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Difference of Gall Formation Rates and Parasitic Rates of *Thecodiplosis japonensis* (Diptera: Ceidomyiidae) Larvae in Pine Forests around Urban and Mountain Villages

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Abstract

This study analyzed and compared the damage rate, natural parasitic rate, and the morphological characteristics of *Thecodiplosis japonensis* larvae, which inhabit forest areas as control areas to large urban areas in 2018 and 2019. This research was conducted to provide basic data for the management of *Thecodiplosis japonensis*, which harm pine needles, and the results were as follows. First, the gall formation rate of *Thecodiplosis japonensis* collected from urban areas was upper-crown 35.59% and 34.25%, mid-crown 25.57% and 27.95%, and lower-crown 25.34% and 26.61%; the gall formation rate of *Thecodiplosis japonensis* was in the order of upper-crown>mid-crown>lower-crown in 2018 and 2019, respectively. In the control areas, the gall formation rates of *Thecodiplosis japonensis* in mountain villages in 2018 and 2019 were upper-crown 17.72% and 21.78%, mid-crown 13.85% and 16.97%, and lower-crown 15.12% and 15.79%; thus, in the order of upper-crown>lower-crown>mid-crown. The number of larvae in the galls of needles damaged by *Thecodiplosis japonensis* was as follows: the average number of larvae in the pine trees of urban areas was 9 and 8 in the upper-crown, 7 and 8 in the mid-crown, and 6 and 7 in the lower-crown respectively. This shows that the number of larvae was fewer in the lower-crown than the upper-crown, and that the number of larvae was higher in 2018 than in 2019. For natural parasitic rate of *Thecodiplosis japonensis*, the gall formation rate and natural parasitic rate of *Thecodiplosis japonensis* were surveyed; the natural parasitic rate was 12.5% and 11.8% in urban areas while the rate was 21.7% and 20.9% in mountain villages in respectively in 2018 and 2019.

Key Words: *Thecodiplosis japonensis*, gall formation rate, *Thecodiplosis japonensis*-damaged needles, natural parasitic rate

Introduction

In South Korea, the damages caused by *Thecodiplosis japonensis* (Uchida et Inouye) as well as related biological and ecological phenomena were difficult to predict and quantify. *Thecodiplosis japonensis* are widespread in South Korea and Japan, and its damages were identified in the palace garden of Seoul and the water sources of Mokpo, Jeollanam-do for the first time in 1929 (Takaki 1929). From these two points of source, *Thecodiplosis japonensis* spread gradually, making entire South Korea an infected region and causing damages nationwide. South Korea's research on the research on *Thecodiplosis japonensis* is now unparalleled as to study the ecology and control of this pest from the mid-1950s to the present to combat the phenomenon unique to South Korea.

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Thecodiplosis japonensis forms galls in the Japanese red pine and Japanese black pine, inhibiting arboreal growth. While damaged needles wither in the same year in pine trees and survive until the following year in Japanese black pines, continued damage causes trees to die due to impeded arboreal growth by declined photosynthesis, weaker tree energy, and the formation of secondary pests (Yim et al. 1981).

Many quantitative and qualitative studies have been conducted on *Thecodiplosis japonensis*. Examples in South Korea and abroad include studies on the history, ecology, and control method of *Thecodiplosis japonensis* as well as the hydrothermal fluctuation of forest lands damaged by *Thecodiplosis japonensis* (Lee et al. 2000) as well as on materials to use pine trees damaged by *Thecodiplosis japonensis* (Hwang et al. 2001) which are underway. One study examined the characteristics of dead and survived trees in areas damaged by *Thecodiplosis japonensis* (Chung et al. 2000). Another study demonstrated that when *Thecodiplosis japonensis* invade a new area, the gall formation rate increases rapidly for 6 generations, causing a marked growth impediment or even death of pine trees and then showing a sharp decrease in density afterwards; the change process in the gall rate occurs over 12 generations (Park and Hyun 1983). *Thecodiplosis japonensis* causes severe damage when it newly invades and proliferates in undistributed areas. Although the trees become stable once the damage is recovered, it is imperative to research the causes of recovery in the recovered areas, morphological characteristics of larvae, and the recurrence of the damage.

