9	Gamma-cyhalothrin	100 ± 0.00 a	90.9 ± 7.85 a
10	Acetamiprid + indoxacarb	100 ± 0.00 a	100 ± 0.00 a
11	Acetamiprid + etofenprox	100 ± 0.00 a	100 ± 0.00 a
12	Acetamiprid	100 ± 0.00 a	93.2 ± 6.80 a
13	Dinotefuran	60.1 ± 8.43 cd	34.1 ± 3.96 c
14	Pyridaben	75.6 ± 11.17 bc	100 ± 0.00 a
15	Pyriproxyfen	8.1 ± 10.18 e	9.1 ± 3.90 ef
16	Spiromesifen	3.2 ± 2.79 e	15.9 ± 3.96 de

[a)]Means followed by the same letter within a column are not significantly different at p=0.05 by Tukey's Studentized Range Test (SAS Institute, 2008).

3.2. Toxicity of environmental-friendly pesticides

The effects of 22 environment-friendly pesticides on the mortality of *I. koebelei* under laboratory conditions are presented in Table 4. Most biological or botanical pesticides were more toxic to larvae than adults of *I. koebelei* similar to synthetic pesticides. Several botanical or biopesticides (e.g., Barogaru alpha, Bijin alpha, Eungaetan alpha, Eungsami, Nobug, Suncho) were highly toxic to larvae, or adult, or both stages of *I. koebelei*. However, Barogaru, Bijin alpha and Neem seed oil showed low toxic effects on adult *I. koebelei*. Among the biological or botanical pesticides tested, six commercial products (Barojin alpha, BT one, Daeyou ecocide, Q pact, Solbitchae, and Top seed) showed low mortality against the larvae and adults of *I. koebelei,* with < 20% mortality.

3.3. Residual toxicity

The effects of residual toxicity of several pesticides, which are less toxic to *I. koebelei,* on *I. koebelei* adults are presented in Table 5. The synthetic pesticides pyriproxyfen EC and spiromesifen SC showed low residual toxicity to *I. koebele,* in terms of mortality and pre-oviposition period. However, pyriproxyfen EC reduced fecundity significantly. The biopesticides BT one and Solbitchae did not show significant residual toxicity.

The effects of residual toxicity of several pesticides, which are less toxic to *I. koebelei,* on *I. koebelei* larvae are presented in Table 6. The synthetic pesticide pyriproxyfen EC showed significant residual toxicity, resulting in unsuccessful

pupation. Another synthetic pesticide spiromesifen SC showed a rather low residual toxicity, resulting in 70% pupation rate. In addition two environment-friendly pesticides, BT one and Solbitchae, did not affect survival and showed low residual toxicity on fecundity of *I. koebelei*. Meanwhile, Solbitchae somewhat shortened the pre-oviposition period and reduced the fecundity of adults.

Table 4. Effects of commercial environment-friendly pesticides on mortality of *Illeis koebelei* under laboratory conditions

No.	Pesticide	Mortality (%, mean ± SE)	
		Larvae	Adults
1	Barogaru alpha	95.1 ± 4.25 ab [a)]	20.5 ± 7.91 ghi
2	Barojin alpha	9.7 ± 4.25 h	3.0 ± 2.64 i
3	Barotok alpha	85.4 ± 7.30 abc	31.8 ± 6.80 fgh
4	Bijin alpha	100 ± 0.00 a	0 ± 0.00 i
5	BT one	7.3 ± 4.25 h	0 ± 0.00 i
6	Daeyou ecocide	2.5 ± 8.43 h	3.0 ± 2.64 i
7	Daeyou eungjinssak	82.9 ± 4.25 bcd	93.4 ± 6.81 ab
8	Daeyou plazmaneem	53.6 ± 4.25 g	65.9 ± 6.80 cd
9	Ddook plus	70.7 ± 7.30 cdef	36.3 ± 3.96 efg
10	Dyna	75.6 ± 8.49 cde	70.4 ± 10.41 cd
11	Eungaetan alpha	100 ± 0.00 a	43.2 ± 7.85 ef
12	Eungsami	100 ± 0.00 a	100 ± 0.00 a
13	Jinap	63.4 ± 7.30 efg	50.0 ± 10.41 def
14	Jinsami	82.9 ± 4.25 bcd	56.8 ± 10.41 cde
15	Neem seed oil	68.3 ± 11.17 defg	11.4 ± 6.80 hi
16	Nobug	100 ± 0.00 a	20.5 ± 3.96 ghi
17	Onsami	75.6 ± 8.49 bc	77.3 ± 7.91 bc
18	Q pact	0 ± 0.00 h	0 ± 0.00 i
19	Solbitchae	1.6 ± 2.79 h	5.3 ± 5.69 i
20	Suncho	100 ± 0.00 a	100 ± 0.00 a
21	Toggagi power	56.1 ± 7.35 fg	65.9 ± 11.81 cd
22	Top seed	0 ± 0.00 h	0 ± 0.00 i

