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**A THESIS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

**Spatio-temporal dynamics of *Ricania shantungensis*
(Hemiptera: Ricaniidae) in Korea**

한국에서의 갈색날개매미충의 시·공간 동태 연구

BY

Sunghoon Baek

ENTOMOLOGY PROGRAM

DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY

SEOUL NATIONAL UNIVERSITY

August 2019

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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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**ENTOMOLOGY PROGRAM
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**Spatio-temporal dynamics of *Ricania shantungensis* (Hemiptera:
Ricaniidae) in Korea**

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ABSTRACT

Since the first report on its occurrence in 2010, *Ricania shantungensis* has quickly spread and its damage has exponentially increased in Korea. Thus, efficient management of *R. shantungensis* was required nationally and locally. The objectives of this study were (1) to predict current and future distribution of *R. shantungensis* to minimize impacts of *R. shantungensis* in Korea, (2) to estimate the optimal timing of pesticide application for *R. shantungensis*, and (3) to figure out population dynamics and distribution characteristics of *R. shantungensis* within a field to develop efficient management tactics for *R. shantungensis*.

To predict current and future distribution of *R. shantungensis* in

Korea, CLIMEX and the Maximum Entropy Model (MaxEnt) were applied. Weights of the variables used in both CLIMEX and MaxEnt were determined using the spatial association indices of spatial analysis by distance indices (SADIE). In MaxEnt, the maximum temperature of the warmest month, annual mean temperature, mean temperature of the coldest month, and precipitation of the driest month were determined to be the most important variables affecting the distribution of *R. shantungensis*. The results of this study indicated that *R. shantungensis* had a higher probability of occurrence in western areas than in eastern areas of Korea, and showed great potential to spread eastward. These results are expected to be helpful for managing *R. shantungensis* in Korea and selecting relevant environmental variables for species distribution modeling.

The most efficient control time of *R. shantungensis* is known to be when its first instar nymphs occur. Thus, the occurrence of first instars of *R. shantungensis* was studied. Effects of temperature on development and survival of *R. shantungensis* eggs were examined at seven constant temperatures (12.4, 16.4, 20.4, 24.8, 28.3, 32.4, and 36.9 °C). Lower developmental threshold, thermal constant, optimal developmental temperature, and upper developmental threshold were estimated to be 12.1 °C, 202 DD, 31 °C, and 36.9 °C, respectively. Survivorship was highest at 23.3 °C.

The egg hatching model well predicted occurrence of the first instar nymphs at three sites (Buyeo, Gwangyang, and Habcheon) in Korea. The model provided reliable and accurate control timing for *R. shantungensis* in chestnut fields of Korea. However, suggested time for insecticide application of *R. shantungensis* appeared to largely overlap with the flowering season of chestnut when honey bees' foraging is active. Moreover, all current, registered and applicable pesticides for *R. shantungensis* are toxic to honeybees. Thus, more sophisticated management tactics for *R. shantungensis* should be developed.

As more sophisticated management tactics for *R. shantungensis*, applicability of the site-specific management, effects of pruning and abutting forested areas with chestnut fields on populations of *R. shantungensis* were evaluated. By observing selected, marked, and geo-referenced 115 trees in a commercial chestnut field, survey for each developmental stage of *R. shantungensis* was conducted in 2017 and 2018. Site-specific management for *R. shantungensis* would be applicable because spatial distributions of this pest were generally aggregated and consistent regardless of years and developmental stages. In addition, pruning and management of abutting forested areas with commercial fields could decrease density of *R. shantungensis*. Integration of site-specific management, pruning, and

management of abutting forested areas into the control program of *R. shantungensis* should increase the control efficiency in chestnut fields.

In summary, this study provided current and future distribution of *R. shantungensis* in Korea, estimated optimal application timing of pesticides for *R. shantungensis*, and suggested management tactics of *R. shantungensis* in chestnut fields.

Key words: *Ricania shantungensis*, species distribution model (SDM), spatial distribution, site-specific management, optimal application timing

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General introduction

An invasive species, *Ricania shantungensis* (Hemiptera: Ricaniidae), might be invaded from China (Rahman et al., 2012). In China, *R. shantungensis* was recorded as an important economic pest of various fruit plants and roadside trees in Zhejiang province, China (Chou et al., 1985). In Korea, the host of *R. shantungensis* was known as 138 species belonging to 62 families including chestnut, peach, persimmon, apple, black locust, Japanese angelica tree, snowbell, pussy willow, paper mulberry, silk tree, tree of heaven, Japanese cornelian cherry, and so on (Kim et al., 2015). Broad host range and shortage of natural enemies for *R. shantungensis* allow this pest to spread out quickly.

In Korea, the populations of *R. shantungensis* was first discovered at the forest and orchard areas in Gongju and Yesan of Chungcheong-do in 2010 (Jo, 2014). In 2011, the populations spread out southwardly to Jeonra-do and northwardly to Gyunggi-do (Jo, 2014). Small number of *R. shantungensis* was found in Eastern parts of Gyung-sang-do in 2013 (Jo, 2014), and then the number was increasing and slowly spreading toward middle parts of Gyung-sang-do (Kang et al., 2016). The populations of *R. shantungensis*

already spread out to all Korean areas except for the Eastern part of Korea, and are still spreading. Especially, the occurrence of *R. shantungensis* at agricultural areas in 2017 was increased by 109.6 % compared to 2016 (Hong et al., 2017). Therefore, the demands of information on current and future distribution of *R. shantungensis* are increasing due to its increasing economic importance.

Information on current and future nation-wide distribution of *R. shantungensis* is important and valuable for developing its management policy. However, information on distribution of *R. shantungensis* in local fields would be more helpful for local farmers. The site-specific pest management, which can be defined as management of pests based on localized insect density within a field rather than average densities throughout the area (Park et al., 2007), is worthy for considering for control of *R. shantungensis*. The site-specific management is theoretically possible because most insects are spatially aggregated within fields (Davis, 1994). Therefore, it is need to know whether the distribution of *R. shantungensis* is aggregated in a field, or not.

There has been a few studies to develop environment friendly pesticides for management of *R. shantungensis* instead of pesticides (Jeon et al., 2016; Kim et al., 2016a; An et al., 2017; Choi et al., 2017). In 2017, egg

parasitoids of *R. shantungensis* were discovered in 14 regions of Korea, and suspected as a species in *Telenomus* genus (Kim et al., 2017b). These environment friendly pesticides and natural enemies were not actively used yet because most of these pesticides were not tested at field conditions, the mass-rearing techniques of natural enemies were not developed, and farmers want quick and cheap management tactics to control *R. shantungensis*. Therefore, the management of *R. shantungensis* mainly depends on pesticides. The active ingredients of registered pesticides for *R. shantungensis* are currently Acetamiprid, Dinotefuran, Imidacloprid, Etofenprox, Deltamethrin, Sulfoxaflor, Bifenthrin, and Abmectin (Kim et al., 2016b). Among them, Acetamiprid, Dinotefuran, Imidacloprid, and Etofenprox showed more than 90% lethality (Kim et al., 2016b). In these conditions, development of the strategies of applying the site-specific management in a large agricultural field such as chestnut producing areas should be required to increase the efficiency of management and decrease the side effects of pesticides.

To increase the management efficiency, the optimal application timing is also important. In Korea, the first instar nymphs of *R. shantungensis* begin to occur in the early May (Kim et al., 2016a). Once *R. shantungensis* become late instar nymphs, it is difficult to control because they actively evade the pesticides and their habitats are not limited to arboreal plants (Choi

et al., 2016a). Adults of *R. shantungensis* occur from the middle of July through five moltings from the first instar (Kim et al., 2016a). Adult stage of *R. shantungensis* is not also optimal developmental stage for the management because the mobility of the adult stage is highest among all developmental stages. Even though adults occur from the middle of July in Korea, their oviposition was observed from the early August (Kim et al., 2016a). Adults generally lay eggs inside cracks, which are made by their legs, of newly developed twigs (Kim et al., 2016a; Kim et al., 2017a) by covering their eggs with mother made wools. Thus, eggs are protected not only from environmental factors such as rain, snow, cold, and so on but also pesticides. *R. shantungensis* overwinters as eggs (Kim et al., 2016a). Therefore, the optimal control timing is very limited to the period when eggs are hatching to the instar nymphs which are aggregated with limited movement activity. In this regard, the occurrence model of the first instar nymphs of *R. shantungensis* (i.e., egg hatching model of *R. shantungensis*) should be developed and applied for the control of *R. shantungensis*.

The purposes of this study were (1) to predict current and future distribution of *R. shantungensis* in Korea, (2) to estimate the optimal application timing of pesticides for *R. shantungensis*, and (3) to figure out the distribution characteristics of *R. shantungensis* within a field to develop

efficient management tactics of *R. shantungensis*.

Chapter I.

Current and future distribution of *Ricania shantungensis* in Korea: Application of spatial analysis to select relevant environmental variables in MaxEnt and CLIMEX modeling

1-1. Abstract

Since the first report on the occurrence of *Ricania shantungensis* in Korea, it has quickly spread, and its associated damage has been exponentially increasing. This study was conducted to predict the potential habitat, current and future distribution of *R. shantungensis* in Korea using CLIMEX and MaxEnt, and to suggest a new parameter selection method in both of these modeling programs. The weights of both of the variables used in CLIMEX and those in MaxEnt were determined by using the spatial association indices of SADIE. The Climate Matching in CLIMEX compared the weather data of Zhejiang in China and that of all Korean territory. MaxEnt was applied and evaluated with 295 presence and absence data of *R. shantungensis* and eight environmental variables preselected by spatial and correlation tests. In the MaxEnt, the maximum temperature of the warmest month, annual mean temperature, mean temperature of the coldest month, and precipitation of the driest month were determined to be the most important variables affecting *R. shantungensis* distribution in Korea. The results of this study indicated that *R. shantungensis* had a higher occurrence possibility in western areas than eastern areas in Korea, but a high potential to spread eastward. These results are expected to be helpful for managing *R.*

shantungensis in Korea and selecting relevant environmental variables for species distribution modeling.

Key words: *Ricania shantungensis*, CLIMAX, MaxEnt, SADIE, species distribution model

1-2. Introduction

Since *Ricania shantungensis* was first reported in Gongju and Yesan of Chungcheong-do in 2010 in Korea, this insect pest has quickly spread southward to Jeonla-do and northward to Gyunggi-do (Jo, 2014), and is now slowly spreading toward the middle parts of Gyungsang-do (Kang et al., 2016). Overall, the population of *R. shantungensis* in agricultural areas has increased by over 100 % each year from 2015 to 2017, which has led to serious economic damage (Hong et al., 2017). This pest directly causes damage from sucking and egg-laying, and indirectly induces sooty mold disease on the leaves by its excretions (Jo, 2014). Accordingly, there has been an increasing demand for knowledge on the current and future distribution of *R. shantungensis* in order to determine how environmental conditions influence its occurrence and improve the management efficiency for the species. There has been a report (Kim et al., 2015) on the habitat suitability of *R. shantungensis* in Korea estimated using MaxEnt (Maximum Entropy Model), in which predicted habitats could explain the distribution of *R. shantungensis* at that time. However, the current distribution of *R. shantungensis* appeared to be mismatched with their prediction in newly spread areas of Korea, and they did not explain how the variables selected in

the model affected its occurrence. Moreover, the future distribution of *R. shantungensis* has not been predicted until now.

MaxEnt estimates the distribution of a species by finding the distribution, which is closest to the geographically uniform distribution, determined by the environmental characteristics of its presence locations at given environmental variables (Phillips et al., 2006). The characteristics of MaxEnt are use of presence and pseudo-absence data, determination of environmental and/or biological factors related to target species' distribution, and correlative parameter selection process (Phillips et al., 2006; Elith et al., 2011; Pearson, 2018). The correlative parameter selection process inevitably leads to major criticisms: ecologically unrelated variable selection with target species distribution (Zeng et al., 2016), relatively low prediction possibility in spreading species (Elith and Leathwick, 2009), and highly biased results at unsampled areas (Pearson et al., 2007).

Due to inherent issues of correlative methods for species distribution models (SDMs), deterministic methods are also popularly used for SDMs. Among deterministic methods, CLIMEX is one of the most popular due to their good prediction of target species' distribution and relative ease of use (Sutherst et al., 2007; Seo et al., 2008; Jung et al., 2016). CLIMEX can estimate the potential distribution and risk of a species in relation to climate by using the deterministic method, which requires pre-determined parameters

regardless of the target species distribution in the areas of interest, which is the opposite of the parameter selection process in MaxEnt (Sutherst et al., 2007). CLIMEX is composed of CLIMEX Model and Climate Matching (Sutherst et al., 2007; Jung et al., 2016). Both CLIMEX Model and Climate Matching in CLIMEX requires biological information to determine the model parameters and the weights of the parameters, respectively (Sutherst et al., 2007). For many invasive species, their biological information is limited because they have not been studied well because they are often less economically important in their areas of origin. This is likely to be the case for *R. shantungensis*. There is no known detailed information on the distribution of *R. shantungensis* except for in Korea to estimate the CLIMEX Model parameters and only limited information is available for determining the parameters themselves. However, Climate Matching requires relatively less information such as the climate information of the areas of origin and interest, and the weights of the model parameters compared to CLIMEX Modeling (Sutherst et al., 2007). The origin of *R. shantungensis* was assumed to be Zhejiang or Shandong provinces in the eastern coastal areas of China (Kwon et al., 2017). However, *R. shantungensis* has been recorded and managed as an important economic pest in only Zhejiang province (Chou et al., 1985). Thus, the occurrence suitability of *R. shantungensis* in Korea could

be estimated by comparing the climate information of Zhejiang province of China and the areas of Korea.

Climate matching in CLIMEX could provide potential habitats of *R. shantungensis* in Korea, but may not exactly match the actual distribution of *R. shantungensis* because the model prediction is only determined by climate factors, and the model predicts its potential habitats using the deterministic process. By contrast, MaxEnt evaluates both climate and non-climate factors, and selects correlative variables with its distribution. Thus, the distribution predicted by MaxEnt should be well matched with the actual distribution even though the reliability of its selected parameters remain to be proven. In other words, prediction by CLIMEX and MaxEnt is closed to the fundamental and realized niches of a target species, respectively (Zeng et al., 2016). Therefore, the results of CLIMEX could be helpful by predicting spreading directions of *R. shantungensis* in Korea from the current distribution expected from MaxEnt in the near future. Moreover, both results CLIMEX and MaxEnt could minimize drawbacks of each modeling process and support each other by considering the ecological aspects from CLIMEX and current distribution from MaxEnt for *R. shantungensis* if both CLIMEX and MaxEnt are applied together even though both models have no standardized process for determining the relevant variables in MaxEnt and the weights of the parameters in CLIMEX, respectively.

Therefore, this study was conducted to evaluate the potential habitat of *R. shantungensis* with CLIMEX, to predict the current distribution of presence possibility of *R. shantungensis* with MaxEnt, to suggest a method to select parameters in MaxEnt and parameters' weights in CLIMEX, and finally to predict the future distribution of presence possibility of *R. shantungensis* in Korea.

1-3. Materials and Methods

1-3-1. Data collection for presence and absence of *R. shantungensis*

In order to collect data on the presence and absence of *R. shantungensis*, Korea was divided into 30 km by 30 km grids, and at least one location point per each grid was surveyed for the occurrence of *R. shantungensis* to minimize unsampled areas (Fig. 1). First, presence data were obtained from previously published data (Kim et al., 2015), as well as unpublished reports in Gyunggi and Chungnam Agricultural Research and Extension Services in 2017. Then, the grid locations where the presence of *R. shantungensis* were unchecked were surveyed in 2017. The survey was mainly conducted along the rest areas of the highways within a grid because *R. shantungensis* had been mainly found first at rest areas of the highways in newly invaded areas (Kim et al., 2015). If the rest areas were not present within a grid, then major host plants (i.e., *Robinia pseudoacacia*, *Diospyros kaki*, *Castanea* spp., *Prunus serrulata*, and so on) at temporary parking available places abutting forested areas were randomly observed within the grid. The absence of *R. shantungensis* was determined after checking at least more than 20 trees. Whole branches within observers' reach were observed in each tree. In total, 295 data points (149 previously reported points and 146

newly surveyed points) were collected, and *R. shantungensis* was found at 175 points among them (Fig. 1).