A study examined intra-generational and inter-generational density fluctuations by comparing the regions in the early stage and the recovery stage of *Thecodiplosis japonensis*, and reported that the differences in larval mortality rates between regions were due to larval fatality and its natural enemy, parasitic wasp (Lee 1986). Considering its distribution status in South Korea, the *Thecodiplosis japonensis* in the southern and northern regions have passed through about 60 generations in isolation from each other since the first occurrence, implying the possibility of genetic differentiation. This was reported by an ecological variation survey of *Thecodiplosis japonensis* in two regions, Muan, Jeonnam and Hoengseong, Gangwon (Hwang and Yim 1990). In a study on the population dynamics of *Thecodiplosis japonensis*, its mortality rate for each ecologically identical growth stage was 23.1% for the reduction rate of dendritic population density in warm season and the larval mortality rate inside the gall was 14.2%; the population reduction rate of soil inhabitation period was 25.0% before wintering, 52.9% in the mating stage, and 32.0% in the pupal stage. Here, the study reported that the moisture content of soil and its variation are the most important as the lethal factors, and that the larvae become smaller and the eclosion rate decreases in places with the severe damage by this pest (Park and Hyun 1977). A study (Han 1985) on the several biological characteristics of Korean *Thecodiplosis japonensis* by region examined the biological variations in the region. *Thecodiplosis japonensis*, which are parasitic on and damage pine trees and black pine trees, are considered to appear in a variety of forms compared to the pine forest that grows soundly around urban areas, in terms of habitat distribution and morphological features.

This study analyzed and compared the damage rate, natural parasitic rate, and morphological characteristics of larvae, focusing on pine needles damaged by *Thecodiplosis japonensis*, and by dividing pine forest sites into large urban areas and mountain villages. The study was conducted to provide basic data for establishing the prevention and control measure of *Thecodiplosis japonensis*.

Materials and Methods

Survey selection and overview

This study selected the urban forest area located in Geumjeong-gu, Busan, as well as Wang mountain aera of Geumseo-myeon, Sancheong-gun, Gyeongnam as the control area. The survey district selected three urban areas and

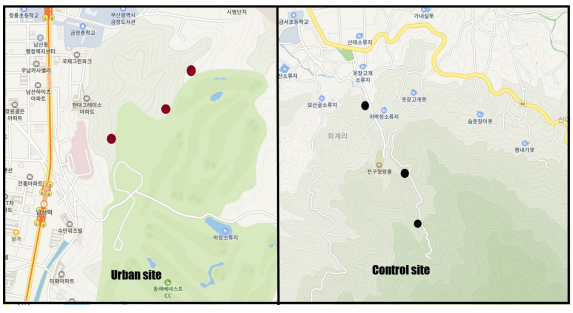

Fig. 1. The location map of the sampling sites.

three control areas, and five pine trees per survey site based on the four directions of east and west and north and south from the pine trees (Fig. 1).

Research methods and classification

This study first analyzed and compared the gall formation rate of *Thecodiplosis japonensis* in the selected urban areas and mountain villages. The study randomly determined the gall formation rate from 5 survey trees in each of the 6 sites, and divided the crowns of trees selected from the survey areas for two years in 2018 and 2019 into upper, mid, and lower-crown. After, the study randomly collected the new shoots growing in each of the two pine trees in the same year for four directions, and calculated the gall formation rate as the ratio of the number of needles forming galls to the total number of needles. Then, the study examined the length of needles damaged by *Thecodiplosis japonensis* and the number of larvae and natural enemies in the larvae body after extricating the larvae from the gall. This study used a stereomicroscope and a digital caliper as the experimental tools.

100 sample needles forming galls which were collected from the survey sites of *Thecodiplosis japonensis* were separated to examine the lengths of larvae and damaged needles using the caliper, and the number of larvae inside the gall of damaged trees was examined using the stereomicroscope. Here, the body length and width of the larvae extricated from the needles forming galls were examined using the stereomicroscope, and about 20 larvae samples were used in this study. 1,000 larvae in the galls that were collected from each of the survey sites were randomly selected to investigate the natural parasitic rate of Inostemma seoulis that are parasitic on *Thecodiplosis japonensis*, using the stereomicroscope.