[a)]Means followed by the same letter within a column are not significantly different at p=0.05 by Tukey's Studentized Range Test (SAS Institute, 2008).

Table 5. Effects of residual toxicity of several pesticides on the survival and fecundity adult *Illeis koebelei*

Pesticide	Survival rate (% ± SE)	Pre-oviposition period (day ± SE)	Fecundity (eggs/female ± SE)
Pyriproxyfen	90.0 ± 10.00 ns	9.7 ± 1.15 ns	68.7 ± 25.77 d [a)]
Spiromesifen	93.3 ± 5.77	9.3 ± 1.04	134.1 ± 31.09 bc
BT one	100	8.3 ± 1.08	144.0 ± 29.55 ab
Solbitchae	96.7 ± 5.77	9.0 ± 0.42	131.2 ± 28.93 c
Control	100	10.0 ± 0.40	149.7 ± 27.15 a

[a)]Means followed by the same letter within a column are not significantly different at p=0.05 by Tukey's Studentized Range Test (SAS Institute, 2008).

Table 6. Effects of residual toxicity of several pesticides on the survival and fecundity of *Illeis koebelei* larva

Pesticide	Pupation rate (% ± SE)	Emergence rate (% ± SE)	Pre-oviposition period (day ± SE)	Fecundity (eggs/female ± SE)
Pyriproxyfen	0 c [a)]	0 b	0 c	0 b
Spiromesifen	70.0 ± 10.00 ab	57.1 ± 12.30 a	8.8 ± 0.25 a	144.3 ± 12.50 ab
BT one	86.7 ± 11.55 a	71.7 ± 15.88 a	8.7 ± 0.56 a	131.3 ± 17.56 b
Solbitchae	66.7 ± 11.55 b	59.7 ± 8.69 a	7.3 ± 0.36 b	127.3 ± 17.95 b
Control	86.7 ± 5.77 a	73.2 ± 5.77 a	9.4 ± 0.55 a	155.0 ± 15.52 a

[a)]Means followed by the same letter within a column are not significantly different at p=0.05 by Tukey's Studentized Range Test (SAS Institute, 2008).

4. Discussion

This study first to reports the toxicity of pesticides to the mycophagous predator, *I. koebelei*, of powdery mildew of agricultural crops. The pesticides tested are 16 synthetic and 22 environment-friendly products being used for controlling insect or microbial pests on cucumber in Korea. Many studies on toxicity against the multicolored Asian ladybeetle, *Harmonia axyridis* Pallas, have been conducted with various insecticides, biopesticides, fungicides, herbicides, and insect-resistant transgenic crops (Koch, 2003). Cho et al. (1997) reported that synthetic pyrethroid