1-3-2. Environmental, Traffic, Footprint, and Landcover data collection

Numerical climate data composed of monthly temperatures (i.e., maximum, minimum, and mean) and precipitation of 1981-2010 in 73 meteorological stations were obtained from the Korea Meteorological Administration (KMA) (<http://www.kma.go.kr>). The climatic data were interpolated throughout all of Korea by using inverse distance weighting with an option of estimating a point value with the nearest five meteorological station data. The estimated monthly temperature and precipitation data were transformed to 19 bioclimatic variables format of WorldClim (<http://www.worldclim.org>) in DIVA-GIS 7.5 (Hijman et al., 2018). All of the variables were created in the same resolution (i.e., 1 km by 1 km) and file format (i.e., ASCII) by considering resolution of projected map and environmental data availability. Future climate data were also downloaded from the KMA, and the data were developed by the RCP (Representative concentration pathway) 8.5 scenario for temperature and precipitation based on historical data (1986-2005) in Korea. In this study, the 2030s (2031-2040), 2050s (2051-2060), 2070s (2071-2080), and 2090s (2091-2100) climate data

(i.e., temperature and precipitation) of RCP 8.5 were used to estimate the future distribution of *R. shantungensis* in Korea.

The digital elevation model (DEM) was downloaded from the website of the National Spatial Data Infrastructure Portal in Korea to create elevation data (<http://www.nsd.go.kr>). Because these elevation data were created at a different resolution (i.e., 0.8 km by 0.8 km) unlike 19 bioclimatic variables, the data were resampled by the numeric value of the nearest neighbor cell by using the resample tool in ArcGIS 10.1 (ESRI; Redlands, CA, USA).

The traffic data were downloaded from the website of the Traffic Monitoring System in Korea (<http://www.road.re.kr>). For analysis, the downloaded traffic amount at 3,477 points was interpolated with ordinary Kriging and mapped in the ASCII file format with a 1 km by 1 km resolution in ArcGIS 10.1. Footprint data were also downloaded from the website of NASA's Earth Observing System Data and Information System (<http://earthdata.nasa.gov>). Worldwide data were clipped with the mask of Korean territory in ArcGIS 10.1.

Landcover data, furnished by Environmental Geographic Information in Korea, was downloaded (<http://egis.me.go.kr>) and resampled by the attribute value with the largest area within a 1 km by 1 km cell in

ArcGIS 10.1 because the resolution of the downloaded data was much higher (i.e., 5 m by 5 m) than that of the other variables.

In total, 23 variables (Table 1) including 19 bioclimatic variables were prepared to describe the current distribution of *R. shantungensis* in Korea.

1-3-3. Spatial association between environmental variables and distribution of *R. shantungensis*

In order to accurately predict the distribution of *R. shantungensis* in Korea, the variables selected for modeling should be biologically related with *R. shantungensis*. If the distributions of the selected variables are spatially related with the distribution of *R. shantungensis*, the possibilities of biological relationships between *R. shantungensis* and its surrounding environmental variables are expected to be increased (Baek, 2015). Thus, spatial analysis by distance indices (SADIE) (Perry, 1995) was used to measure the spatial associations between the occurrence data of *R. shantungensis* and 22 environmental variables. The Landcover variable could not be applied for spatial analysis because it was in the form of categorical data. SADIE quantifies the contribution of counts at each location to a patch (i.e., a region of relatively large counts close to one another) and a gap (i.e., a region of relatively small counts close to one another) (Perry and Dixon, 2002). For the

spatial association test in SADIE, the variables compared should have coincident coordinates, and all data should have clustering indices (i.e., degree of contribution at each location) (Perry and Dixon, 2002). To match the coordinates of each point of *R. shantungensis* occurrence data and the 22 variables, point data were extracted at the middle points of each grid (Fig. 1) in ArcGIS 10.1. For this process, the krigged map for *R. shantungensis* occurrence was used. The spatial indices of each point of 23 sets were estimated in SADIE. The overall spatial association (X) is the mean of the local correlation coefficient between the clustering indices of the two sets; $X > 0$ for a positive spatial association, $X = 0$ for no spatial association, $X < 0$ for a negative spatial association (Perry and Dixon, 2002); positive X values indicate the coincidence of a patch cluster for one set with a patch cluster for the other or the coincidence of two gaps and negative X values are indicated by a patch coinciding with a gap (Perry and Dixon, 2002). The associated possibility (P) was also calculated based on randomization tests (Perry and Dixon, 2002). The null hypothesis is that the spatial arrangement of the count data between two data sets is random (Park et al., 2006). In this study, $X > 0$ indicates a positive spatial association, $X = 0$ indicates no spatial association, $X < 0$ indicates a negative spatial association between *R. shantungensis* occurrence and its surrounding environments. All of the SADIE statistics

were calculated using SADIEShell version 1.22 (Rothamsted Experimental Station, Harpenden, Herts, UK).

1-3-4. Prediction of habitat suitability of *R. shantungensis* with CLIMEX

The Climate Matching function in CLIMEX predicts the habitat suitability of a target species in a country of interest (i.e., Korea in this study and Away in CLIMEX) by comparing the climate of the origin area (i.e., Zhejiang in this study and Home in CLIMEX) with that of Away (Sutherst et al., 2007). Metadata provided by the CLIMEX program and 30 years (1981-2010) climate data from the KMA were used for the climate data of Zhejiang in China and Korea, respectively. The similarity level of climates between Home and Away was determined by the Composite Match Index (CMI), which is calculated using seven components (i.e., Maximum temperature (I_{tmax}), Minimum temperature (I_{tmin}), Average temperature (I_{tav}), Total rainfall (I_{rain}), Rainfall pattern (I_{rpat}), Relative humidity (I_{hum}), and Soil moisture (I_{smst}) and the individual weight of each component (Sutherst et al., 2007). The closer to 1 from 0 the CMI value is, the more similar to the Home area the climates of Away are (Jung et al., 2016).

Among the seven components, only the five related with temperature and precipitation were used for Climate Matching in this study. Between I_{tav} and I_{tmin} , I_{tav} was selected and used for the analysis because I_{tav} was highly

correlated (correlation coefficient (r) > 0.9) with I_{\min} and the X value of I_{tav} in the spatial association tests was higher than I_{\min} (Table 2). The weights of the four remaining variables were determined by using the X values of the related environmental factors in the spatial association tests between the environmental factors and *R. shantungensis* occurrence as follow:

$$\text{Weight of } I_{\text{tav}} = [X_{\text{Bio1}} / (X_{\text{Bio1}} + X_{\text{Bio5}})] * [\{(X_{\text{Bio1}} + X_{\text{Bio5}}) / 2\} / \{(X_{\text{Bio12}} + X_{\text{Bio13}} + X_{\text{Bio14}} + X_{\text{Bio16}} + X_{\text{Bio17}} + X_{\text{Bio18}} + X_{\text{Bio19}}) / 7\}] \quad (1)$$

$$\text{Weight of } I_{\text{max}} = [X_{\text{Bio5}} / (X_{\text{Bio1}} + X_{\text{Bio5}})] * [\{(X_{\text{Bio1}} + X_{\text{Bio5}}) / 2\} / \{(X_{\text{Bio12}} + X_{\text{Bio13}} + X_{\text{Bio14}} + X_{\text{Bio16}} + X_{\text{Bio17}} + X_{\text{Bio18}} + X_{\text{Bio19}}) / 7\}] \quad (2)$$

$$\text{Weight of } I_{\text{rain}} = [X_{\text{Bio12}} / \{X_{\text{Bio12}} + (X_{\text{Bio13}} + X_{\text{Bio14}} + X_{\text{Bio16}} + X_{\text{Bio17}} + X_{\text{Bio18}} + X_{\text{Bio19}}) / 6\}] * [\{(X_{\text{Bio12}} + X_{\text{Bio13}} + X_{\text{Bio14}} + X_{\text{Bio16}} + X_{\text{Bio17}} + X_{\text{Bio18}} + X_{\text{Bio19}}) / 7\} / \{(X_{\text{Bio1}} + X_{\text{Bio5}}) / 2\}] \quad (3)$$

$$\text{Weight of } I_{\text{rpat}} = [\{X_{\text{Bio12}} + (X_{\text{Bio13}} + X_{\text{Bio14}} + X_{\text{Bio16}} + X_{\text{Bio17}} + X_{\text{Bio18}} + X_{\text{Bio19}}) / 6\} / X_{\text{Bio12}}] * [\{(X_{\text{Bio12}} + X_{\text{Bio13}} + X_{\text{Bio14}} + X_{\text{Bio16}} + X_{\text{Bio17}} + X_{\text{Bio18}} + X_{\text{Bio19}}) / 7\} / \{(X_{\text{Bio1}} + X_{\text{Bio5}}) / 2\}] \quad (4)$$

where, $X_{\text{Bio}i}$ indicates the X value of bioclimatic factor $\text{Bio}i$ in the spatial

association test with *R. shantungensis* occurrence. Bio1, Bio5, Bio12, and Bio13, 14, 16, 17, 18, 19 were related with I_{tav} , I_{tmax} , I_{train} , and I_{rpat} , respectively. These bioclimatic factors were not only related with the components of the Climate Matching in CLIMEX but also spatially associated with *R. shantungensis* occurrence. From these processes, the actual weights used of each component were 0.61, 0, 0.39, 0.48, 0.49, 0, and 0 for I_{tmax} , I_{tmin} , I_{tav} , I_{rain} , I_{rpat} , I_{hum} , and I_{smst} , respectively.

1-3-5. Prediction of current distribution for *R. shantungensis* with MaxEnt

Because MaxEnt requires presence data (Phillips and Dudík, 2008), of the 295 data points, 175 presence data points were used for prediction. The 132 data points from the new field survey were used as training data and the other (from the published paper) 43 data were used as test data because the selected parameters should explain both the past (i.e., test in this study) and current data (i.e., training in this study) to avoid a criticism of MaxEnt (i.e., suitable for species of equilibrium status (Elith and Leathwick, 2009)). Thus, the test data in this study were not used to evaluate model performance, but were used to select and exclude unrelated variables with the presence of *R. shantungensis* in Korea. In the average nearest neighbor test in ArcGIS 10.1, the training data showed significantly ($P < 0.01$) clustered pattern which

could cause overfitting problems (Kramer-Schadt et al., 2013) against environmental variables. Thus, by reducing the spatial cluster of the presence data of *R. shantungensis*, spatial filtering (Boria et al., 2014) was executed by expected mean distance (0.138124 decimal degrees) in the average nearest neighbor test (hypothetical random distribution in interest region) using tool ‘spatially rarefy occurrence data’ in the SDM toolbox in ArcGIS 10.1. Ultimately, 72 points among 132 occurrence points were used as training data for MaxEnt modelling, and 43 points were used for test data.

In order to eliminate multi-collinearity variables, which can lead to overfitting problems (Dormann et al., 2013) or ambiguous interpretation, the correlation test was executed in ArcGIS 10.1. In the case that the correlation coefficient (r) was greater than 0.9 ($r > 0.9$, $r < -0.9$), only one variable was used for further analyses among the pairs of correlated variables by considering the X values of the spatial association tests among correlated variables (Table 2). Through these procedures, 13 variables (i.e., Bio1, Bio2, Bio3, Bio5, Bio6, Bio7, Bio12, Bio14, Bio17, Bio18, Footprint, Traffic volume, and Landcover) among of the original 23 were preferentially selected. To avoid another criticism of MaxEnt (i.e., variable selection irrelevant with target species’ distribution due to MaxEnt’s processor) (Zeng et al., 2016), spatially associated variables (i.e. Bio1, Bio5, Bio6, Bio12, Bio14, Bio17, Bio18, and Landcover) were used for MaxEnt modeling.

MaxEnt software version 3.4.1 (Phillips et al., 2018) was initially executed with default parameter settings (e.g., feature type, regularization multiplier, and 10,000 background points) by using training data, test data and eight pre-selected variables. In all executions of MaxEnt modelling, the jackknife test was conducted to measure the variable importance and exclude unrelated variables. Variables were excluded from the model in two conditions so as to reduce model complexity and eliminate relatively unrelated variables: First, the value of test gain was less than zero. Second, the gain value without one variable was higher than that in the case where all variables were used. These processes were repeated until there was no variable to be discarded. Through these stepwise procedures, seven variables (i.e., Bio1, Bio5, Bio6, Bio12, Bio14, Bio 17, and Bio18) were finally selected, and the presence probability model for *R. shantungensis* was built.

1-3-6. Comparison between expected occurrence by MaxEnt and actual occurrence of *R. shantungensis*

In order to compare the absence of *R. shantungensis* between the observed and predicted values by the MaxEnt model, the predicted values of the MaxEnt model were extracted with 120 observed points (i.e., observed absence points in this study) in ArcGIS 10.1. The AUC score of MaxEnt provides predictive ability of developed model. However, actual absence

points of *R. shantungensis* in Korea were aggregated in western parts and not used for the MaxEnt modeling process. Thus, the absence data need to be evaluated to verify the prediction reliability of suggested MaxEnt model in this study. If the extracted value were more than 0.5 at one point, the occurrence of *R. shantungensis* was considered as presence at that point. The prediction abilities of the MaxEnt model for observed occurrence were statistically compared to random possibility (i.e., 50 %) using Chi-square test (SAS institute, 2011).

1-3-7. Prediction of future distributions of *R. shantungensis* in Korea

MaxEnt was also used to predict the future probability of *R. shantungensis* presence in Korea with the seven selected variables (i.e., Bio1, Bio5, Bio6, Bio12, Bio14, Bio 17, and Bio18) of the RCP 8.5 climate change scenario at the 2030s (2031-2040), 2050s (2051-2060), 2070s (2071-2080), and 2090s (2091-2100).

1-4. Results

1-4-1. Habitat suitability of *R. shantungensis* with CLIMEX

In Korea, the CMI values were higher than 0.7, indicating that the climatic conditions in all locations of Korea were similar to those of Zhejiang in China. However, the similarity of climatic conditions was relatively lower in the eastern part than in the western part of Korea, where *R. shantungensis* mainly occurred (Fig. 2).

1-4-2. Prediction of current and future distribution of *R. shantungensis* with MaxEnt

The AUC score (i.e., 0.78) of the training data in the MaxEnt model in this study was larger than 0.75, indicating reliable predictive ability (Fig. 3). The AUC score (i.e., 0.79) of the test data was slightly higher than that of the training data. The contributions in this model were 41.2, 15.9, 15.7, 12.6, 7.1, 5.5, and 2.0 % for Bio5, Bio1, Bio6, Bio14, Bio18, Bio 17, and Bio2, respectively. According to the response curves for the variables related to the presence of *R. shantungensis* in Korea, each variable affected the presence possibility of *R. shantungensis* in a different way (Fig. 4): The Maximum temperature of the warmest month (i.e., Bio5) showed a generally sigmoidal

effect. The Minimum temperature of the coldest month (i.e., Bio6) showed a sigmoidal effect until -6 °C, a negative effect between -6 °C and 2 °C, then an exponential effect after 2 °C. The precipitation of the warmest quarter (i.e., Bio18) showed two peaks around minimum and 825mm. The precipitation of the driest month (i.e., Bio 14) showed endurance at a certain level of precipitation, and then an exponential effect, but the precipitation of the driest quarter (i.e., Bio 17) showed a negative effect. Both the annual mean temperature (i.e., Bio1) and annual precipitation (i.e., Bio12) were favorable for the occurrence of *R. shantungensis* in Korea by maintaining approximately 61% occurrence possibility.