Results and Discussion

Gall formation rate of Thecodiplosis japonensis

This study analyzed the gall formation rate on pine needles from an area of forests damaged by *Thecodiplosis japonensis*, by dividing them into upper-crown, mid-crown, and lower-crown. The study also collected the new shoots of pine needles that formed *Thecodiplosis japonensis* gall by classifying urban areas and mountain villages. The findings showed in terms of the gall formation of *Thecodiplosis japonensis*, gall formation rate increased for 6 generations when the imago of *Thecodiplosis japonensis* invades a new region and rapidly decreases afterwards. Such process of changes in the damage rate of the gall formation rate tends to progress for over 12 generations. Thus, this research selected pine forests in urban areas, which were severely damaged by *Thecodiplosis japonensis* during the early 1980s, and clean mountain villages where pine tree forests grow spontaneously in groups, and investigated the gall for-

Table 1. Gall formation rate of pint needle gall midge in the needle leaves of pine tree by year and survey sites (mean±SE)*

Site	Shoots location	Year					
		2018			2019		
		No. of samples	No. of gall-formed pine needles	Gall formation rate (%)	No. of samples	No. of gall-formed pine needles	Gall formation rate (%)
Urban (Busan)	Upper	826	294	35.59±2.17^{a}*	987	338	34.25±2.07^{a}
	Middle	927	237	25.57±2.03^{b}	973	272	27.95±2.32^{ab}
	Low	872	221	25.34±3.01^{b}	1,007	268	26.61±2.02^{b}
	Mean	875	250.67	28.83	989	29.60	29.60
Control (Sancheong)	Upper	1,027	182	17.72±2.01^{a}	1,088	237	21.78±1.89^{a}
	Middle	989	137	13.85±1.87^{b}	1,102	187	16.97±1.77^{b}
	Low	1,002	457	15.12±1.97^{ab}	1,089	172	15.79±1.98^{b}
	Mean	1,006	152.33	15.12	1,093	198.67	18.18

*Means with the same letter are not significantly different at the 5% level for Duncan's multiple range test.

mation rate of *Thecodiplosis japonensis* in the regions. The results were shown as below (Table 1).

The gall formation rate of *Thecodiplosis japonensis* collected from pine forests in urban regions averaged upper-crown 35.59% and 34.25%, mid-crown 25.57% and 27.95%, and lower-crown 25.34% and 26.61% in 2018 and 2019, respectively. Therefore, the gall formation rate was in the order of upper-crown > mid-crown > lower-crown, and it was higher in 2019 when compared by year. The gall formation rate of *Thecodiplosis japonensis* in mountain villages was upper-crown 17.72% and 21.78%, mid-crown 13.85% and 16.97%, and lower-crown 15.12% and 15.79% in 2018 and 2019 respectively. The order was upper-crow n > lower-crown > mid-crown as a result, and the gall formation rate was higher in 2019. The overall average gall formation rate for urban areas and mountain villages was 28.83% and 29.60%.

The gall formation rate of urban areas and mountain villages in 2018 and 2019 was 28.83% and 29.60% respectively in urban areas while it was 15.12 and 18.18% respectively in mountainous villages, showing that the rate was 1.9 and 1.6 times higher in urban areas than mountain areas, the control site. These are the results of decline in pine forests due to air pollution around the Ulsan Chemical Industrial Complex, as air pollutants are introduced into soil, inhibiting root growth and development in the pine forest. This was similar to a research finding that the acidification of forest soil due to acid rain led to the destruction of wax layer in pine needles and thus decline in the resistance of pine trees, increasing the gall formation rate of *Thecodiplosis japonensis* (Han 1984; Bae et al. 1987; Seo et al. 1995).

Pine trees are known as a very vulnerable species to air pollutants. A study reported that due to the influx of many air pollutants into the forest ecology and acidic deterioration, chemical and physical properties of the soil have degraded, caused decline in the vitality of pine forests, and reduced growth, thus increasing the formation of *Thecodiplosis japonensis* (Park and Hyun 1983). Such weaker resistance against pests in forest ecology has made pests to inhabit and thrive in forests, which has also increased the gall formation rate of *Thecodiplosis japonensis*. In recent years, *Thecodiplosis japonensis* tends to grow in places with a high nitrogen and calcium content, such as in urban and industrial complex areas, waste storage sites, and pollutant landfills. The rising population of *Thecodiplosis japonensis* in nearby pine forests was caused by an increase of sulfur oxides and nitrogen oxides emitted from petrochemical factories or urban areas,