insecticides were less toxic to *H. axyridis* than to aphids. Insect ecdysone aganists, halofenozide and methoxyfenozide are known to cause premature larval molting, interruption of feeding, and incomplete pupation (Carton et al., 2003). Several terpenoids derived from plants, such as camphor, menthol, catnip, and grapefruit, are known to repel ladybeetles (Riddick et al., 2008). In the case of ladybeetle *Hippodamia variegata* (Goeze), Almasi et al. (2013) selected pirimicarb and pymetrozine as a low toxic pesticide in contrast to proteus that showed high mortality rate. Rahmani et al. (2013) reported that a new neonicotinoid insecticide, thiamethoxam, shortened the pre-adult developmental period, while it didn't showed effect on the adult developmental period. In the case of ladybeetle, imidacloprid and deltamethrin have been reported to be relatively harmful (Bozsik, 2006) to ladybeetle *Coccinella septempunctata* L., while the two biopesticides Bioshower (a.i. 100% fatty acid) and insecticidal soap (a.i. 20% fatty acids) showed no toxicity (Raudonis et al., 2010). Radha (2013) reported the comparative toxicity of biopesticides and synthetic pesticides against cowpea aphid (*Aphis craccivora*) and its natural enemy ladybeetle *Micrapis discolor*. Botanical insecticides (neem seed extracts) and microbial pesticides (spinosad) as were recommended as alternatives to chemical insecticides for the IPM of *A. craccivora* in cowpea.

In this study, the toxicities of various synthetic insecticides and environment-friendly pesticides to *I. koebelei* were significantly different (Table 3, 4). In particular, bifenthrin + imidacloprid WP, deltamethrin EC, gamma-cyhalothrin CS, acetamiprid

+ indoxacarb WP, acetamiprid + etofenprox WP, and acetamiprid WP showed strong toxicity to *I. koebelei*. Based on the IOBC classification, the three insecticides, bifenthrin + imidacloprid WP, acetamiprid + indoxacarb WP, and acetamiprid + etofenprox WP, were classified as toxicity Class 4 (harmful). Pyriproxyfen EC and spiromesifen SC caused < 20% mortality and are classified as toxicity Class 1 (harmless).

This study showed that *I. koebelei* is very sensitive to neonicotinoid insecticides. Neonicotinoid insecticides are commonly used against a wide range of herbivorous insect pests such as aphids, mealybugs and whiteflies in greenhouses or farms in Korea (KCPA, 2012). This result is consistent with the results of Lucas et al. (2004) who reported that imidacloprid is highly toxic to both adult and larval stages of ladybeetle *Coleomegilla maculata* under laboratory conditions. Neonicotinoid insecticides, including proteus, are toxic to both larvae and adults of coccinellids (Almasi et al., 2013). Bifenthrin + imidacloprid WP, acetamiprid + indoxacarb WP, and acetamiprid + etofenprox WP classified as toxicity Class 4 need to be tested in semi-field or field conditions to determine their effects on mycophagous *I. koebelei.* Meanwhile, spiromesifen SC, which was placed in Class 1, could be used as a part of the cucumber powdery mildew IPM program in combination with *I. koebelei*. According to the IOBC, if insecticides were harmless in the laboratory test, it is not necessary to perform further semi-field or field studies (Almasi et al., 2013). However, we suggest that performing further residual toxicity tests may be important

for pesticides that showed low contact toxicity to natural enemies in laboratory tests. For example, pyriproxyfen EC, which had low toxicity in the laboratory test, showed markedly high residual toxicity to *I. koebelei* by decreasing fecundity.

Many environment-friendly pesticides (e.g., Barogaru alpha, Barotok alpha, Daeyou eungjinssak, Eungaetan alpha, Eungsami, Iinsami, Nobug and Suncho) were highly toxic to *I. koebei* larvae. Daeyou eungjinssak (a.i. natural seed extracts), Eungsami (a.i. *Azadirachta indica* + *Sophora flavescens* + microorganism) and Suncho (a.i. *Azadirachta indica*) were highly toxic to *I. koebelei* adults and classified as toxicity Class 3 or 4 in the IOBC category. However, Barojin alpha (a.i. Plant extracts) and BT one (a.i. *Bacillus thuringiensis*) were less toxic to *I. koebelei* larva. Q pact (a.i. *Ampelomyces quisqualis* 94013) and Top seed (a.i. *Paenibacillus polymyxa* AC-1) are microbial fungicides for controlling powdery mildew disease of various agricultural crops (Lee et al., 2004; Kim et al., 2013). The microbial fungicides Q pact, Top seed, and BT one were less toxic to *I. koebelei*. However, the toxicities of pesticides could differ according to the developmental stages of insects. In this study, *I. koebelei* larvae were more susceptible to pesticides than adults were. Although BT one did not show residual toxicity to *I. koebelei* adults, the fecundity of adults from third instar larvae, which had been exposed to BT one, decreased (Table 5, 6). These results show that many botanical or microbial pesticides could decrease the population of *I. koebelei*.