The correctly predicted rate of MaxEnt in actually absent points was 86.7 % by matching 102 points among 120, which was statistically ($\chi^2 = 64.5$, $P < 0.001$) meaningful. The future presence probability maps of *R. shantungensis* in Korea were also developed with MaxEnt by using the 2030s, 2050s, 2070s, and 2090s climatic data under the RCP 8.5 scenario (Fig. 5). In the future distribution of *R. shantungensis*, Gyung-sang-do and Gangwon-do were expected to have high occurrence possibility. Currently, *R. shantungensis* were newly found in only few points in Gyung-sang-do and Gangwon-do in the survey of 2017.

1-5. Discussion

This study predicted the potential habitat suitability and current distribution possibility of *R. shantungensis* in Korea using CLIMEX and MaxEnt. Both models generally well described the observed distribution pattern of *R. shantungensis* in Korea. Accordingly, spatial analysis could be a considerable tool for finding reliable variables to explain the distribution of a target species. Moreover, this study forecasted the future occurrence of *R. shantungensis* and found the relevant environmental variables with the occurrence of *R. shantungensis* in Korea. These results would be helpful for preparing management strategies for *R. shantungensis* and national long-term agricultural plans in Korea.

The predicted current distribution of *R. shantungensis* in this study was generally similar to that of the previous study (Kim et al., 2015), but showed opposite results in a few areas (e.g., Gangwon-do parts bordering with Gyunggi-do, eastern parts in Gyeongsang-do, northern parts of Gyunggi-do, etc.) despite the fact that both studies used the same program (MaxEnt) and similar environmental variables. These areas were newly invaded areas by *R. shantungensis* within Korea after the study (2015) of Kim et al. These differences could potentially be caused by the correlative parameter selection

process of MaxEnt (Elith and Leathwick, 2009). The MaxEnt model for this spreading insect could have the high possibility of non-relevant variable selection caused by the correlative modeling process in MaxEnt (Zeng et al., 2016). Kim et al. (2015) reported that the precipitation and mean temperature of the warmest quarter, forest type, and landcover were the most important variables affecting *R. shantungensis*. These variables could predict *R. shantungensis* well at that time, but failed to accurately describe its distribution in 2017. Moreover, Kim et al. (2015) did not explain why these variables were selected and how these variables affected the distribution of *R. shantungensis*. However, the important variables in this study, i.e., maximum temperature of the warmest month, annual mean temperature, mean temperature of the coldest month, and precipitation in the driest month, could explain the past data of Kim et al. (2015) and the current data, indicating strong relationship of these variables with the occurrence of *R. shantungensis*. Thus, the results of this study will be more reliable for predicting the presence possibility of *R. shantungensis* in Korea.

The correlative models including MaxEnt have the greatest advantage of having a high matching rate between the observed and predicted data as compared to the models using deterministic processors. However, there are also inevitable criticisms of correlative models (Pearson et al., 2007; Elith and Leathwick, 2009; Zeng et al., 2016). This study attempted to

minimize the disadvantages of MaxEnt. By using spatial analysis in the variable selection processes, only variables that were relevant to *R. shantungensis* occurrence were used in the modeling process. Moreover, it was shown that the selected variables and their parameters could explain its occurrence in both the past and present by using two separate sets of data even though *R. shantungensis* is still spreading in Korea (Jo, 2014; Kang et al., 2016; Hong et al., 2017). There were multiple studies (Braunisch et al., 2013; Bradie and Leung, 2017; Petitpierre et al., 2017) to find suitable variables related with occurrence of a target species. Bradie and Leung (2017) reviewed published results of MaxEnt, and found that temperature and precipitation among environmental variables were generally related with target species' occurrence. Petitpierre et al. (2017) concluded that the variables, a direct impact on species physiology and fitness, were related with species' occurrence from reviews of MaxEnt studies. Braunisch et al. (2013) suggested that variables related with target species occurrence should be selected in MaxEnt modeling even though they discussed the methods to select variables in case of uncertain relationship between species' occurrence and its environments. The selected variables in this study were also satisfied with the criteria of previous studies (Braunisch et al., 2013; Bradie and Leung, 2017; Petitpierre et al., 2017). This study also showed high accuracy, 86.7 %, at points where the data were not used in MaxEnt modeling. It is possible that

the model prediction could be increased by absence data (Brotons et al., 2004) even though there is the potential for false absence (Hirzel, 2002). However, the results of this study could also predict the presence and absence of *R. shantungensis* well. Finally, the results of CLIMEX, one of the deterministic models, also showed that the western parts of Korea were relatively more suitable for *R. shantungensis* than the eastern parts as did those of MaxEnt.

The results of this study indicated that seven environmental variables (i.e., Bio1, Bio5, Bio6, Bio12, Bio 14, Bio 17, and Bio18) were important for predicting the occurrence of *R. shantungensis*. It is reasonable to conclude that temperature-related variables (i.e., Bio1, Bio5, and Bio6) positively affect *R. shantungensis* occurrence because metabolic rate with increasing temperature was normally increased up to its upper lethal temperature, and this is reflected in the developmental rate in insects due to their ectodermal characteristic (Chapman, 1982). Elevation negatively correlated with Bio5 was also proven that elevation negatively affected the occurrence of *R. shantungensis* (Choi et al., 2017b). Kim et al. (2017b) reported that high humidity was helpful for increasing the survival of *R. shantungensis* eggs during winter. Thus, Bio14 and 17 would be expected to positively affect *R. shantungensis* occurrence in Korea, but long-term rain during winter was expected to negatively affect *R. shantungensis* occurrence in the MaxEnt modeling. Long-term and frequent rain during winter might cause death of

overwintering eggs due to its ecological characteristics; eggs are laid between cracks of branches and covered with wools made by their mothers. Precipitation during summer (i.e., Bio18) showed two peaks at low precipitation (i.e., 500 – 530 mm) as well as around 825 mm precipitation. These two peaks could be caused more by the frequency of heavy rain in Korea during summer rather than absolute amounts of precipitation because heavy rain could affect the flight activities and survival of adults of *R. shantungensis*.

The results of this study indicated that the maximum temperature of the warmest month was the most important variable for predicting the occurrence of *R. shantungensis* by occupying a 41.2 % contribution rate in the MaxEnt model. This month (i.e., August in Korea) is matched with the pre-ovipositional period of *R. shantungensis* in Korea. The female adults lay eggs inside cracks and cover their eggs with wool they make themselves (Jo, 2014). Moreover, they should find a suitable habitat for the development of their progenies. These oviposition behaviors of *R. shantungensis* are expected to consume a high energy. Thus, *R. shantungensis* adults may require significant feeding activity before oviposition, and a high temperature would be helpful for their activities and ovary development. The high energy requirement is also proven by its relatively long pre-oviposition period (i.e., over one month) by considering its body size and temperature (Jo, 2014). The

annual mean temperature (15.9 % in contribution rate) and precipitation (2% in contribution rate) were shown to constantly and positively contribute to *R. shantungensis* occurrence. This result is consistent with the CLIMEX results, which showed that all areas in Korean territory were suitable for the habitat of *R. shantungensis*. Both the mean temperature of the coldest month (15.7 % in contribution rate) and the precipitation of the driest month (12.6 % in contribution rate) are related with overwintering of *R. shantungensis*. These variables were proven to be highly related with the occurrence of *R. shantungensis* in MaxEnt and SADIE, as well as its ecology. Thus, the occurrence prediction of *R. shantungensis* would be enabled in other non-invaded countries by using these major variables related with its occurrence (Phillips and Dudík, 2008; Duque-Lazo et al., 2016; Petitpierre et al., 2017).

As an invasive species, the occurrence of *R. shantungensis* was expected to be highly related with human-related variables (i.e., Traffic and Footprint in this study) because insect spreading is generally correlated with human-related factors (Lockwood et al., 2007) and there was a report showing a high contamination rate of *R. shantungensis* at the rest areas of highways and roadsides (Kim et al., 2015). However, this study showed that these human-related variables were not significantly ($p < 0.05$) related with the occurrence of *R. shantungensis* in Korea. This could be related to the high mortality of *R. shantungensis* eggs in cut trees (Kim et al., 2017b). Kang et

al. (2016) also reported that *R. shantungensis* was slowly spreading toward the middle parts of Gyung-sang-do along the coastal areas of southern Korea even though the spreading direction was toward Busan-Si with very high Traffic and Footprint values. Thus, *R. shantungensis* would be expected to spread continuously except for in conditions of the transplanting of trees contaminated with *R. shantungensis*.

Even though rapid spreading would not be expected, the spreading of *R. shantungensis* to Gyung-sang-do appeared to be inevitable because the habitat suitability of this region for *R. shantungensis* in CLIMEX was high (i.e., CMI value > 0.7), and the distribution possibility of these areas in MaxEnt was the highest in Korea in the 2030s. Gyung-sang-do is economically and agriculturally important in Korea, and there are huge apple, persimmon, and chestnut export agricultural complexes, as these are known as good host plants for *R. shantungensis*. Thus, the management of *R. shantungensis* should be required to minimize the economic impacts and slow its spreading in Korea based on the current and 2030s' distribution possibility of *R. shantungensis*.

An increasing number of invasive species are expected to continue occurring internationally due to increased trades, transportation, and human activities, as well as global warming (Liebhold and Tobin, 2008). Thus, demands for species distribution models (SDMs) would be increased to

delineate current distribution, predict future distribution and habitat suitability, and identify the associations between occurrence and the surrounding environment related to the occurrence for a target species. For more reliable SDMs, the selection of variables should be related to the characteristics of a target species. As an example, spatial association analysis was applied in this study. The parameters selected by spatial association analysis lead to increased prediction accuracy and reliability for the occurrence of *R. shantungensis* in Korea. The results of CLIMEX and MaxEnt predicted that *R. shantungensis* had a higher occurrence possibility in western areas than eastern areas in current Korea, but a high potential to spread eastward in the future. These results will be helpful for developing relevant management strategies and national long-term agricultural plans in Korea.

Table 1. List of 23 variables including 19 bioclimatic variables

Variables	Unit	Range
Bio1 = Annual mean temperature	°C	2.6 – 16.9
Bio2 = Mean diurnal range (Mean of monthly (max temp. – min temp.))	°C	5.0 – 12.9
Bio3 = Isothermality (Bio2 / Bio7 * 100)	-	19.6 – 32.9
Bio4 = Temperature seasonality (SD * 100)	-	708 – 1,072
Bio5 = Max temperature of warmest month	°C	17.7 – 30.7
Bio6 = Min temperature of coldest month	°C	-18.8 – 4.1
Bio7 = Temperature annual range (Bio5 - Bio6)	°C	25.2 – 40.4
Bio8 = Mean temperature of wettest quarter	°C	13.0 – 25.4
Bio9 = Mean temperature of driest quarter	°C	-11.2 – 10.5
Bio10 = Mean temperature of warmest quarter	°C	13.0 – 25.9
Bio11 = Mean temperature of coldest quarter	°C	-11.2 – 8.3
Bio12 = Annual precipitation	°C	995 – 1,918
Bio13 = Precipitation of wettest month	mm	208 – 403
Bio14 = Precipitation of driest month	mm	15 – 47
Bio15 = Precipitation seasonality (CV)	-	59.5 – 108.0
Bio16 = Precipitation of wettest quarter	mm	557 – 1,023
Bio17 = Precipitation of driest quarter	mm	54 – 178
Bio18 = Precipitation of warmest quarter	mm	530 – 910
Bio19 = Precipitation of coldest quarter	mm	54 – 182
Elevation	m	-3 – 1,819
Traffic volume	ea	834 – 176,191
Footprint	-	12 – 100
Landcover	-	-

Table 2. Spatial association between 22 environmental variables and the presence possibility of *R. shantungensis* in Korea

Environmental variables	X^*	P
Bio 1	0.2466	0.0006
Bio 2	-0.0984	0.8373
Bio 3	-0.1692	0.9442
Bio 4	0.0413	0.3443
Bio 5	0.3881	< 0.0001
Bio 6	0.2491	0.0046
Bio 7	0.0498	0.3169
Bio 8	0.3461	< 0.0001
Bio 9	0.1235	0.1078
Bio 10	0.3812	< 0.0001
Bio 11	0.2293	0.0151
Bio 12	0.3011	0.0011
Bio 13	0.2142	0.0207
Bio 14	0.4088	< 0.0001
Bio 15	-0.016	0.5625
Bio 16	0.2688	0.0035
Bio 17	0.2937	0.0027

Bio 18	0.3835	< 0.0001
Bio 19	0.2715	0.0052
Elevation	-0.2611	0.9956
Traffic	0.0304	0.3830
Footprint	0.0833	0.2153

* Index of association (X) with its associated probability (P). For a two-tail test at 95% confidence level, $P < 0.025$ indicates significant positive association and $P > 0.975$ indicates significant negative association.

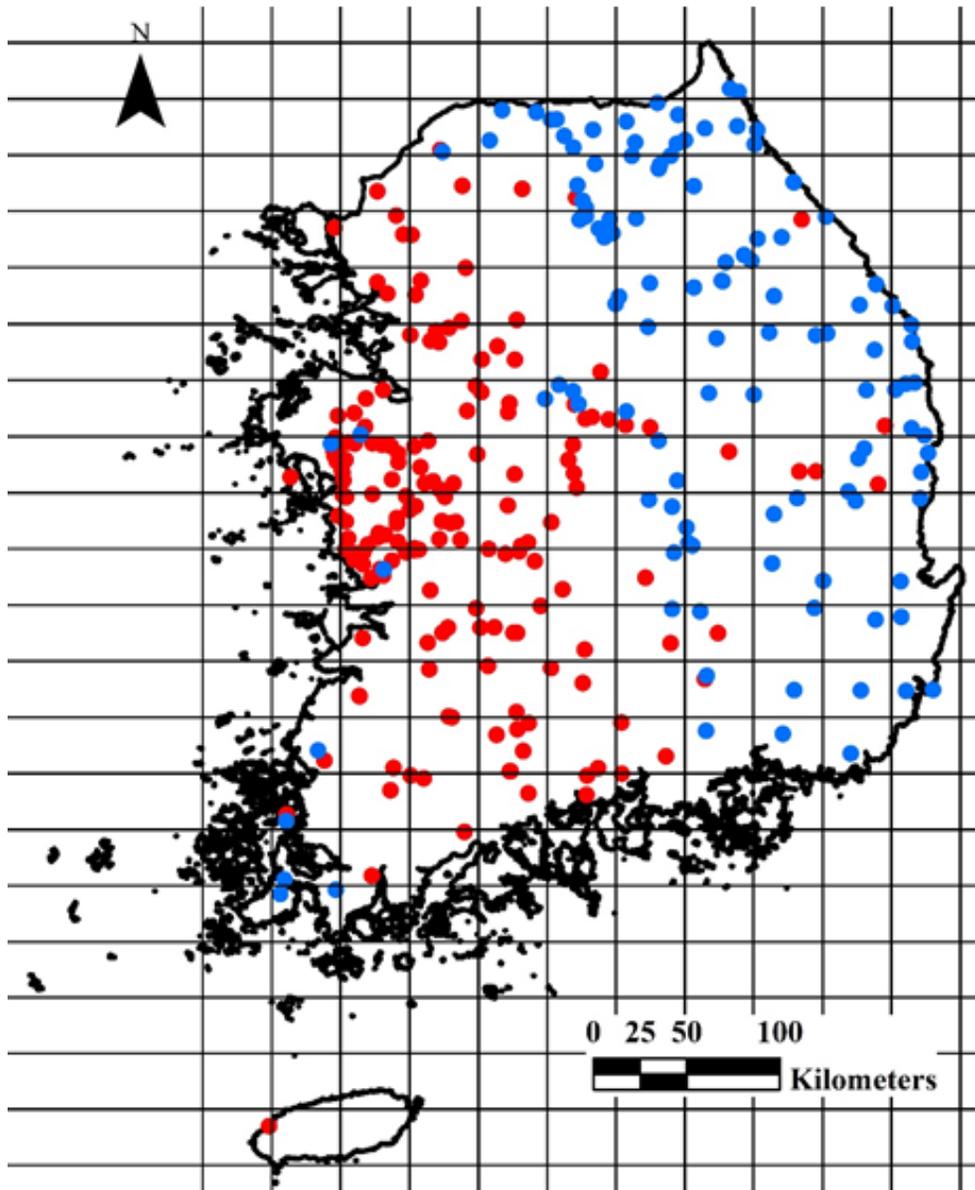


Fig. 1. Imaginary grids (30 km by 30 km) and observed points for *R. shantungensis* presence in Korea (●, Presence; ●, Absence)