Table 2. Comparison on damaged and healthy needles of pine trees by survey sites

Site	Shoots location	Year			
		2018		2019	
		Length of damaged needles (cm)	Length of healthy needles (cm)	Length of damaged needles (cm)	Length of healthy needles (cm)
Urban (Busan)	Upper	4.5	9.2	4.9	9.8
		3.8-5.5	8.7-9.8	4.2-4.8	9.7-9.9
	Middle	4.6	9.4	4.7	9.9
		4.0-5.8	9.1-9.7	4.5-4.9	9.7-10.1
	Low	4.9	10.5	4.8	10.7
		4.4-5.4	10.1-10.9	4.5-5.1	10.2-10.9
	Mean	4.67	9.70	4.70	10.13
Control (Sancheong)	Upper	4.9	11.0	5.1	11.5
		4.8-5.2	10.9-11.0	4.9-5.2	11.4-11.6
	Middle	5.2	11.5	5.2	11.6
		5.0-5.4	11.4-11.6	5.0-5.4	11.5-11.7
	Low	5.2	11.7	5.2	11.5
		5.1-5.4	11.5-11.9	5.1-5.4	11.4-11.6
	Mean	5.10	11.40	5.17	11.53

thereby increasing the gall formation rate of *Thecodiplosis japonensis*.

Needles damaged by Thecodiplosis japonensis

The results from collecting and examining *Thecodiplosis japonensis*-damaged needles in urban areas and mountain villages show that (Table 2) the damage of pine needles by *Thecodiplosis japonensis* tend to not to have significant changes from when new shoots grow in the spring until the end of June. Thus, the lengths of pine needles were compared according to Ko's method (1968), using the length of the healthy pine needles and the *Thecodiplosis japonensis*-damaged needles from early July when gall starts forming until mid-October when the larvae growth is almost finished. Based on the findings that the damaged needles do not grow after gall is formed at the pine needle base, this study calculated the lengths of healthy needles and damaged needles by collecting the new shoots of pine trees that have the signs of damages occurred in the pine forest for two years.

The lengths of damaged and healthy needles of pine trees collected from urban area pine forests for two years in 2018 and 2019 were measured. According to the results, the average length of damaged needles was 4.67cm and 4.70cm respectively, while the average length of healthy needles was 9.70cm and 10.13cm respectively. In mountain villages, the measurements showed that the length of damaged needles averaged 5.10 cm and 5.17 cm per year, respectively, and the length of healthy needles averaged 11.4 cm and 11.53 cm per year, respectively. The results show that in urban areas and mountain villages, there were length differences of damaged needles that formed *Thecodiplosis japonensis* gall. A study that examined the morphological characteristics of *Thecodiplosis japonensis* in pine forests around the industrial complex areas reported that the length of healthy needles was 0.9 times higher than the length of needles damaged by *Thecodiplosis japonensis* (Lee 2017).

Another study found that there were no significant length differences between healthy leaves and damaged needles caused by *Thecodiplosis japonensis* according to the gall formation rate between the two areas (Hwang and Yim 1990). However, when compared to the regions where pollutants are released into the air due to automobile exhaust and urban housing heating to form urban environmental pollution and smog, the difference of damaged needles was 0.9 times in healthy regions and the length of healthy needles had a difference of 0.7 times. These differences suggest that the damage symptoms exacerbate in both damaged and healthy needles due to the decline of pine forests in urban areas, which affect the growth of pine trees as well as the needles damaged by *Thecodiplosis japonensis*.

Number of larvae in the galls of needles damaged by Thecodiplosis japonensis

The following table shows the number of *Thecodiplosis japonensis* larvae inhabiting the gall of base and damaging the pine needles collected from urban areas and mountain villages (Table 3).

The number of *Thecodiplosis japonensis* larvae inhabiting the pine needle base was examined for two years in 2018 and 2019. The results indicate that the average number of larvae in the urban area pine forests was 9 and 8 in the upper-crown, 7 and 8 in the mid-crown, and 6 and 7 in the lower-crown respectively, showing that the average number of larvae was lower in the mid and lower-crown than in the upper-crown and was higher in 2019. The average number of larvae in the mountain pine forests was 8 and 7 in the upper-crown, mid-crown 5 and 7, and lower-crown 6 respectively; thus, the number of larvae was lower in the