To date, no study has reported the toxicity of biological or botanical pesticides to mycophagous *I. koebelei*. We found that many botanical pesticides made from plant extracts or microorganisms could destroy or reduce the population of *I. koebelei*. The residue of active ingredient on plants can differ according to the formulation or supplement agent of the commercial products. Although, the results of laboratory toxicity test provided some useful information on selective pesticides for *I. koebelei*, the long-term effect of these pesticides on *I. koebelei* populations under field conditions could not be explained. Therefore, further field studies regarding these aspects need to be conducted. Powdery mildew disease is one of the most economically important plant pathogens in agricultural ecosystems worldwide. In this study, we could find the feeding potential of *I. koebelei* against the cucumber powdery mildew. And we developed mass rearing skill (Chapter I) and selected low toxic chemical agent (Chapter III) for IPM and control effect against powdery mildew in agro-ecosystem. Based on these results, we propose an integrated control strategy to controlling of cucumber powdery mildew in autumn season as shown in Fig 1.

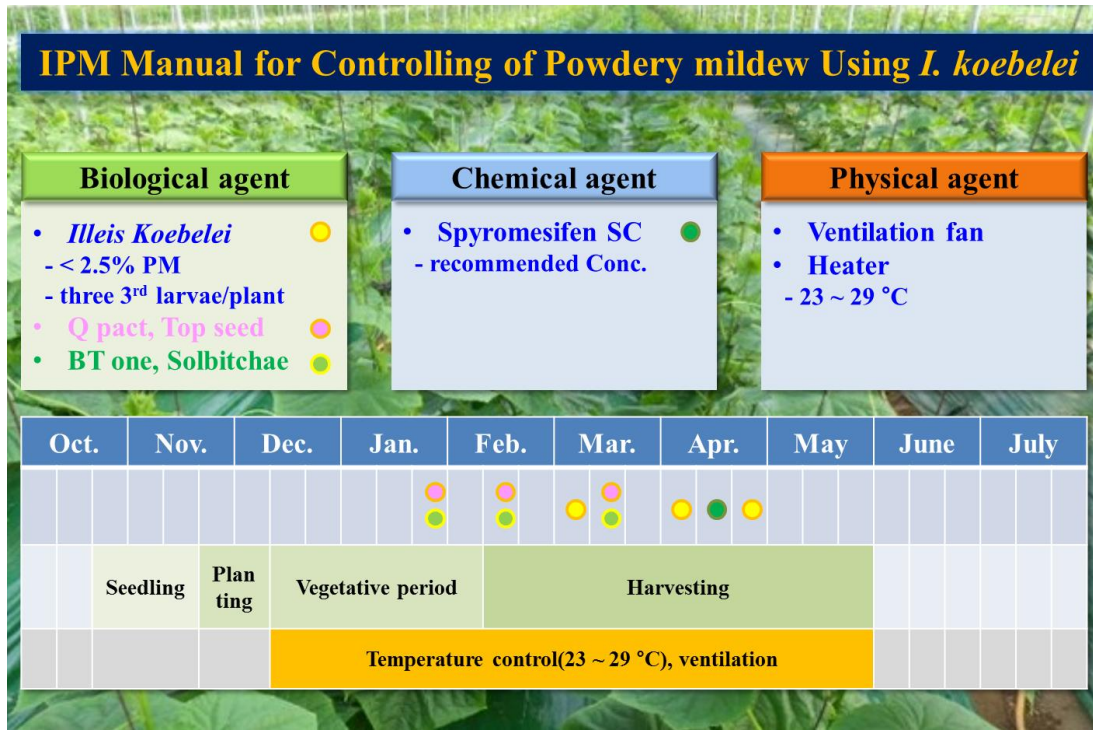


Fig. 1. Integrated control strategy to controlling of cucumber powdery mildew in autumn season.