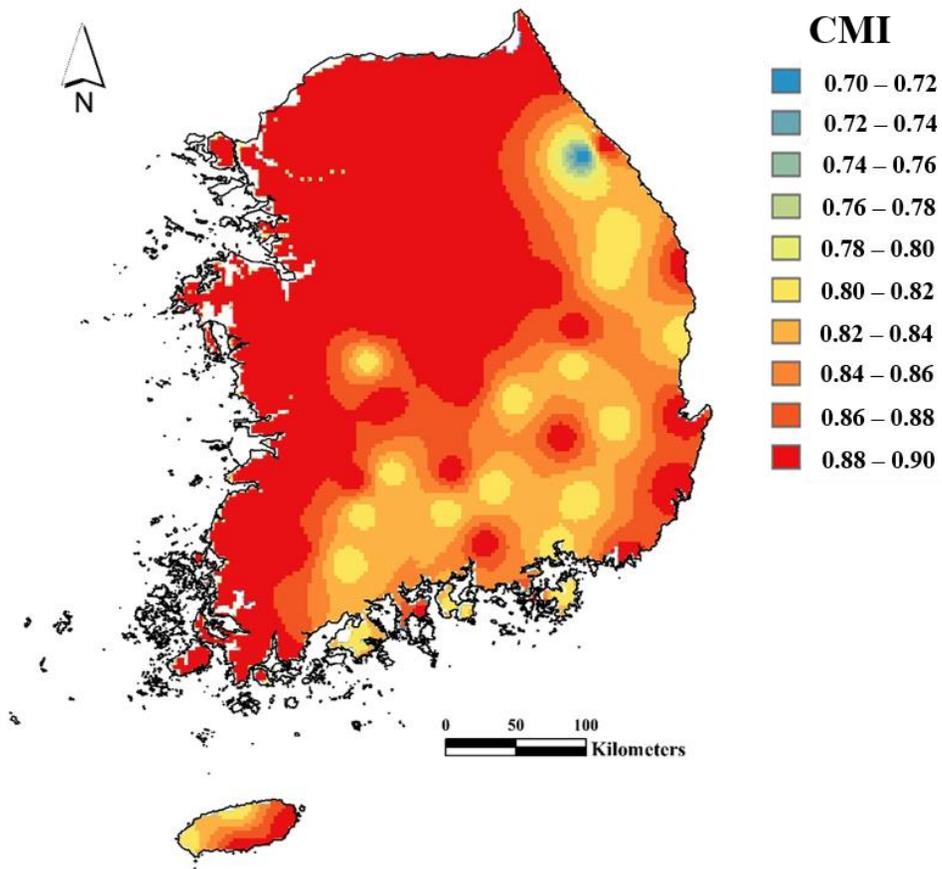


Fig. 2. Climate similarity (i.e. Climate matching index (CMI) in CLIMEX) map in Korea compared to one of the most favorable climatic conditions for *R. shantungensis* populations, Zhejiang in China. CMI > 0.5 indicates a suitable habitat for *R. shantungensis*.

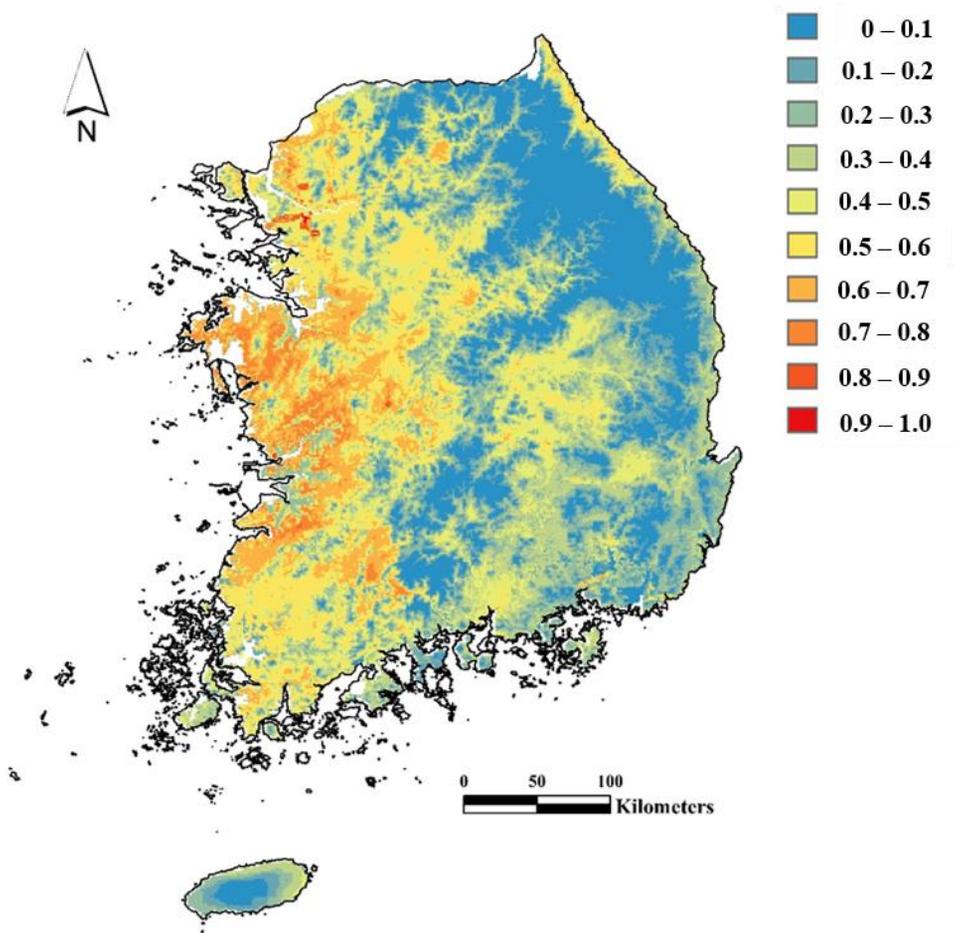
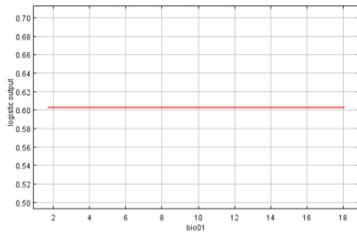
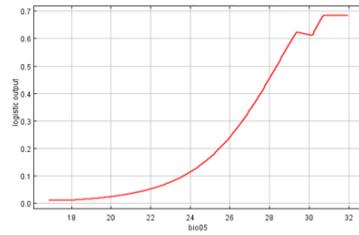


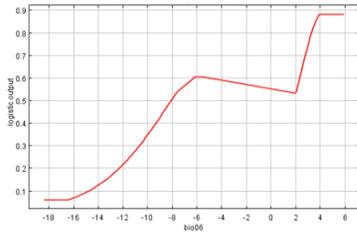
Fig. 3. Presence probability maps of *R. shantungensis* in Korea using MaxEnt



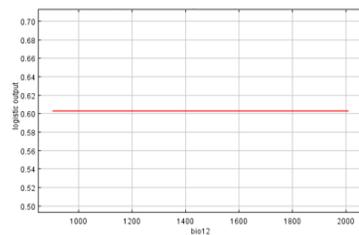
(a) Bio 1



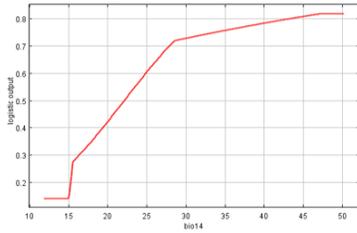
(b) Bio 5



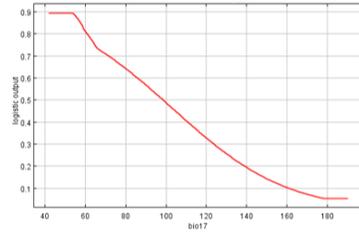
(c) Bio 6



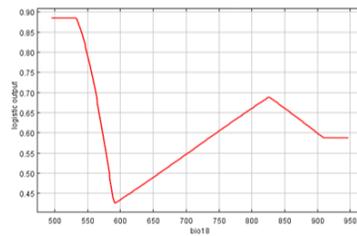
(d) Bio 12



(e) Bio 14



(f) Bio 17



(g) Bio 18

Fig. 4. Response curves for each variable related to the presence of *R. shantungensis* in Korea: (a) Bio 1; (b) Bio 5; (c) Bio 6; (d) Bio 12; (e) Bio 14; (f) Bio 17; (g) Bio 18.

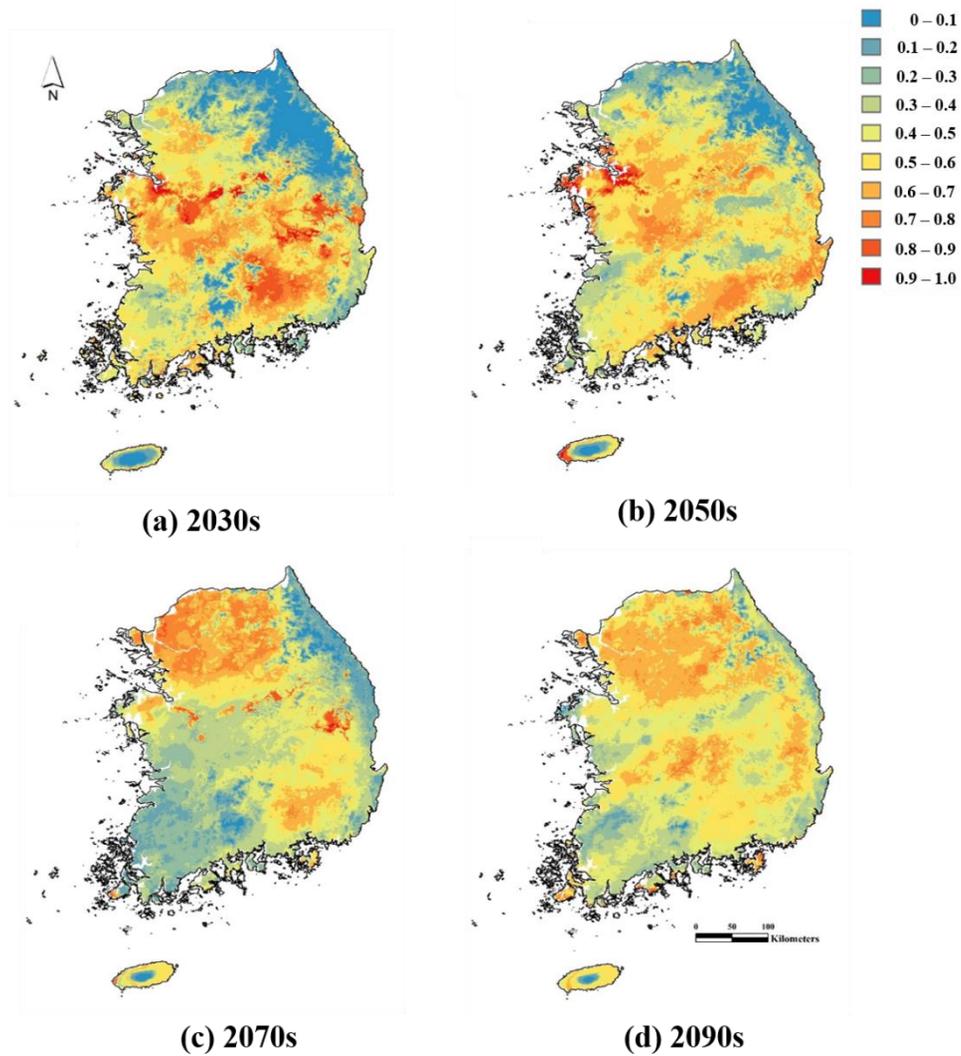


Fig. 5. Future presence probability maps of *R. shantungensis* in Korea using MaxEnt: (a) 2030s; (b) 2050s; (c) 2070s; (d) 2090s

Chapter II.

Occurrence model of first instars of *Ricania* *shantungensis*

2-1. Abstract

One of Ricaniid planthoppers, *Ricania shantungensis*, is an important pest in agriculture and forestry in Korea. Its most efficient control time is known to be when its first instar nymphs occur. Thus, the objective of the present study was to predict the occurrence of first instars of *R. shantungensis*. Effects of temperature on development and survival of *R. shantungensis* eggs were examined at seven constant temperatures (12.4, 16.4, 20.4, 24.8, 28.3, 32.4, and 36.9 °C). Development and survival of *R. shantungensis* eggs were quantitatively described by applying an empirical model as a function of temperature over a wide thermal range. Lower developmental threshold, thermal constant, optimal developmental temperature, and upper developmental threshold were estimated to be 12.1 °C, 202 DD, 31 °C, and 36.9 °C, respectively. Survivorship was the highest at 23.3 °C. The developed model well predicted field occurrences at three sites (Buyeo, Gwangyang, and Habcheon) in Korea. Therefore, results of this study are expected to increase the prediction accuracy of *R. shantungensis* occurrence and management efficiency of *R. shantungensis*.

Key words: *Ricania shantungensis*; egg development; egg survivorship; optimal management timing

2-2. Introduction

Efficient management of *Ricania shantungensis* is speculated to be possible only during a limited period due to its ecological characteristics. Adult stage of *R. shantungensis* has the highest mobility among all developmental stages. They can lay eggs on new developed branches of almost all broadleaf trees (Choi et al., 2016a; Kim et al., 2016). Nymphal stages are also active. Thus, they can easily evade pesticides. Their habitats include not only arboreal plants, but also herbaceous plants (Choi et al., 2016a). Their eggs are protected from surrounding environmental factors and pesticides because their eggs are covered with wools and laid between cracks, which are made by their mothers. Thus, the only optimal pesticide application period is when eggs are just hatched and when just hatched instars are aggregated around their egg masses.

Multiple studies have been performed to determine the timing of the first instar occurrence of *R. shantungensis* for its optimal management (Choi et al., 2012; Kang et al., 2013; Choi et al., 2016b). Choi et al. (2012) predicted that the lower developmental threshold and thermal requirement from eggs to first instars were 4.75 °C and 429.3 degree-days (DD), respectively. Choi et al. (2016b) suggested the lower developmental threshold and thermal requirement as 7.4 °C and 330.2 DD, respectively. Kang et al. (2013) did not

provide lower developmental threshold or thermal requirement in their study. However, lower developmental threshold and thermal requirement calculated from their data were 9.7 °C and 434.8 DD, respectively. Hosts of *R. shantungensis* were Japanese cornelian cherries and blueberries for studies of Choi et al. (2012 and 2016b) and Kang et al. (2013), respectively. These studies (Choi et al., 2012; Kang et al., 2013; Choi et al., 2016b) showed different lower developmental thresholds and thermal requirements from eggs to first instars of *R. shantungensis*. Especially, the studies of Choi et al. (2012) and Choi et al. (2016b) showed 2.6 °C difference in the lower developmental threshold of *R. shantungensis*, although their host plants were the same. Thus, the lower developmental threshold and thermal requirement from eggs to first instars of *R. shantungensis* need to be re-estimated and verified. Moreover, non-linear developmental model, upper developmental threshold, or survivorship model for *R. shantungensis* eggs has not been reported yet. These models are needed to decide habitat suitability of a location, project generation numbers locally and worldwide, and forecast its seasonal occurrence.

Even if these models are developed and verified, it is still difficult for crop growers to apply these results to *R. shantungensis* management. Results of this study could be easily visualized with a map for growers to decide the application timing of *R. shantungensis*. This expected 50 % occurrence map

in Korea might not exactly match with the actual occurrence of *R. shantungensis* in each grower field. However, it could help predict sampling timing and give enough time to prepare application of pesticides.