Table 3. The number of larvae per gall in survey sites

Site	Shoots location	Year	
		2018	2019
		No. of larvae per gall	
Urban (Busan)	Upper	9	8
		2-10	2-10
	Middle	7	8
		2-9	2-10
	Low	6	7
		2-9	2-8
	Mean	7.33	7.67
Control (Sancheong)	Upper	8	7
		2-9	2-9
	Middle	5	7
		2-7	2-8
	Low	6	6
		2-7	2-8
	Mean	6.33	6.67

mid-crown and lower-crown than the upper-crown and was higher in 2019. The average numbers of larvae in the upper, middle, and lower-crown were 7.33 and 7.67 respectively, and the number of larvae in the mountain villages was 6.33 and 6.67 respectively. In addition, the size of *Thecodiplosis japonensis* larvae in urban and mountain villages was 0.86 and 0.91 time respectively, showing that the number of larvae inhabiting urban areas was higher than mountain villages. These results were consistent with a study which found that the damage rate of *Thecodiplosis japonensis* was higher in the pine needles of urban area forests than mountain village forests.

The study results indicate that the number of larvae inhabiting the base of damaged pine needles in the forests of urban areas and mountain villages was higher in urban areas, which were deteriorating due to environmental pollution. More favorable conditions for the growth of *Thecodiplosis japonensis* larvae in the pine needles of pine forests were developed, as a result of weaker resistance in pine forests due to environmental pollution in industrial complex areas; the inhabitation density of *Thecodiplosis japonensis* increases in the pine forests of regions with environmental pollution (Ferrel 1980; Kim et al. 1985). The comparison of results implies it is consistent with a study result that the number of larvae inhabiting urban area forests, which emit higher environmental pollutants than mountain villages.

Length of mature larvae in the needles damaged by Thecodiplosis japonensis

To examine the length of mature larvae of *Thecodiplosis japonensis* at the base of pine needles, the study collected the upper, middle, and lower-crown of pine trees from urban areas and mountain villages, the control area respectively. The study induced the extrication of larvae from the needles which formed *Thecodiplosis japonensis* gall, and measured the body length of 50 extricated mature larvae as follows (Table 4).

The mature larvae, whose growth was completed from the base of pine needles forming the gall of *thecodiplosis japonensis*, was extricated and examined over two years in 2018 and 2019. The average length of mature larvae in urban area pine forests was upper-crown 2.41 mm and 2.42 mm, mid-crown 2.42 mm and 2.41 mm, lower-crown 2.41 mm and 2.42 mm respectively; therefore, there was no statistical difference in the body width of mature larvae in upper, middle, and lower-crown by year. The average length of mature larvae in the upper, middle and lower-crown of pine trees in urban areas was 2.41 mm and 2.42 mm respectively in 2018 and 2019, showing no difference by year. Next, the average length of the mature larvae of pine trees in mountain villages was upper-crown 2.42 mm and 2.42 mm, middle-crown 2.42 mm and 2.43 mm, lower-crown 2.43 mm and 2.42 mm respectively; therefore, there was no statistical difference in the body length of mature larvae in the upper, middle, and lower-crown by year. The average length of mature larvae in the upper, middle, and lower-crown of pine trees in mountain villages was 2.42 mm and 2.42 mm respectively in 2018 and 2019, showing that there was no statistical difference by year. Such results indicated that there was no huge difference in the body length change of mature larvae in polluted and non-polluted zones. It was reported that the maturity of *Thecodiplosis japonensis* larvae was related to the temperature of growth stage (Skuharvy 1994); thus, this study also divided the urban areas and mountain villages into polluted and non-polluted zones. Since thee was no huge temperature difference as the survey areas did not have huge latitude difference, it was considered to have no changes in the size of mature larvae. Future studies should examine the effect on the maturity stage and length of *Thecodiplosis japonensis*, which inhabit southern regions as well as in northern and central regions with relatively higher altitude, to vary the latitudes

Table 4. Larval body length of of pine trees pine needle gall midge in survey sites (mean±SE)

Site	Shoots location	Year	
		2018	2019
		Body length of larvae (mm)	
Urban (Busan)	Upper	2.41±0.12	2.42±0.08
	Middle	2.42±0.14	2.41±0.07
	Low	2.41±0.09	2.42±0.08
	mean	2.41	2.42
Control (Sancheong)	Upper	2.42±0.13	2.42±0.12
	Middle	2.42±0.12	2.43±0.11
	Low	2.43±0.14	2.42±0.11
	Mean	2.42	2.42
Standard deviation		0.008	0.006

of survey sites more significantly.

