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Host plants and Biological Characteristics of *Illeis koebelei* Timberlake (Coleoptera: Coccinellidae: Halyziini) in Gyeonggi-do

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노랑무당벌레의 발생기주 및 생물학적 특성

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ABSTRACT: We investigated mycophagous ladybird, *Illeis koebelei* from 12 species of plants infected with powdery mildew in Gyeonggi-do, Korea. The pear tree, *Pyrus ussuriensis* var. *macrostipes* (Nakai), was most preferred by *I. koebelei*. This species was found from early July to early November in pear orchards. There was no entomophagous trace in the gut of *I. koebelei* without powdery mildew spores in a microscope. All stages except egg and pupa are obligate mycophagous, and the feeding potential is ranked as follows: fourth instar, adults, third instar, second instar, and first instar. Feeding amounts of each stage of *I. koebelei* were 45.6, 144.4, 372.2, 628.1, and 473.7 mm^2 of cucumber powdery mildew per day. Fourth instar larvae showed highest consumption of cucumber powdery mildew. Developmental periods of four larval instars and adults feeding cucumber powdery mildew were 1.2, 2.3, 2.3, 4.6, and 37.7 days, respectively, at 25°C. In this study, we could not determine the feeding potential of *I. koebelei* against the cucumber powdery mildew; therefore, and further studies are required to elucidate the potential of this species as a biological control agent, e.g., mass rearing, selection of low toxic chemical agents for Integrated Pest Management (IPM), and control techniques against powdery mildew in agro-ecosystems.

Key words: *Illeis koebelei*, Powdery mildew, Mycophagous, Biological control

초 록: 경기도 8개 지역에서 2010년부터 2012년 동안 식균성인 노랑무당벌레의 발생기주를 조사한 결과, 흰가루병에 감염된 12종의 식물에서 관찰이 되었다. 특히 가장 밀도가 높았던 배과원에서 노랑무당벌레는 7월 상순부터 11월 상순까지 발견되었다. 식균성인 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, 37.7일 이었고, 발육단계별 오이 흰가루병 섭식량은 45.6, 144.4, 372.2, 628.1, 473.7 mm^2로 4령, 성충, 3령, 2령, 1령 순으로 많았다. 본 연구를 통해 노랑무당벌레의 오이 흰가루병에 대한 섭식능력을 바탕으로 향후 유용 토착천적으로써 대량사육기술, 저독성 약제 선발 등 작물 흰가루병 종합방제기술(IPM)에 대한 연구가 필요하리라 사료된다.

검색어: 노랑무당벌레, 흰가루병, 식균성, 생물적 방제

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

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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, 2009; Hegazi and El-Kot, 2010).

The family Coccinellidae is comprised of approximately 6,000 species worldwide (Hodek et al., 2012). Most Coccinellid species are commonly entomophagous, and the major prey is the 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. 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 ladybird 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).

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), and Korea (Kim et al., 1994). 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.

Materials and Methods

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. *I. 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).

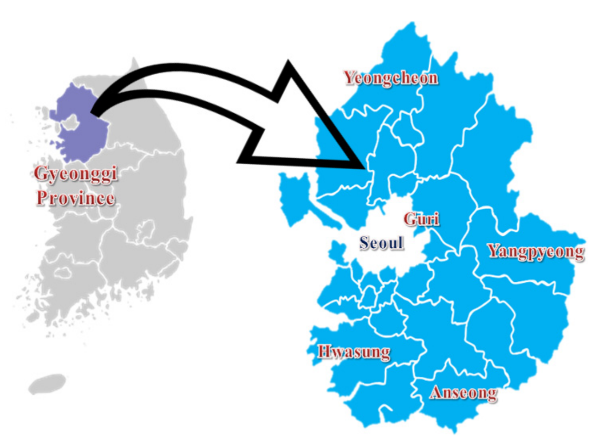

Fig. 1. Study area investigated for the natural occurrence of *Illeis. koebelei*.

Rearing of *I. koebelei*

Cucumis sativuss seeds were planted in plastic pots (100 cm diameter, 89 cm height) and grown in a thermostatic chamber at 25℃, 70 ± 10% RH, and a photoperiod of 16:8 (L:D) h. Powdery mildew was collected from cucumbers plants for inoculum. Collected powdery mildew, *Sphaerotheca fuliginea* 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*.

The beetles were maintained in the rearing room at 25 ± 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.