Therefore, this study was conducted (1) to develop linear, non-linear developmental, and survivorship models of *R. shantungensis* eggs, (2) to compare results of theoretical models with actual occurrence in the field, and (3) to provide an optimal application timing map of pesticides for *R. shantungensis* in Korea.

2-3. Material methods

2-3-1. Developmental and survivorship models of *R. shantungensis* eggs

R. shantungensis eggs were collected on April 10th, 2018 from a commercial chestnut field in Buyeo, Chungcheongnam-do, Korea. In most cases, branches with one or two egg masses were collected by cutting them with pruning shears to minimize confusion according to synchronized occurrence of first instars from eggs. Collected branches with *R. shantungensis* egg masses were cut obliquely to help water absorption and meet the length of clear plastic vials (2.85 cm diameter × 9.5 cm height) (Stockwell Scientific; Scottsdale, AZ, USA). Floral foams (Smith Oasis Korea; Chunan-si, Korea) were also cut with the clear plastic vials. The cut floral foam was then pushed down to the bottom of each vial to maintain water longer and hold a branch. One branch with egg masses was put in a plastic vial through the cut floral foam. Stilled water was provided egg masses without submerging them. Twenty prepared vials were put a S/T wire test tube rack with 50 holes (Korea Material Scientific; Seoul, Korea). Each rack was randomly allocated to one temperature among seven (12.4, 16.4, 20.4, 24.8, 28.3, 32.4, and 36.9 °C). These vials were checked daily for egg hatching and for replenishing water until eggs were not hatched anymore

during seven successive days from the last hatching at each temperature. After finishing the developmental experiment, barks of the branches were carefully taken off to count individual eggs of each egg mass under a microscope. All experiments were conducted under a photoperiod of 16:8 (L:D) h with relative humidity (RH) of 50-80 % using DS-8CL environmental chambers (Dasol Scientific; Hwaseong-si, Korea). Temperature and RH inside chambers were monitored using HOBO data loggers (U12-012, OnSet Computer Corp.; Pocasset, MA, USA). Average temperatures, not setting temperature, during experimental periods were used for data analyses.

Effect of temperature on development of eggs was tested with PROC GLM in SAS (SAS Institute, 2011). Means were separated by Tukey's honestly significant difference (HSD) test ($P < 0.05$; SAS Institute, 2011). Development rates of eggs were expressed as the reciprocal of developmental times (in days) of each stage by applying a linear model (Davidson, 1994) and a non-linear model (Brière et al., 1999). A linear model was applied to fit the linear portion of developmental rate using PROC REG in SAS (SAS Institute, 2011). The linear model was:

$$y = ax + b \quad (1)$$

where y was the developmental rate at temperature x ($^{\circ}\text{C}$), a was the slope,

and b was y -intercept. Lower developmental threshold was calculated as $-a/b$ (Arnold, 1959). 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 rate data over the entire temperature range. The non-linear model (Brière et al., 1999) used in this study was:

$$R(T) = n \times T(T - T_b)(T_L - T)^{1/2} \quad (2)$$

where $R(T)$ was the rate of development at temperature T ($^{\circ}\text{C}$), n was an empirical constant, T_b was the lower developmental threshold ($^{\circ}\text{C}$), and T_L was the upper developmental threshold. The optimum temperature (T_{opt}) at which the maximal rate of development occurred was estimated using the equation suggested by Brière et al. (1999). The operative thermal range (B_{80}) indicating ≥ 80 % performance of maximal rate was determined using the protocol of Lutterschmidt and Hutchison (1997).

Variation in developmental time for eggs was described by normalized cumulative distribution of the frequency of developmental time. Development times were standardized by physiological age (p_x) of immatures using Eq. (3):

$$p_x = \sum_{i=1}^n r(T_i) \quad (3)$$

where $r(T_i)$ was the developmental rate at temperature T ($^{\circ}\text{C}$) at day i . Physiological age (p_x) was transformed as degree-days (DD) by using thermal constant estimated from Eq. (1) and cumulated DD from January 1st to April 10th in Buyeo field conditions at the lower developmental threshold estimated from Eq. (1). Two-parameter Weibull function was then applied:

$$p(DD(p_x)) = 1 - \exp^{-(DD(p_x)/\alpha)^\beta} \quad (4)$$

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

Egg survival rate (%) was calculated by dividing the number of individuals survived to first instars by the initial number of individuals at each temperature. Effect of temperature on the survivorship of eggs was tested with PROC FREQ in SAS (SAS Institute, 2011). Means were separated by all pairwise Chi-square tests ($P < 0.05$; SAS Institute, 2011). The following model (Eq. 5) suggested by Sanchez-Ramos et al. (2015) was used:

$$S(T) = 100 - \exp^{a+bT+cT^2} \quad (5)$$

where $S(T)$ was the survival rate (%) at temperature T ($^{\circ}\text{C}$), and a , b , and c were fitted parameters. Operative thermal range (B_{80}) with ≥ 80 % performance of maximal rate was determined using protocols recommended by Lutterschmidt and Hutchison (1997).

2-3-2. Observation of first instar occurrence under field conditions

To validate the estimated degree-days required for *R. shantungensis* eggs from findings of our laboratory studies, development was monitored under field conditions at three chestnut commercial fields (Buyeo, Gwangyang, and Habcheon) in Korea in 2018. In these sites, one branch with *R. shantungensis* egg masses was covered with a meshed fabric pouch (45×10 cm, Eggthirty; Namyangju, Korea). The pouch was tied two times with fabric stings of the pouch and a water hose clamp (Home plus Co.; Seoul, Korea) to prevent hatched nymphs' escape from the pouch. Hatched *R. shantungensis* nymphs were checked every week from the middle of April until first instar nymphs did not occur anymore during three successive weeks from the last occurrence at each place.

For each site, accumulated degree-days were calculated from January 1st, 2018 to the last occurrence of *R. shantungensis* first instars using 11.6 $^{\circ}\text{C}$ and 36.9 $^{\circ}\text{C}$ as lower and upper developmental thresholds, respectively. An online

computer program (Degree-Day Utility, UC IPM Online) was utilized for degree-day calculations equipped with single sine and vertical upper-cutoff methods. Daily maximum and minimum air temperature data were obtained from Buyeo, Gwangyang, Habcheon weather stations of Korea Meteorological Administration located < 20 km away from the experimental sites. Based on previous studies (Choi et., 2012; Choi et al., 2016b; Kang et al., 2016), accumulated degree-days from January 1st, 2018 to the last occurrence of first instars of *R. shantungensis* in the commercial fields were also calculated using lower developmental threshold of each of previous studies to compare accuracy of prediction at 50 % first instar occurrence of *R. shantungensis* with this study.

Survivorships of populations between laboratory and field experiments were also compared to verify the reliability of laboratory experiments. Four egg survivorships, one laboratory population at 16.4 °C, and three field populations were tested with Chi-square test (SAS Institute, 2011). The reason for using the laboratory population at 16.4 °C was that the average of daily mean temperature in April and May of three field experimental sites was the closest to 16.4 °C among seven laboratory experimental temperatures. The reason that April and May temperature data were used was that degree-days for *R. shantungensis* egg were started to be accumulated from early April and first instar occurrence ended in May at all three sites.

2-3-3. Mapping 50 % occurrence timing of *R. shantungensis* first instars in Korea

Daily mean maximum and minimum air temperature data from 1981 to 2010 were collected from all weather stations (73 sites) and geo-coordinates of each weather station were obtained from Korea Meteorological Administration. For each site, accumulated degree-days were calculated from January 1st to thermal constant (DD) of *R. shantungensis* first instars in this study using 11.6 °C and 36.9 °C as lower and upper developmental thresholds, respectively. A computer program (Degree-Day Utility, UC IPM Online) was utilized for degree-day calculations by single sine and vertical upper-cutoff methods. From the calculated accumulated degree-days, Julian-days, 50 % occurrence of the first instar, were estimated for each site. The 50% occurrence timing of *R. shantungensis* first instars in Korea was visualized with ArcGIS 9.4 (Environmental Systems Research Institute; Redlands, CA, USA) by using inverse distance weighing as an interpolation method.

2-4. Results

2-4-1. Developmental and survivorship models of *R. shantungensis* eggs

R. shantungensis eggs could hatch from 16.4 to 32.4 °C (Table 1). Survivorship of eggs was significantly affected by temperature ($X = 213.7$; $df = 6$; $P < 0.001$). Development was also significantly affected by temperature ($F = 1,165.9$; $df = 4$; $P < 0.001$). Linear portions of developmental rate data were well fitted to the linear model ($F = 320.0$; $df = 1, 5$; $P < 0.001$) (Fig. 1, Table 2). Lower developmental threshold and thermal constant (in degree days) for eggs were estimated to be 12.1 °C and 202, respectively. The non-linear model provided a good fit for development rate of *R. shantungensis* eggs ($F = 363.2$; $df = 2, 5$; $P < 0.001$) (Fig. 2, Table 2). Upper developmental threshold and optimal temperature of eggs were 36.9 and 31.0 °C, respectively. Thermal ranges (B_{80}) with $\geq 80\%$ of the maximum value of egg development was 25.4 to 34.8 °C.

The survival model well described the relationship between egg survival and temperatures ($F = 8.7$; $df = 2, 5$; $P = 0.032$) (Fig. 3, Table 2). Temperature with the maximal survival (%) for egg was estimated to be 23.3 °C. Thermal range (B_{80}) with $\geq 80\%$ of the maximum value of egg survival was 17.6 to 29.0 °C. Egg survivorship at 16.4 °C of the laboratory experiment was similar

to that of field populations ($X = 5.9$; $df = 3$; $P = 0.117$).

2-4-2. Validation of laboratory experiment results

Developmental variation (i.e., distribution of developmental time) of *R. shantungensis* eggs according to degree-days was well described by the two-parameter Weibull function ($F = 266.9$; $df = 1, 18$; $P < 0.001$) (Fig. 4, Table 2). Average deviation between model prediction in this study and field occurrence was 3.5 days (Table 3). The deviation was decreased from early and final occurrence toward 50% occurrence of first instars (Fig. 5). Average deviations between model predictions and actual occurrences at 50% occurrence were 3 ± 1.0 (mean \pm SD), 18 ± 3.5 , 13 ± 3.1 , and 11 ± 1.5 days in this study, study of Choi et al. (2012), study of Choi et al. (2016b), and study of Kang et al. (2013), respectively (Table 4).

2-4-3. Mapping 50 % occurrence timing of *R. shantungensis* first instars in Korea

As an example of application of results of the present study, expected timing of 50 % occurrence of *R. shantungensis* first instars was mapped (Fig. 6). Occurrence timing was delayed from southern parts toward northern part of Korea while 50% occurrence of *R. shantungensis* first instars might occur from the end of May to early June in most areas of Korea peninsula (Fig. 6).

2-5. Discussion

Temperature played a pivotal role in determining the development and survival of *R. shantungensis* eggs. However, suitable thermal range of egg development (i.e., B_{80}) was much higher than that of egg survivorship. As temperature was increased from the optimal temperature (23.3 °C) for survival of *R. shantungensis* egg to the optimal temperature (31.0 °C) for development, egg survivorship was decreased and development rate was accelerated as a trade-off. At overlapping temperature range of 25.4-29.0 °C between optimal thermal ranges for development and survival of eggs, *R. shantungensis* eggs could show rapid development and high survivorship. Populations of *R. shantungensis* might increase quickly at local regions with these temperature ranges in spring.

Lower developmental threshold and thermal constants (DDs) obtained by linear models coupled with weather data can be used to predict phenology of *R. shantungensis* in the field (Pruess, 1983; Higley et al., 1986). There are multiple studies (Choi et al., 2012, Kang et al., 2013; Choi et al., 2016b) on egg development of *R. shantungensis* because egg hatching period is an optimal timing for pesticide application to control *R. shantungensis*. When deviations between 50% occurrence dates predicted from the lower developmental threshold and thermal constants of four studies (including our

study) and those observed in three sites of our study were estimated, our study showed the highest accuracy of prediction. These differences in the accuracy among these four studies could be caused by experimental problems and/or biotic factors such as food source, quantity, quality, and origin (Leather and Dixon, 1982; Kaakeh and Dutcher, 1993; Yamashita et al., 2005). Previous studies (Choi et al., 2012, Kang et al., 2013; Choi et al., 2016b) might have used setting temperatures for environmental chambers, not actual temperatures for the analysis and modeling to predict lower developmental threshold and thermal constants of *R. shantungensis* eggs because all temperatures in the previous studies were expressed as integral and there was no description in the papers on how temperatures were recorded during their experiments. As described in Table 1 of this study, differences between setting and actual temperatures in environmental chambers were not small, ranging from 0.3 to 0.9 °C. Egg development of *R. shantungensis* should be predicted with actually measured temperatures during the experiment, not initial setting temperatures. However, differences of results among these four studies could also be caused by biotic factors such as food source, quantity, quality, and origin (Leather and Dixon, 1982; Kaakeh and Dutcher, 1993; Yamashita et al., 2005). Branches with *R. shantungensis* eggs were collected from Japanese cornelian cherries, blueberries, and chestnut fields in the studies of Choi et al. (2012 and 2016b), the study of Kang et al. (2013), and our study,

respectively. Moreover, deviations between expected and observed ones were estimated from observed 50 % occurrence of *R. shantungensis* first instars in chestnut fields. Thus, application of these results in our study to other crops and trees needs to be careful.

This study provided reliable and accurate pesticide application timing for *R. shantungensis* in chestnut fields of Korea. However, results of this study indicated that occurrence periods of first instar nymphs of *R. shantungensis* were largely overlapped with flowering season of chestnut (Kim et al., 2003). Chestnut flowers are one of important honey sources in Korean apiculture. Currently, 50 products of pesticides are registered for *R. shantungensis* in Korea. Among them, 12 products among them are allowed to be applied in chestnut fields (RDA, 2018). All registered and applicable pesticides whose effective ingredients are gamma-cyhalothrin, deltamethrin, dinotefuran, chromafenozide, and etofenprox, have been reported to be toxic to honeybees. Thus, current pesticide application should be limited at highly contaminated areas by *R. shantungensis* considering their effects on bees. Moreover, non-toxic or low-toxic pesticides for honeybees need to be developed as soon as possible to increase the efficiency of pesticide application for *R. shantungensis*.

Up to date, the upper developmental threshold is not required to predict field occurrence of *R. shantungensis* first instars because spring temperature

has not been above the upper threshold of eggs in spring. According to thermal constant in the study of Choi et al. (2016b) to complete *R. shantungensis* development from eggs to adults, there is high possibility of occurrence of another generation when temperature is increased. In that case, upper threshold estimated in this study is needed to increase accuracy of prediction for the next generation under field conditions because upper developmental threshold of insect development has been commonly used to more accurately calculate degree-days from weather data among high temperature cut-off techniques (Roltsh et al., 1999).

Development and survival models coupled with climatic data are fundamental for prediction phenology in the field (Baek et al., 2008). They can be used to simulate the impact of global warming on distribution, abundance, and occurrence pattern in the future (Hance et al., 2007; Dixon et al., 2009). They can also be used to estimate the establishment potential of *R. shantungensis* if they are introduced into non-native locations (Hart et al., 2002). As an example of application of temperature-dependent experiment, 50 % occurrence timing for *R. shantungensis* first instars was predicted and mapped for all areas of Korea peninsula. This prediction could be applied from local areas to worldwide locations as described in this study if climatic data are acquired.

In summary, findings in this study quantitatively demonstrated thermal

development and survival of *R. shantungensis* eggs. In addition, theoretical results in the laboratory experiment were verified and validated with actual occurrence of *R. shantungensis* in fields. Moreover, the optimal application timing of pesticides for *R. shantungensis* was suggested based on results of this study.