Width of mature larvae in the needles damaged by Thecodiplosis japonensis

To examine the width of mature larvae of *Thecodiplosis japonensis* at the base of pine needles, the study collected the upper, middle, and lower-crown of pine trees from urban areas and mountain villages, the control area respectively. The study induced the extrication of larvae from the needles which formed *Thecodiplosis japonensis* gall, and measured the body width of 50 extricated mature larvae as follows (Table 5).

The mature larvae, whose growth was completed from the base of pine needles forming the gall of *thecodiplosis japonensis*, was extricated and examined over two years in 2018 and 2019. The average body width of mature larvae in urban area pine forests is upper-crown 0.71 mm and 0.72 mm, mid-crown 0.72 mm and 0.71 mm, and lower-crown 0.71 mm and 0.72 mm respectively; thus, there was no statistical difference in the body width of mature larvae in upper, middle, and lower-crown by year. The average body width of mature larvae in the upper, middle and lower-crown of pine trees in urban areas was 0.71 mm and 0.72 mm respectively, showing no difference by year. Next, the average body width of mature larvae of pine trees in mountain villages is upper-crown 0.71 mm and 0.73 mm, mid-crown 0.72 mm and 0.72 mm, and lower-crown 0.71 mm and 0.72 mm, respectively; thus, there was no statistical difference in the body width of mature larvae in upper, middle, and lower-crown by year. The average body width of mature larvae in the upper, middle, and lower-crown of pine trees in mountain villages was 0.71 mm and 0.72 mm respectively, showing that there was no statistical difference by year. Such results reported that there was no huge difference in the body width change of mature larvae found in Douglas fir in the region with air contamination (Wickman 1981). This study also classified the urban areas and mountain villages into polluted and non-polluted zones but found no changes in the body width of larvae.

Table 5. Larval body width of pine needle gall midge in pine trees (mean±SE)

Site	Shoots location	Year	
		2018	2019
		Body width of larvae (mm)	
Urban (Busan)	Upper	0.71±0.11	0.72±0.12
	Middle	0.72±0.08	0.71±0.09
	Low	0.71±0.07	0.72±0.09
	Mean	0.71	0.72
Control (Sancheong)	Upper	0.71±0.11	0.73±0.09
	Middle	0.72±0.07	0.72±0.08
	Low	0.71±0.07	072±0.07
	Mean	0.71	0.72
Standard deviation		0.005	0.008

Natural parasitic rate of Thecodiplosis japonensis

To examine the natural parasitic rate of Isostasius seoulis which are parasitic on *Thecodiplosis japonensis*, this study randomly selected 1,000 larvae from the galls collected from each survey site and calculated the parasitic rate of Isostasius seoulis with a stereoscopic microscope (Table 6).

Table 6 shows the results of investigating the gall formation rate and natural parasitic rate of *Thecodiplosis japonensis* at survey sites. The natural parasitic rates were 12.5% and 11.8% respectively in 2018 and 2019 in urban areas, and 21.7% and 20.9% respectively in mountain villages. Such results of examining pests on the stereomicroscope demonstrate that the natural parasitic rate of *Thecodiplosis japonensis* is less than 10%. This suggests the necessity of the transplantation of natural enemy and the prevention and control of *Thecodiplosis japonensis*. Next,

Table 6. Natural enemy parasitism rate and gall formation rate of infested pine needle gall midge

Sites and year	Natural enemy parasitism rate (%)		Gall formation rate (%)	
	2018	2019	2018	2019
Urban (Busan)	12.5	11.8	28.83	29.60
Control (Sancheong)	21.7	20.9	15.12	18.18

the gall formation rate was 28.83% and 29.60% respectively in 2018 and 2019 in urban areas, which was a "medium" damage rate, while the rate was 15.12% and 18.18% respectively in mountain villages, which was a "light" damage rate, suggesting no need for the prevention or control of *Thecodiplosis japonensis* in the future. The natural parasitic rate of *Thecodiplosis japonensis* was less than 10% in forests with "middle" or higher damage rate, implying that natural enemy transplantation and medicine prevention and control must be carried out as biological methods. Therefore, since the damage rate is increasing year by year despite the stable natural parasitic rate in the pine forests of urban areas, it is necessary to examine the damage rate and natural parasitic rate of *Thecodiplosis japonensis* by continuous monitoring to conduct pest prevention and control.

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