Results

Natural Occurrence

Illeis koebelei was found on 12 species of plants infected with powdery mildew in Gyeonggi-do (Table 1). Of them, *I. koebelei* was most abundantly found on the pear tree, P*yrus ussuriensis* var. *macrostipes* (Nakai) on which powdery mildew belonging to Phyllactinia was heavily infected. 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 2). All stages of *I. koebelei* were found from August to September, when the monthly mean temperature

Table 1. Host plants infected with powdery mildew and the abundance of *Illeis koebelei* in Gyeonggi-do

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: Anseong, G: Guri, H: Hwasung, Y1: Yeoncheon, Y2: Yangpyeong.

Table 2. Seasonal occurrence of *Illeis koebelei* on pear powdery mildew in Gyeonggi-do

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

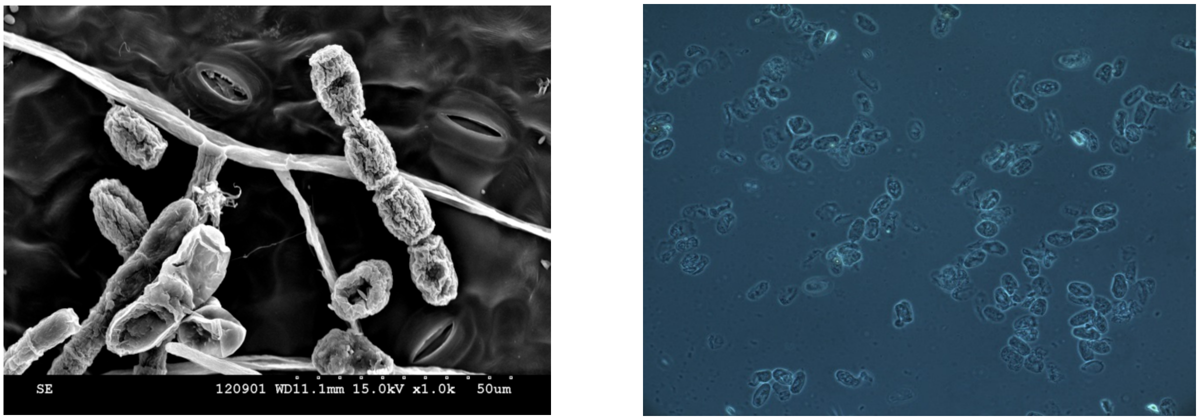

Fig. 2. Powdery mildew spores on the cucumber leaf (left) and those from the gut of *Illeis koebelei* adult (right) after feeding the cucumber powdery mildew.

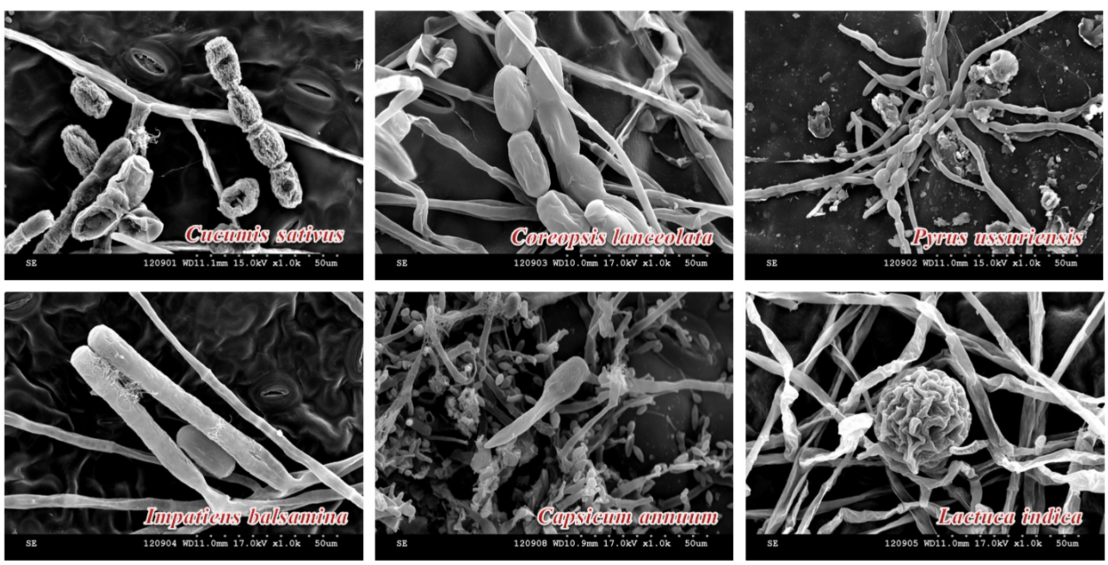

Fig. 3. Spore structures of nine species of powdery mildew that *Illeis koebelei* feeds on.

was 25.6℃ and 20.7℃, respectively.