Table 1. Developmental time (day, mean \pm SD) and survivorship of *R. shantungensis* eggs at constant temperature

Setting temperature (°C)	Actual temperature (°C)	Developmental time (day, mean \pm SD)	Survivorship (%)
12	12.4	-****	0d** (0/493)***
16	16.4	38.9 \pm 5.66a*	16.1a (78/483)
20	20.4	22.2 \pm 1.67b	11.4b (61/533)
24	24.8	15.0 \pm 3.51c	17.6a (89/507)
28	28.3	11.4 \pm 0.71d	9.7b (56/576)
32	32.4	10.4 \pm 0.50d	1.9c (11/594)
36	36.9	-	0d (0/488)
Total			(295/3,674)

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

** Survivorships within a column followed by the same letter are not significantly different ($P > 0.05$; Chi-square test)

*** Numbers in parentheses indicate the numbers of individuals that survived / total number of individuals tested

**** No individuals survived

Table 2. Parameter estimates (\pm SEM) of models for *R. shantungensis* eggs

Models	Parameters	Estimated values
Linear developmental model	a	$4.9300 \times 10^{-3} \pm 2.8347 \times 10^{-4}$
	b	$-5.6980 \times 10^{-2} \pm 6.6500 \times 10^{-3}$
Non-linear developmental model	n	$6.400 \times 10^{-5} \pm 4.052 \times 10^{-6}$
	T_b	12.1233 ± 0.78683
	T_L	$36.900 \pm 1.345 \times 10^{-8}$
Survival model	a	4.9859 ± 0.2024
	b	-0.0458 ± 0.0178
	c	$9.84 \times 10^{-4} \pm 3.55 \times 10^{-4}$
Distribution model of developmental time	α	240.8000 ± 0.8190
	β	17.4780 ± 1.2917

Table 3. Comparisons (days) of observed and predicted dates for the occurrence of *R. shantungensis* first instars at each observed time

Sites	Occurrence (%)	Actual DD	Expected DD	Difference (days)
Buyeo	50*	232.3	264.3	4
	75	246.2	269.3	4
	90.4	291.8	277.4	2
	98.1	353.0	285.8	8
	100	409.3	≥ 317	0
Gwangyang	18.2	192.6	241.2	10
	42.4	217.7	255.5	6
	50*	234.1	264.3	3
	75.8	289.6	269.6	2
	97.0	344.3	283.8	7
	100	425.1	≥ 317	0
Habcheon	29.4	222.7	248.9	4
	50*	241.2	264.3	2
	94.1	294.9	280.4	1
	100	348.4	≥ 317	0
Average				3.5

* 50% occurrence was estimated from the nearest two points around 50% through the linear regression

Table 4. Differences (days) between model predictions and observed data at 50 % cumulative occurrence of *R. shantungensis* first instars in four studies

	This study	Choi et al. (2012)	Choi et al. (2016b)	Kang et al. (2016)
Lower developmental threshold (°C)	12.1	4.75	7.4	9.7
Thermal constants (DD)	264.3	429.3	330.2	434.8
Buyeo	4	15	10	13
Gwangyang	3	22	16	10
Habcheon	2	18	14	11
Average (Mean±SD)	3±1.0	18±3.5	13±3.1	11±1.5

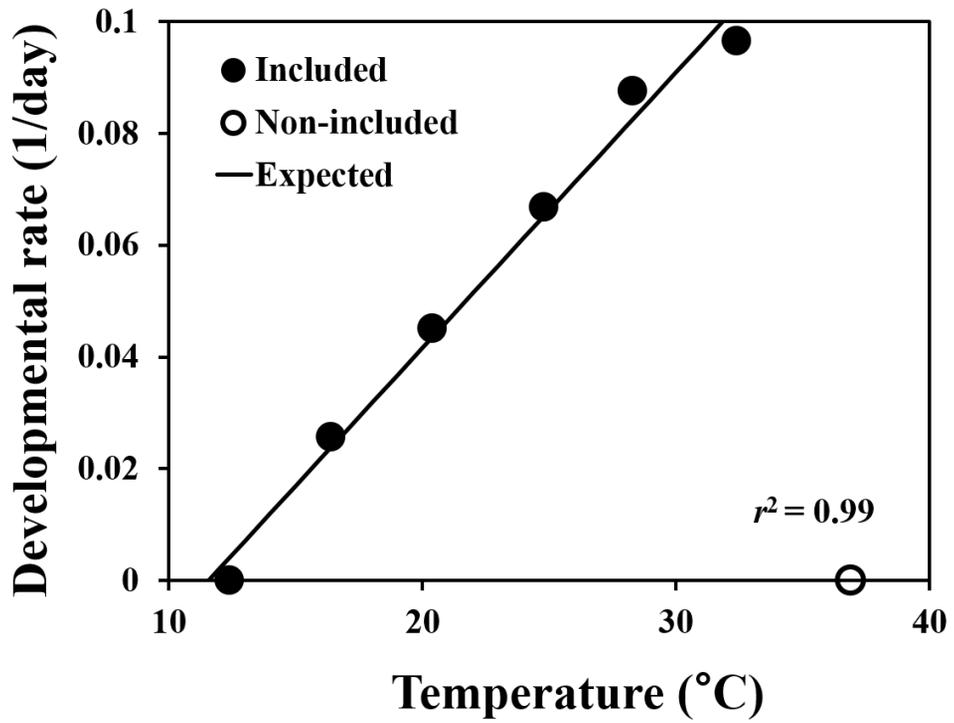


Fig. 1. Linear model of developmental rate (1/day) of *R. shantungensis* eggs at constant temperature.

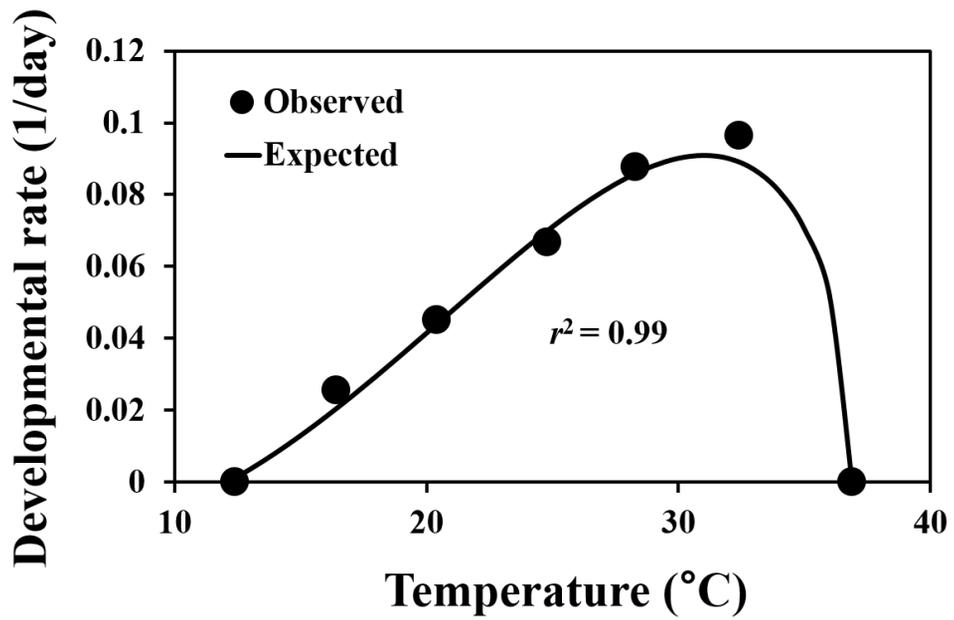


Fig. 2. Nonlinear model of developmental rate (1/day) of *R. shantungensis* eggs at constant temperature.

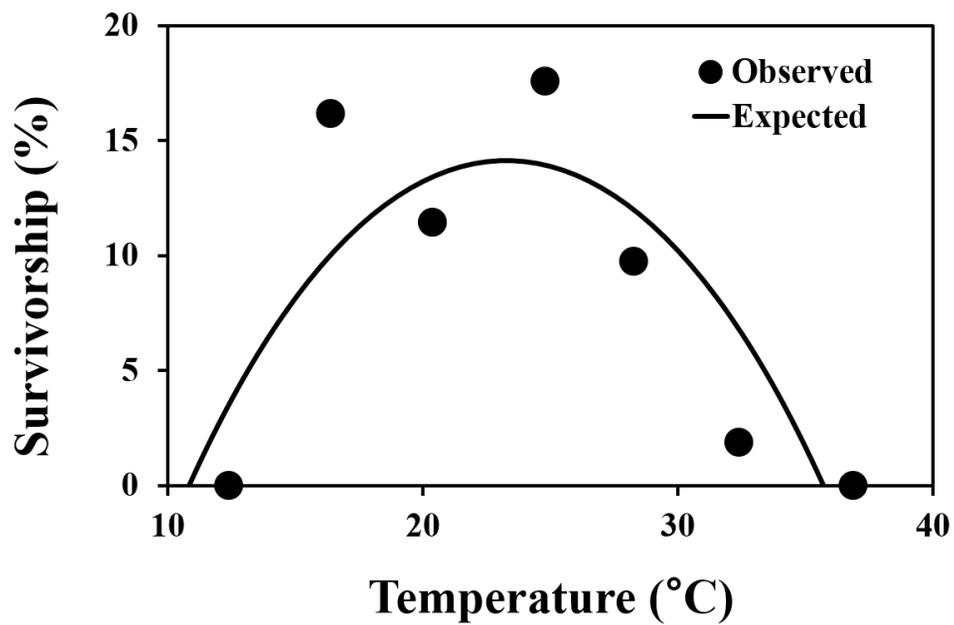


Fig. 3. Temperature-dependent model for survival (%) of *R. shantungensis* eggs.

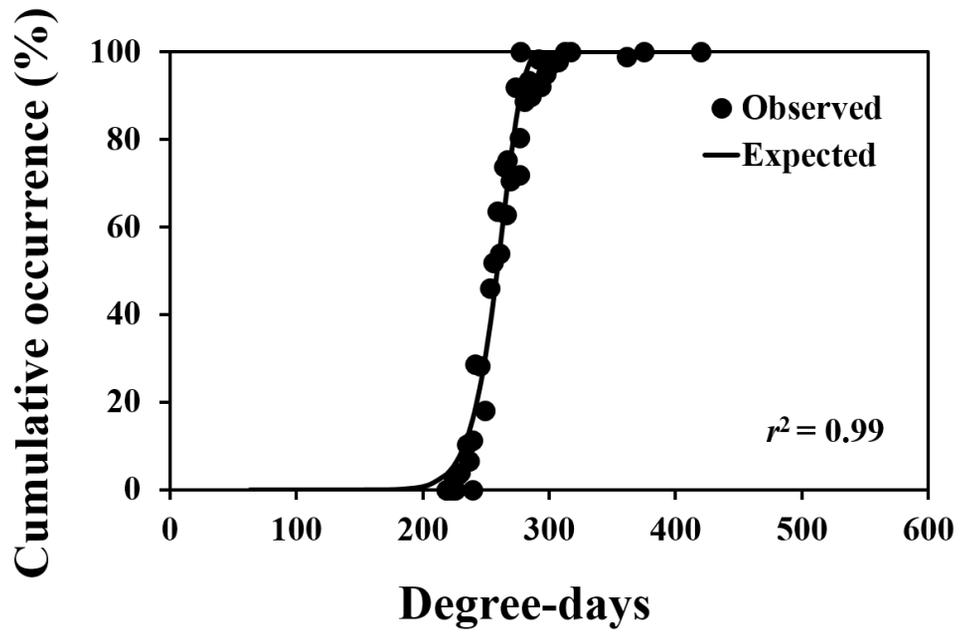


Fig. 4. Cumulative distribution of development of *R. shantungensis* eggs against accumulated degree-days.

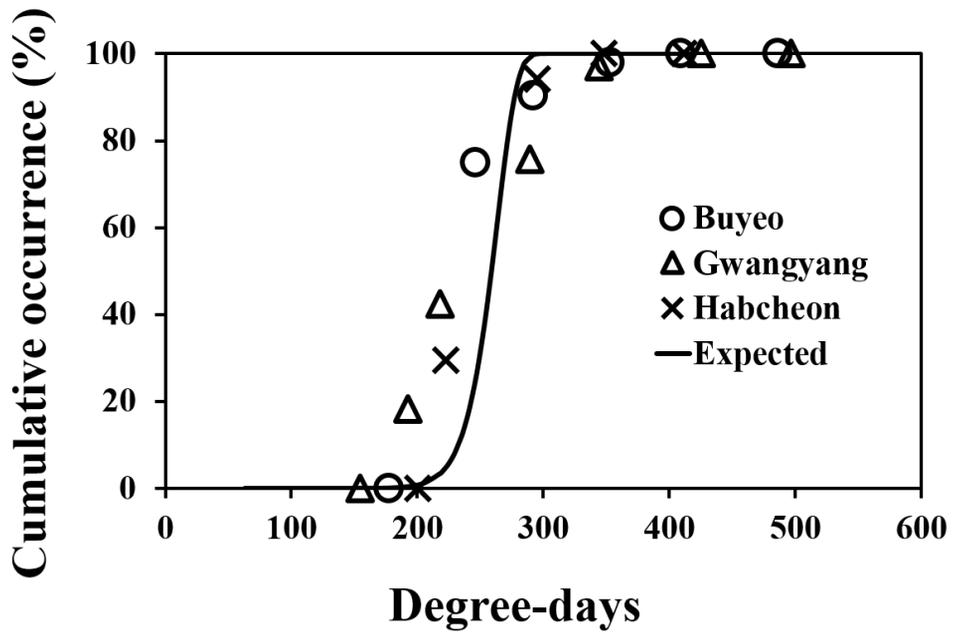


Fig. 5. Accumulative actual and predicted occurrence of *R. shantungensis* first instars. Total numbers of first instars observed in chestnut fields in Buyeo, Gwangyang, and Habcheon were 52, 33, and 17, respectively.

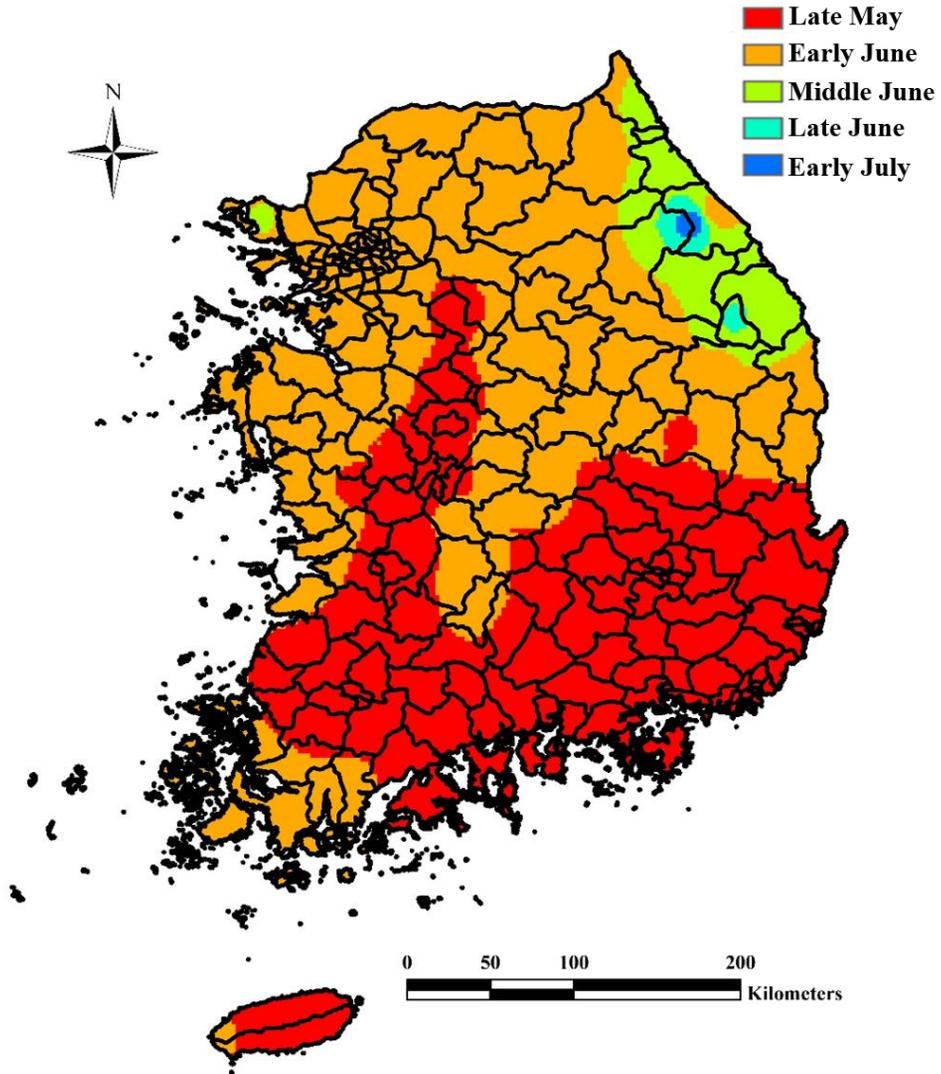


Fig. 6. Expected 50 % occurrence timing of *R. shantungensis* first instars based on 30-year average daily maximum and minimum data from 1980 and 2010 in Korea (suggested optimal application timing of pesticides for management of *R. shantungensis*)

Chapter III.