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Abstract in Korean

식균성 노랑무당벌레의 생태 및 약제 감수성에 관한 연구

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초록

식균성 노랑무당벌레 (*Illeis koebelei* Timberake)는 흰가루병에 감염된 12종의 식물에서 관찰되었다. 특히 배과원에서 가장 밀도가 높았으며, 7월 상순부터 11월 상순까지 발견되었다. 노랑무당벌레의 장내에서는 흰가루병 균사나 포자 외에 다른 먹이의 흔적이 발견되지 않았고, 알과 번데기를 제외한 전 발육단계에서 균을 섭식하는 특성을 볼 때 절대적 식균성 곤충으로 생각된다. 25℃에서 오이 흰가루병균을 섭식한 노랑무당벌레의 발육기간은 알, 유충, 번데기, 성충이 각각 3.9, 10.4, 4.1, 37.7일이었고, 발육단계별 오이 흰가루병 섭식량은 4령, 성충, 3령, 2령, 1령 순으로 많았다.

노랑무당벌레의 발육과 산란모델을 개발하기 위해 15.4℃에서 39.5℃까지 8 개 등급의 항온조건에서 시험을 수행하였다. 발육률은 선형과 비선형 모델에 의해 잘 적용되었는데, 영기별 발육영점온도는 알, 1 령, 2 령, 3 령, 4 령, 전용, 용 및 전체 유충기에서 각각 3.6, 12.7, 12.1, 11.3, 11.3, 12.8, 14.7, 14.2℃이었고, 각 영기별 발육 완료에 필요한 온일도(DD, Degree-days)는 각각 86.6, 16.0, 22.5, 30.2, 49.3, 14.5, 43.8, 217.4DD 였다, 유충 생존율은 25.1℃에서 가장 높았다. 평균 산란수는 29.3℃에서 18.6 개로 가장 적었으며, 20.3℃에서 205.3 개로 가장 많았다. 노랑무당벌레 성충의 생존율과 누적 산란율은 각각 시그모이드 함수와 2 파라미터 Weibull 함수에 의해 잘 묘사되었다. 이 연구의 결과는 향후 상품화 되었을 경우 대량사육 및 운송을 위한 최적 환경조건을 설정하는데 이용이 가능하며, 또한, 노랑무당벌레와 흰가루병 사이의 최적 발생 조건을 비교하고, 현장에서 노랑무당벌레의 발생소장과 개체군동태를 예측하는 데에도 이용이 가능하리라 생각된다.

오이에 등록된 살충제와 유기농업자재를 대상으로 노랑무당벌레의 유충과 성충에 대한 독성을 검정한 결과, bifenthrin + imidacloprid (WP), acetamiprid + indoxacarb (WP), acetamiprid + etopheprox (WP)약제들은 IOBC 기준을 적용할 경우 Class 4

(harmful)에 속하는 높은 독성을 나타내었다. 단기간 독성평가시 저독성 살충제였던 spiromesifen (SC)은 노랑무당벌레 3령 유충과 갓 우화한 성충이 흰가루병원균과 동시에 섭식하더라도 생존율과 번식력에는 영향이 적었던 반면, pyriproxyfen (EC)의 경우는 유충의 용화율과 성충 번식력을 크게 떨어뜨리는 것으로 나타났다. 한편 유기농업자재인 큐팩트 (a.i. *Ampelomyces quisqualis* 94013)와 탑시드 (a.i. *Paenibacillus polymyxa* AC-1)는 3령 유충과 성충에 독성을 보이지 않았으며, 비티원 (*Bacillus thuringiensis*)은 갓 우화한 성충의 생존율과 번식력에 영향을 미치지 않았다. 따라서, 천적에 대한 독성 평가시 노출 이후 생존율과 번식력에 대한 장기적 검토가 필요하며, 위의 저독성 농자재들은 오이 흰가루병 종합적 방제에 노랑무당벌레와 함께 이용이 가능할 것으로 생각된다. 아울러 위 결과들을 종합하여 오이 흰가루병 방제를 위한 종합관리전략을 제시하였다.

검색어: 노랑무당벌레, 식균성, 기주, 흰가루병, 섭식량, 생물적 방제, 온도발육모델, 발육영점온도, Weibull 함수, 독성

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