Spores of cucumber powdery mildew were found in the gut of *I. koebelei* (Fig. 2). 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 and the preference of *I. koebelei* (Fig. 3, Table 1).

Biological characteristics

Table 3 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. 4). After embryonic development, the egg color changed to dark yellowish and finally dark greyish before hatching. There are 4 instars, all similar in appearance. Gray whole body length of first instar with black hairs was measured 1.38 mm, and yellowish second instar larvae with blackish dots and white hairs on the whole body was more elongate flattened and measured 2.89 mm. From the third instar, their dots become much darker and body length was 3.96 mm. Fourth instar was similar to the third, but 5.31 mm in its body length. Pupa was somewhat smaller than adult in body size and yellow with black spots on the whole (Fig. 4). The female adult was 4.51 mm in length and it is elongated yellow oval convex appears to be shielded beetle, and has two black spots appeared on the

Table 3. Developmental characteristics and feeding amount of each stage of *Illeis koebelei* that fed on cucumber powdery mildew

Stages	Body length (mm ± SD)	Developmental periods (day, Mean ± SD)	Feeding amount (mm^2/day ± SD) (%)
Egg	10.2 ± 0.04	3.9 ± 0.6	-
1st instar	1.38 ± 0.44	1.2 ± 0.4	45.6 ± 20.1 (10)
2nd instar	2.89 ± 0.54	2.3 ± 0.7	144.4 ± 97.8 (21)
3rd instar	3.96 ± 0.50	2.3 ± 1.3	372.2 ± 226.9 (79)
4th instar	5.31 ± 0.57	4.6 ± 1.4	628.1 ± 340.0 (133)
Pupa (♀)	3.68 ± 0.19	4.1 ± 0.4	-
Egg-pupa	-	18.4 ± 1.3	-
Adult (♀)	4.51 ± 0.25	37.7 ± 31.9	473.7 ± 257.9 (100)

※ Sample size comprised 30 eggs, larvae, pupae, and adults, 3 replications, at 25°C and 16:8 (L:D) h.

Fig. 4. Stages of *Illeis koebelei* : A. eggs, B. third instar larvae, C. pupa, D. adults.

pronotum (Fig. 3).

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℃. Feeding amounts of each stages of *I. koebelei* were 45.6, 144.4, 372.2, 628.1, 473.7 mm^2 of cucumber powdery mildew per day. Fourth instar larvae most consumed cucumber powdery mildew.

Discussion

Takeuchi et al. (2000) found that *I. koebelei* feeds on 11 species of powdery mildews (e.g., *Sphaerotheca fusca*, *S. cucurbitae*, and *Phyllactinia moricola*), and the kind of feed (powdery mildew) effects on their developmental duration of immature stage and survival rate. It seems to be positive correlation between powdery mildew severity and *I. koebelei* abundancy (Table 1). However, in spite of the abundant density of cucumber powdery mildew, the *I. koebelei* density was relatively low. It is supposed to be caused by the chemical or physical control of cucumber powdery mildew for the more harvest.

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

I. 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 the surface of pear leaves that powdery mildew occurred severely (Table 1). Although most of ladybirds could be found easily before July in Gyeonggi-do, Korea, we could not find *I. koebelei* in these seasons. Various circumstances (e.g. species of prey, prey density, temperature) effect on the oviposition selection of adult ladybird (Hodek et al., 2012). It seems that *I. koebelei* is alive in these seasons by feeding substitution diet e.g. pollen. Because pollen can be the good alternative food source for many ladybeetles (Lundgren, 2009).

We reconfirmed the mycophagous habits through investigating the feeding cucumber powdery mildew amount of each stages of *I. koebelei.* Feeding capacity of each stages can be ranked at 25℃ as follows: fourth instar, adults, third, second and first instar. The spores of cucumber powdery mildew in the gut of *I. koebelei* were found easily with no trace of arthropod food without fungal materials (Fig. 2). For the mass rearing of *I. koebelei* using cucumber powdery mildew for food, the adequate temperature may be 24℃ in the range from 16℃ to 26℃ which is the optimum temperature for powdery mildews (Yarwood et al., 1954).

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 further 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 effect against powdery mildew in agro-ecosystem.

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