Spatial and temporal distribution of *Ricania shantungensis* in a chestnut field: implications for site-specific management, pruning, and management of neighboring forested areas

3-1. Abstract

Chestnut is one of the most important forest products in Korea, but economic damage by an invasive species, *Ricania shantungensis* has been increasing. To develop new management tactics for *R. shantungensis*, applicability of the site-specific management, and effects of pruning and management of abutting forested areas with chestnut fields on populations of this pest were evaluated. By observing selected, marked, and geo-referenced 115 trees in a commercial chestnut field, survey was conducted for each developmental stage of *R. shantungensis* (i.e., spring egg, nymph, adult, and winter (or fall) egg) in 2017 and 2018. Site-specific management for *R. shantungensis* would be applicable because spatial distribution of *R. shantungensis* was generally aggregated and consistent regardless of years and developmental stages. Moreover, pruning and management of abutting forested areas with commercial fields could decrease density of *R. shantungensis*. Integration of site-specific management, pruning, and management of abutting forested areas into the control program of *R. shantungensis* should increase the control efficiency in chestnut fields.

Key words: *Ricania shantungensis*; SADIE; semivariogram; spatial distribution; spatial association; spatial dependence; site-specific management

3-2. Introduction

Chestnuts are distributed from the middle subtropical zones to the northern temperate zones (Choo et al., 2001) and the main chestnut production countries are China, Korea, Italia, and Tukey (Son et al., 2004a). In Korea, chestnut was one of the most important export items among forest products by occupying 35% in the forest product export (Son et al., 2004b). These economically important chestnuts in Korea have been seriously damaged by *Ricania shantungensis*, an invasive insect (Kim et al., 2015).

After the first discovery in forests and orchards in Gongju and Yesan of Chungcheong-do in 2010, *R. shantungensis* quickly spread out to most of regions except for eastern parts of Korea, and its spreading is still in progress (Jo, 2014; Kang et al., 2016). Especially, the occurrence of *R. shantungensis* in agricultural areas in 2017 was increased by 109.6 % compared to 2016 (Hong et al., 2017). *R. shantungensis* is much more problematic in mid-mountain areas (Im et al., 2016) where most chestnuts are growing. Thus, the economic damage by *R. shantungensis* in chestnut fields would be growing continuously if proper management is not attempted.

However, the management of *R. shantungensis* is difficult due to the locations and large size of commercial chestnut fields. Although aerial

insecticide spray is preferred by chestnut growers for control of *R. shantungensis*, economic and environmental cost may not be justified. Moreover, all registered and applicable pesticides for *R. shantungensis* have been reported to be toxic to honeybees (RDA, 2018). In these circumstances, site-specific management for *R. shantungensis* could be a solution to optimize management cost and efficiency, and minimize environmental problem. The site-specific management is defined as a management of pests based on localized insect densities within a field rather than average densities throughout the area (Park et al., 2007). The site-specific management is theoretically possible because most insects are spatially aggregated within fields (Davis, 1994). Moreover, site-specific management should be more effective in case that pest distributions are consistent from year to year because pest distributions can be predicted (Park and Tollefson, 2006).

To evaluate applicability of site-specific management for *R. shantungensis*, spatial distribution of *R. shantungensis* should be identified. Insect distribution in any geographical areas can be divided into three spatial patterns: uniform, random, or aggregated (Krebs, 1999). There are multiple methods to characterize spatial distribution of an insect species such as Taylor's power law (TPL) (Taylor, 1961), Iwao's patchiness regression indices (Iwao, 1972), Ripley's *K* and *L* functions (Ripley, 1977; Diggle, 1983; O'Sullivan and Unwin, 2010), spatial analysis by distance indices (SADIE)

(Perry et al., 1995), Moran's I (O'Sullivan and Unwin, 2010), variogram (Isaaks and Srivastava, 1989; Rossi et al., 1992), Kriging (Cressie, 1993) and so on. Although these TPL and Iwao's indices are convenient and easy to implement, application of the site-specific management requires additional information (e.g., aggregated locations and consistency of spatial pattern). In these aspects, SADIE can be a good method to characterize distribution patterns of *R. shantungensis* in chestnut fields. SADIE can be used to show relative aggregation degree in each sample point, to analyze spatial association between different sampling timing, to provide statistical significance for the analyses of spatial aggregation and association, and to visualize the results of analyses (Perry et al., 1995; Perry et al., 1999; Perry et al., 2002).

As other fruit trees, chestnuts also require pruning processes to optimize production of chestnut fruits. Moreover, pruning could be an assistant method to decrease the population size of *R. shantungensis* because its eggs are readily observable on the branches. Thus, effects of pruning on *R. shantungensis* populations need to be evaluated.

R. shantungensis feeds on diverse hosts (Kim et al., 2015), and chestnut fields are generally located in mid-mountain areas (Im et al., 2016). Thus, presence of alternative hosts is inevitable in surroundings of chestnut fields. However, the effects of forested areas abutting chestnut fields on

population of *R. shantungensis* in chestnut fields has not been studied yet.

Therefore, this study was conducted to figure out effects of pruning and forest areas on *R. shantungensis* populations, and application possibility of the site-specific management for *R. shantungensis* in chestnut fields.

3-3. Material methods

3-3-1. Study site and sampling of *R. shantungensis*

A two-year field study was conducted in 2017-2018 to survey *R. shantungensis* populations in a chestnut field located in Jangyam-myeon, Buyeo-si, Chungcheongnam-do, South Korea (N 36.203759, W 126.856220). Approximately 8,000 chestnut trees were planted from the down to the ridge of hills at roughly 108,000 m² areas inside of a commercial field. There was a rice field, which is not a habitat of *R. shantungensis*, in front of the experimental field (Fig. 1). However, there were diverse shrubs and broadleaf trees suitable for *R. shantungensis* over the ridge of the hills (Fig. 1). Western parts of the field were neighboring with another chestnut field and there were no physical barriers between two fields.

A randomized fixed sampling was conducted. A chestnut tree was randomly selected and marked with pink-colored taffeta flagging tape (9WKP4, Presco Product Co.; Sherman, TX, USA) from east end toward west of the chestnut field. Selected trees were approximately 30 m away each other. Total 103 trees was selected. Trees located at steeped, inaccessible, or abutting areas with another chestnut field were excluded from the selection. In 2018, 12 shrubs or broadleaf trees were additionally selected and marked

along the road located on borders between the chestnut field and forest areas on the ridge of hills to examine effects of abutting forest areas with chestnut fields on *R. shantungensis* populations. Coordinates of each tree were measured using a differentially-corrected global positioning system (GPSMAP64, Garmin Ltd.; Olathe, KS, USA). Two branches per selected tree were randomly selected, and marked at approximately 50 cm away from the branch tip with the flagging tape. Sample unit, 50 cm from the branch tip, was selected based on Baek et al. (2015), in which *Adelgid tsugae* showed the lowest coefficient variations among three sample units (25 cm, 50 cm, and 100 cm branches). The ecology of *A. tsugae* is similar with *R. shantungensis*. Both hemipteran species feed plant saps on only newly developed branches of trees. According to Baek et al. (2015), the average density of *A. tsugae* was decreased and variation was increased beyond 50 cm from the branch tip because the new shoots of hemlock trees were rarely found beyond 50 cm from the tip within a branch. These characteristics of new shoot developments were similarly observed in chestnuts.

Sampling was conducted for each developmental stage of *R. shantungensis* (i.e., egg, nymph, and adult). Sampling dates in 2017 were April 4 (i.e., 2017 spring egg), June 27 (i.e., 2017 nymph), August 30 (i.e., 2017 adult), November 21 (i.e., 2017 winter egg). In 2018, sampling was taken on April 3 (i.e., 2018 spring egg), July 11 (i.e., 2018 nymph), August

14 (i.e., 2018 adult), and September 4 (i.e., 2018 fall egg).

Data of 2017 winter egg and 2018 spring egg were used to analyze the effects of branch pruning on egg density of *R. shantungensis*. Moreover, 2018 data were used to compare between densities of *R. shantungensis* in the chestnut field and neighboring forested areas.

3-3-2. Statistical analysis for effects of branch pruning and abutting forest areas in populations of *R. shantungensis*

The effect of pruning on egg density of *R. shantungensis* was analyzed by using *t*-test (SAS Institute, 2011). The comparison between densities of *R. shantungensis* in the chestnut field and neighboring forested areas was also conducted by using *t*-test (SAS Institute, 2011).

3-3-3. Characterization of spatial distribution

To calculate relative contribution to aggregation in each point and test the significance of spatial distribution patterns of each data set, SADIE (Perry, 1995) was used. SADIE measures the degree of clustering with patches and gaps. A patch is a region with relatively large number of *R. shantungensis*, and a gap is a region with relatively small number. To quantify the degree of clustering of *R. shantungensis* in the chestnut field, the overall aggregation index, I_a expressed as $I_a = D / E_a$, was used. D is distance to regularity which

is defined as the minimum total distance that individual samples in an observed arrangement would need to move to result in uniform or regular spatial distribution (Perry et al., 1999). Therefore, the higher D value, the higher degree of clustering of *R. shantungensis* in distribution. E_a is the mean expected distance to regularity. $I_a = 1$ means a spatially random pattern, $I_a > 1$ means a more aggregated pattern, and $I_a < 1$ means a more regular pattern (Perry et al., 1999). The significance test for spatial patterns was executed with formal randomization tests (Perry et al., 1999; Perry and Dixon, 2002) under the null hypothesis, the observed counts were arranged randomly among the given sample locations. SADIE also quantifies the contribution of each *R. shantungensis* count at each tree to a patch or a gap with unitless clustering indices (Perry et al., 1999). $v_i = 1$ or $|v_j| = 1$ means random distribution of *R. shantungensis* within the field, $v_i > 1$ means a unit belongs to a patch, and $v_j < -1$ means a unit belongs to a gap. In this study, two thresholds, 1.5 for v_i and -1.5 for v_j , were used to map patches and gaps of *R. shantungensis* within the field, representing clustering half as large as that expected by chance alone (Perry et al., 1999). All SADIE statistics were calculated with SADIShell version 1.22 (Rothamsted Experimental Station; Harpenden, Herts, UK). ArcGIS version 10.1 (ESRI; Redlands, CA, USA) was used to visualize the patches and gaps.

3-3-4. Spatial associations between two successive observations

SADIE was also used for spatial association analysis in which SADIE measures spatial associations between two successive observations and between 2017 spring and 2018 fall egg. The overall spatial association (X) is the mean of the local correlation coefficient between the clustering indices of the two sets; $X > 0$ for positive spatial association, $X = 0$ for no spatial association, $X < 0$ for negative spatial association (Perry and Dixon, 2002); positive X values indicate the coincidence of a patch cluster for one set with a patch cluster for the other or the coincidence of two gaps and negative X values indicated by a patch coinciding with a gap (Perry and Dixon, 2002). The associated possibility (P) also was calculated based on randomization tests (Perry and Dixon, 2002) as the spatial aggregation analysis (Perry et al., 1999). The null hypothesis is that the spatial arrangement of the count data between two data sets is random (Park et al., 2006). In this study, $X > 0$ indicates consistent spatial association, $X = 0$ indicates no spatial association, $X < 0$ indicates an opposite change of distribution because samplings were conducted at same site during eight times in two years.

3-4. Results

3-4-1. Population dynamics of *R. shantungensis*

Even though the number of egg masses was lower in 2018 than in 2017, the numbers of nymphs and adults were higher in 2018 than in 2017 (Table 1). The population densities (mean \pm SE) of *R. shantungensis* in forested areas were 10.4 ± 3.59 , 3.3 ± 1.05 , 2.0 ± 0.93 , and 2.2 ± 0.63 per two 50 cm branches for spring egg masses, nymphs, adults, and winter egg masses, respectively. Pruning of chestnut branches marginally reduced the density of egg masses (t value = 1.6; $P = 0.056$) with reduction of approximately 27 % (average 1.0 per two 50 cm branches) of *R. shantungensis* egg masses. The density of *R. shantungensis* in forested areas was not statistically higher than density in the chestnut field except for spring egg masses: spring egg (t value = 4.7; $P < 0.001$), nymph (t value = 1.0; $P = 0.167$), adult (t value = 0.3; $P = 0.376$), and fall egg (t value = 1.6; $P = 0.075$).

3-4-2. Characterization of spatial distribution

The spatial distribution of *R. shantungensis* eggs and adults generally showed aggregation patterns even though random patterns were found in spring and fall eggs in 2018 (Table 1, Fig. 2). However, nymphs showed

random patterns during two years (Table 1, Fig. 2). Distribution maps depicting spatial locations of patches and gaps indicated generally consistent spatial distribution patterns of *R. shantungensis* regardless of developmental stages.

3-4-3. Spatial associations between two successive observations

Spatial associations between two successive developmental stages of *R. shantungensis* showed statistically positive spatial associations ($P < 0.025$) except for the associations between nymph and adult in 2017 and between spring egg and nymph in 2018 ($0.025 < P < 0.975$) (Table 2). Spatial distribution of *R. shantungensis* eggs in 2017 spring was also positively associated with spatial distribution of eggs in 2018 fall ($P < 0.025$) (Table 2).

3-5. Discussion

For site-specific management, the spatial aggregation of target insect pests is required (Park et al., 2007). Consistent distribution pattern would increase the efficiency of site-specific management because distributions of the pest can be predicted (Park and Tollefson, 2006). *R. shantungensis* generally showed spatially aggregated patterns and positive spatial associations in the experimental site except for few exceptional cases during two years. Thus, site-specific management for *R. shantungensis* could be applicable in chestnut fields.

R. shantungensis utilizes diverse host plants in landscapes, and its nymphs and adults are actively mobile. Therefore, understanding their spatial distribution and temporal population dynamics is a key to manage them in chestnut fields. In 2017, adult density was higher than nymphal density, indicating that adult *R. shantungensis* should disperse into chestnut trees from different host plants. Choi et al. (2016a) reported that adults of *R. shantungensis* moved to arboreal plants even though adults during pre-ovipositional period showed statistical ($P < 0.05$) preference on sunflowers than arboreal plants. Sunflowers might serve for nutrition supply for adult *R. shantungensis* before oviposition, and then adults move into arboreal plants

for oviposition.

This study also showed population dynamics of *R. shantungensis* in a chestnut field during two years. Among developmental stages of this pest, egg showed the highest density. Even though this could be a main reason to become a key stage of sampling or management, there are other reasons for us to focus egg stage of *R. shantungensis*. First, nymphs and adults are difficult for sampling and control because they are very active (Kang et al., 2013; Choi et al., 2016a). Second, both nymphs and adults can cause damage by feeding and its honeydew. However, nymphs prefer herbaceous plants rather than trees (Choi et al., 2016a) and their damage is trivial compared to damage by egg-laying processes (Kwon et al., 2017). Third, it is difficult to find relationships between the nymphal densities and their damage on chestnuts due to their ecological characteristics (e.g., activeness, movement between on arboreal and herbaceous plants, higher preference for herbaceous plants than arboreal plants, and so on). If adults are used to develop a fidelity between its densities and damage of chestnuts, its damage could be overestimated because adults can stay for feeding without laying eggs (Choi et al., 2016b). Damage by *R. shantungensis* is mainly caused by egg-laying processes which are to make cracks on newly developed branches and to block currency of plant saps with their eggs (Jo, 2014; Choi et al., 2016b; Kwon et al., 2017). Thus, sampling or management plans should be

developed for egg stage of *R. shantungensis* by considering occurrence density and ecological characteristics of each developmental stage.

Adult *R. shantungensis* showed a higher degree of spatial aggregation, indicating that they would prefer specialized environmental conditions for oviposition. The characteristics such as amount of newly developed branches and hardness of branch pellicles might affect mother's choice because *R. shantungensis* females lay eggs on only newly developed branches (Kim et al., 2016a), prefer higher branches due to relatively high amount of newly developed branches (Kim et al., 2017a), and select scratchable branches for oviposition (Jo, 2014). Eggs also showed spatially aggregated patterns because their spatial distribution was determined by their mothers. However, there was no spatial relationship between nymph and adult occurrence in 2017. This might be caused by the movement of *R. shantungensis* adults toward chestnut trees from herbaceous plants (Choi et al., 2016a). No spatial relationship between spring egg and nymph in 2018 might be caused by nymphal dispersal to adjacent herbaceous plants which would be easier for feeding rather than tree leaves or branches. Nymphal development on herbaceous plants were already reported in previous studies (Jo, 2014; Kim et al., 2016a).

Pruning of branches in ornamental trees are required to control the quality and quantity of fruits. In this study, pruning of chestnut branches

showed the effect of approximately 27 % decrease of egg mass density even though the effect was not statistically significant at 95 % confidence level. Moreover, the hatchability of *R. shantungensis* eggs in pruned braches was less than 1 % in peaches and blueberries due to low moisture content (Kim et al. 2017b) and contaminated branches with eggs masses were easily recognizable. Physical control in insect pest management could be a good option because this can be compatible with other pest management tactics for integrated pest management. Thus, pruning of branches contaminated with *R. shantungensis* egg masses are highly recommended. However, it should be completed as soon as possible to minimize successful hatching of eggs. If then, pruning could be helpful to decrease the density of *R. shantungensis*.

Due to locational characteristics of chestnut fields, chestnut fields are inevitably abutting with forested areas. In this study, the density of only winter egg masses in forested areas abutting the experimental site was higher than the density of chestnut fields. The other developmental stages (i.e., nymph, adult, and fall eggs) of *R. shantungensis* were not significantly different. The statistically higher density of *R. shantungensis* egg masses in springs might be caused that chestnut growers did not manage forested areas abutting their chestnut fields. Therefore, abutting forested areas with commercial chestnut fields need to be managed to increase control efficiency of *R. shantungensis*.

In conclusion, site-specific management, earlier pruning, and management of abutting forested areas are highly recommended to be integrated into control program of *R. shantungensis* in chestnut fields. If then, the control efficiency of *R. shantungensis* should be increased and the populations of *R. shantungensis* would be manageable.

Table 1. SADIE statistics for spatial distribution of *R. shantungensis*

Sample year	Developmental stage	Mean (\pm SE)*	I_a^{**}	P_a
2017	Spring egg	4.3 \pm 1.04	1.7	0.01
	Nymph	0.6 \pm 0.18	1.0	0.46
	Adult	1.0 \pm 0.17	2.2	0.01
	Winter egg	3.7 \pm 0.55	1.7	0.01
	Pooled	9.4 \pm 0.89	2.1	0.01
2018	Spring egg	2.7 \pm 0.38	1.3	0.10
	Nymph	2.2 \pm 0.38	1.1	0.33
	Adult	1.7 \pm 0.26	1.7	0.01
	Fall egg	1.4 \pm 0.17	1.3	0.10
	Pooled	7.8 \pm 0.72	1.6	0.03
Pooled		17.2 \pm 1.35	2.1	0.01

* Overall degree of clustering (I_a) with its associated P values (P_a). $I_a = 1$ suggests a random, $I_a > 1$ suggests an aggregated, and $I_a < 1$ suggests a regular spatial distribution pattern.

** Density of *R. shantungensis* per two 50-cm branches

Table 2. Spatial association of *R. shantungensis* occurrence between two successive developmental stages

Year	Selected times	X^*	P
2017	Spring egg vs. Nymph	0.42	< 0.001
	Nymph vs. Adult	0.18	0.035
	Adult vs. Winter egg	0.22	0.012
Transition	Winter egg vs. Spring egg	0.32	< 0.001
2018	Spring egg vs. Nymph	0.15	0.090
	Nymph vs. Adult	0.28	0.004
	Adult vs. Fall egg	0.51	< 0.001
2017 Spring egg vs. 2018 Fall egg		0.38	< 0.001

* Index of association (X) with its associated probability (P). For a two-tail test at 95% confidence level, $P < 0.025$ indicates significant positive association and $P > 0.975$ indicates significant negative association.

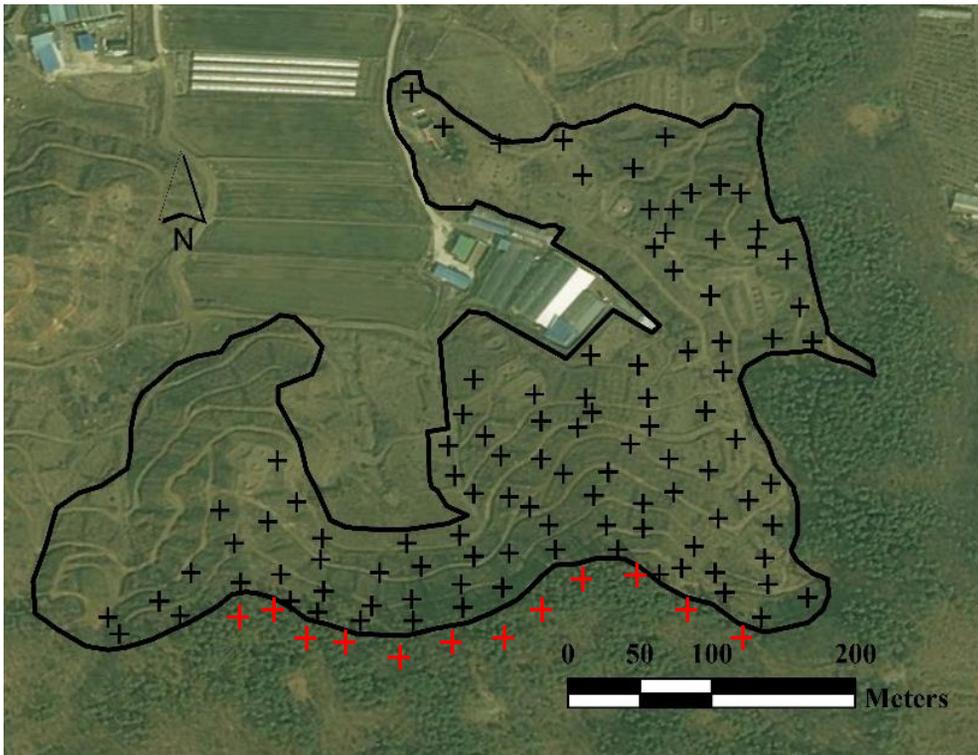


Fig. 1. Location of 115 geo-referenced selected trees in the study site (N 36.203759, W 126.856220) (□ boundary of experimental site, + selected chestnut trees, + selected shrubs in forested areas)

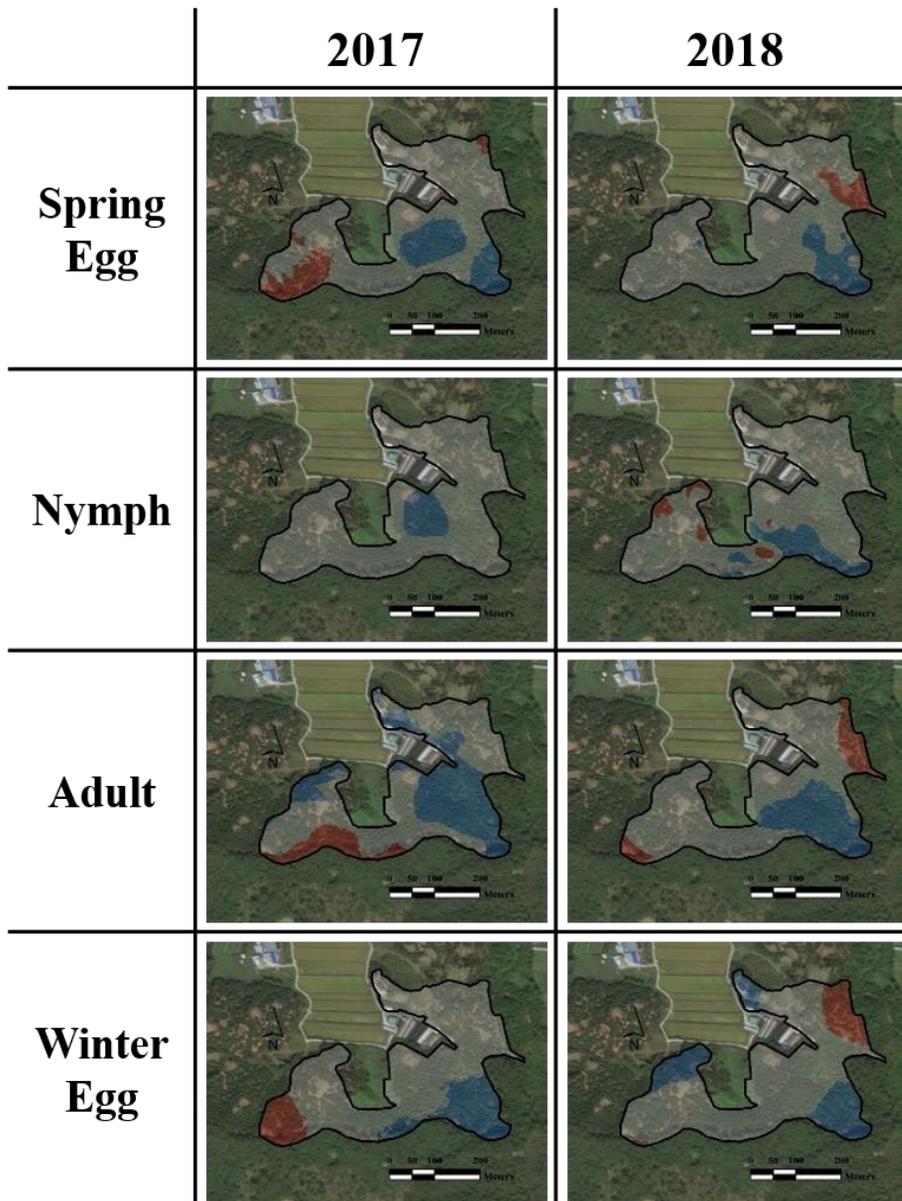


Fig. 2. Distribution maps showing patches and gaps in densities of *R. shantungensis* at each developmental stage in 2017-2018. Red areas indicate patches with clusters of relatively large counts (i.e. $v_i > 1.5$) and blue areas indicate gaps with clusters of relatively small counts (i.e. $v_j < -1.5$).

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한국에서의 갈색날개매미충의 시·공간 통태 연구

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초록

갈색날개매미충은 2010년 충청도 공주와 예산 지역에서 처음 발견된 이후 빠르게 국내 전역으로 확산하여 심각한 경제적 피해를 야기하고 있다. 이에 이 연구는 (1) 국내에서 갈색날개매미충의 피해를 최소화하기 위해 현재 및 미래 분포를 예측하고, (2) 갈색날개매미충의 최적 방제 시기를 예측하며, (3)

갈색날개매미충에 대한 효율적인 방제 전략을 개발하기 위해 이 해충에 대한 개체군 동태 및 공간분포 특징을 규명하였다.

이번 연구는 갈색날개매미충의 현재 분포, 미래분포를 예측하기 위해 CLIMEX와 MaxEnt를 사용하였다. CLIMEX에서 변수의 가중치와 MaxEnt에서 관련 변수 선정에는 SADIE의 환경변수와 갈색날개매미충의 발생 자료의 상관 지수를 이용하였다. CLIMEX의 결과는 한국의 전역이 갈색날개매미충의 발생에 적합한 것으로 나타났다. MaxEnt에서는 가장 더운날의 최고 기온, 연중 평균 기온, 가장 추운달의 평균 기온, 가장 건조한 달의 강우량 등이 갈색날개매미충의 분포에 크게 기여하는 요소로 평가되었다. 이번 연구 결과들은 갈색날개매미충은 국내 서쪽 지역에서 발생 확률이 높지만 동쪽으로 점차 확산될 가능성이 높은 것으로 나타났다.

갈색날개매미충이 국내 전역으로 확산될 가능성이 높기

때문에 갈색날개매미충의 방제 적기에 대한 연구가 반드시 필요하다. 갈색날개매미충의 가장 효율적인 방제 적기는 알에서 1령 약충이 갓 부화한 시기인 것으로 알려져 있다. 이에 1령 발생 시기를 추정하기 위해 12.4, 16.4, 20.4, 24.8, 28.3, 32.4, 36.9°C에서 부여 밤 포장에서 채집한 알의 온도 발육실험을 진행하였다. 갈색날개매미충의 발육영점온도(lower developmental threshold)는 12.1°C로 추정되었으며, 이때 1월 1일부터 1령 50% 발생까지 필요한 누적온열량은 202DD로 예상되었다. 이러한 실내 실험 결과는 부여, 광양, 합천 지역에서 야외 발생을 잘 예측할 수 있었다.

갈색날개매미충의 최적 방제 시기에 대한 연구는 국내 밤재배단지에서 갈색날개매미충의 정확하고 신뢰할 수 있는 최적 방제시기를 제시하였지만, 이 시기는 밤 꽃의 개화시기와 상당부분 겹치는 문제가 있다. 또한 현재 등록된 모든 화학

약제들은 꿀벌에 독성이 강한 것으로 알려져 있다. 이에 갈색날개매미충의 개체군 관리를 위한 새로운 전략들이 필요하다. 이번 연구에서는 이러한 새로운 방제 전략들은 개발하기 위해 정밀방제(site-specific management)의 적용 가능성, 가지치기와 밤 재배단지에 인접한 산림 지역이 갈색날개매미충 개체군에 미치는 영향이 평가되었다. 실험은 부여에 있는 상업적 목적의 밤 포장에서 103그루의 밤나무와 13그루의 활엽수를 대상으로 2년 동안 갈색날개매미충의 발육단계에 따라 갈색날개매미충의 밀도를 조사하였다. 그 결과 갈색날개매미충의 분포는 전체적으로 집중분포를 보였으며 공간분포는 2년동안 변하지 않아, 갈색날개매미충은 정밀방제에 적합한 해충으로 판단되었다. 또한 가지치기와 밤 포장과 접하는 산림지역에서 갈색날개매미충의 관리는 이 해충의 밀도를 관리하는데 도움을 줄 수 있을 것으로 판단되었다. 정밀농업, 이른 가지치기, 포장과 인접한 산림지역을

방제하면 갈색날개매미충의 방제 효율이 급격히 올라갈 수 있을 것으로 기대된다.

이번 연구는 갈색날개매미충의 현재 및 미래 국내분포, 갈색날개매미충의 방제 적기, 밤 재배단지에서 적용가능한 갈색날개매미충 방제를 위한 전략들을 제시하였다. 이 연구 결과들은 갈색날개매미충 관리를 위한 큰 그림을 세우고, 방제 적기에 약제를 살포하는 것과 더불어 새로운 방제 전략들을 적용함으로써 갈색날개매미충의 방제 효율을 올려 줄 수 있을 것으로 기대된다.

검색어: 갈색날개매미충, 종분포모형, 발생특성, 정밀방제, 공간분포,

방제적기